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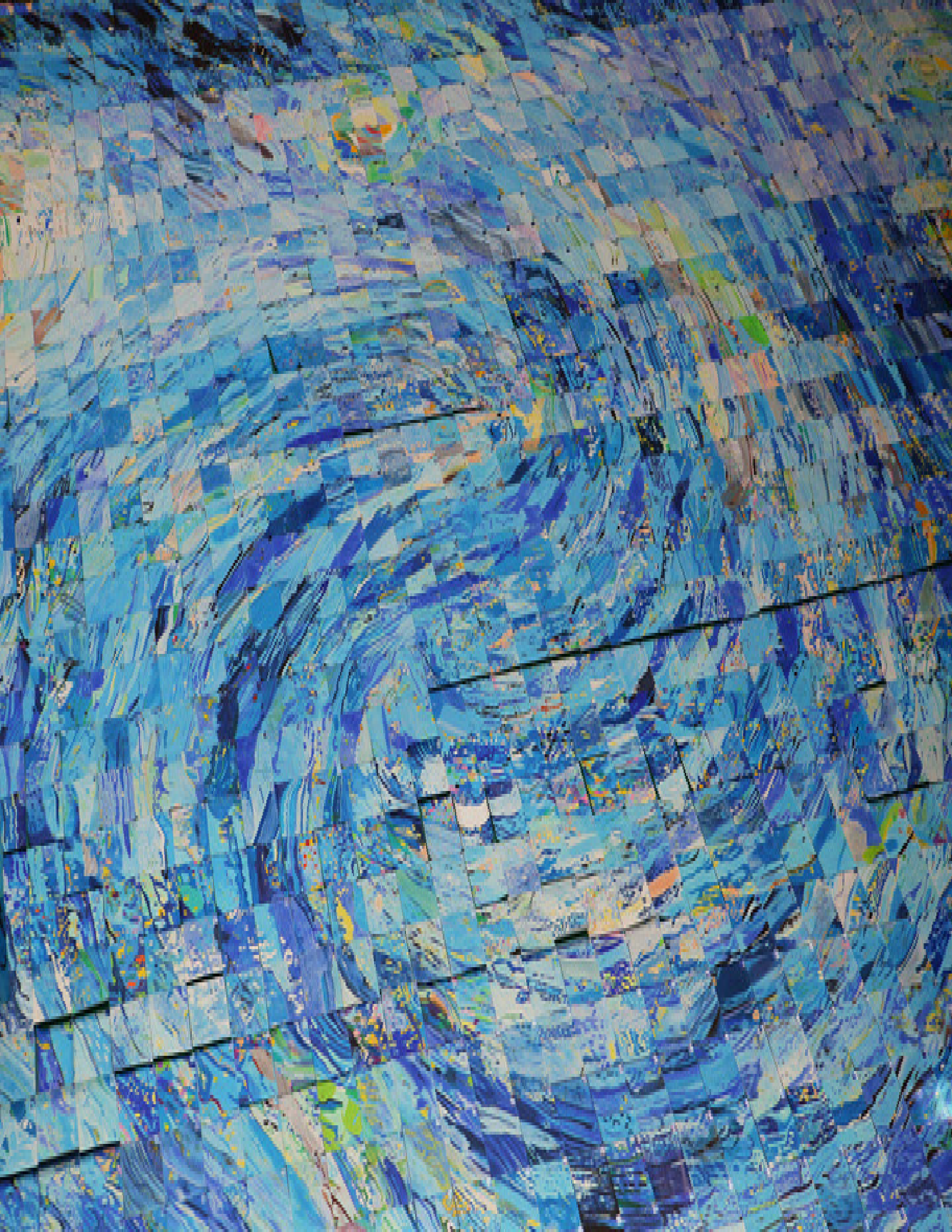
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The Impact of Aspirin on Stem Cells and Growth Factors: Roles in Dentistry

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Abstract

The periodontal ligament (PDL) contains a unique population of mesenchymal stem cells (MSCs), also known as PDL stem cells (PDLSCs). The regenerative properties of PDLSCs offer much potential for stem cell-based therapy, particularly for periodontal or bone regeneration. Aspirin (ASA) is a widely used nonsteroidal anti-inflammatory drug (NSAID) that has been reported to modulate a variety of diseases, such as cardiovascular, diabetes, and cancer. This review article focuses on the impacts of ASA on various stem cells. First, we will explain what constitutes PDLSCs and their derivation from periodontal tissues. Then we will discuss the mechanisms of ASA and its effect on periodontal tissues. Next, we focus on aspirin's effects on the differentiation of various types of stem cells. Finally, we investigate the effects of ASA on growth factors that could enhance the osteoblastic potential of derived stem cells. (**International Journal of Biomedicine. 2023;13(2):188-193.**)

Keywords: acetylsalicylic acid • periodontal ligament • stem cells

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Abbreviations

ASA, acetylsalicylic acid; ARS, Alizarin red S; BMP, bone morphogenetic protein; BMMSCs, bone marrow mesenchymal stem cells; COX, cyclooxygenase; GDF, growth differentiation factor; FGF, fibroblast growth factor; MSCs, mesenchymal stem cells; NSAID, nonsteroidal anti-inflammatory drug; PDL, periodontal ligament; PDLSCs, PDL stem cells; PEG, poly(ethylene glycol); PG, prostaglandin; qPCR, quantitative polymerase chain; SCs, stem cells; TSCs, tendon stem cells; VEGF, vascular endothelial growth factor.

Periodontal Ligament Stem Cells (PDLSCs) derived from Periodontal Ligament (PDL) tissue

Periodontal ligament (PDL) is a soft dynamic connective tissue located between the cementum of the root and the alveolar bone.^(1,2) PDL tissues consist of heterogeneous populations of cells, including fibroblast, endothelial, osteoblast, and cementoblast cells.⁽³⁻⁶⁾ They play a pivotal role in maintaining homeostasis and regeneration of the periodontal tissues.^(7,8) The PDL cells are formed by the cells residing within dental follicle cells during embryogenesis.⁽⁹⁾

The human PDLSCs were first isolated from human-impacted third molars.⁽¹⁰⁾ Numerous studies then indicated

that mesenchymal stem cells (MSCs) isolated from PDL have properties that are similar to bone marrow mesenchymal stem cells (BMMSCs).^(8,10-12) Like BMMSCs, the PDLSCs demonstrated the ability to self-renew and generate clonogenic adherent colonies with spindle and elongated-shaped cells. Moreover, PDLSCs are capable of forming various types of mesodermal origin cells, such as osteoblasts, chondrocytes, cementoblasts, and neural-like cells.^(1,8,10,12)

Periodontal regeneration is a method of regenerative therapy to return the periodontal tissues (including gingiva, root cementum, alveolar bone, and the PDL) to their original healthy condition through the restoration of form and

function of lost structures.⁽¹³⁾ NSAIDs are widely used as an analgesic agent in healthcare. They may be used in managing orthopedic patients pre/post-surgery, to address acute or chronic inflammation. However, such use of NSAIDs may have an undesirable impact on stem cell function, particularly in periodontal or bone regeneration.⁽¹⁴⁻¹⁸⁾

Aspirin and its mechanism

ASA, also known as acetylsalicylic acid, is a NSAID mainly used as an analgesic, antipyretic, and anti-inflammatory. ASA exerts its anti-inflammatory action by suppressing the production of prostanoids (thromboxanes, prostacyclins, and prostaglandins),⁽¹⁹⁾ which are produced by COX-1 and COX-2 enzymes. Prostaglandins (PG), known as prostanoids, are formed when arachidonic acid is released from the plasma membrane by phospholipases and metabolized by the sequential actions of prostaglandin G/H synthase, or COX. COX is a bifunctional enzyme exhibiting both cyclooxygenase and peroxidase activities.⁽²⁰⁾ PG synthesis is triggered by COX activity. The COX enzyme exists in two different forms, namely COX-1 and COX-2. COX-1 has a “housekeeping” role, is constitutively expressed, and plays important roles in physiological functions, such as platelet aggregation, gastric lining protection, and homeostasis.⁽²¹⁾ COX-2 is an inducible enzyme that is implicated in pathophysiological processes, which can lead to inflammation, pain, and fever.⁽²²⁾

COX-1 is expressed in both normal and fractured bones, while COX-2 is upregulated, especially during the initial stages of the bone repair process. Both osteoblasts and osteoclasts produce prostaglandins, where PGE2 is the most abundant PG synthesized. COX and PG play important roles in bone homeostasis,⁽²³⁻²⁵⁾ specifically COX-2, which has a key role in PGE-2 production and is important in osteoblast formation.⁽²⁵⁾ The effect of PGs on bone metabolism is mediated through the PGE receptor types 2 and 4 (EP2 and EP4). In osteoblast formation, PG could increase the proliferation and differentiation of osteoblasts, while in bone resorption, PG could increase the activity of the osteoclast. The inhibition of cyclooxygenase, particularly COX-2, prevents the elevation of PG levels, molecules related to the induction of inflammation, which can lead to various pain sensations.⁽²⁶⁾

Osteogenic differentiation

The good effect of ASA on the enhancement of the osteoblastic potential of derived stem cells has been confirmed by a few studies either *in vitro* or *in vivo*.⁽²⁷⁻³²⁾ In *in vitro* studies, the experiment begins with cell differentiation of osteogenic derived from various sources such as dental stem cells, BMSCs, and adipose stem cells using osteogenic media and accompanied by modulations in the expression of multiple osteogenic gene markers.^(28,29) The confirmation of osteogenic differentiation can be verified via cellular and molecular levels. The mineralized nodule formations were investigated by ARS staining, following which expression levels of osteogenesis markers were assayed by qPCR or western blot.

Beneficial impact of ASA on enhance osteogenic potential (The aspirin's effect on the differentiation of various types of stem cells)

ASA has been reported to modulate a variety of conditions related to human diseases, such as cardiovascular

disease, periodontal health, cancer, and diabetes.^(33,34) The impact of ASA on stem cell properties has been reported in a number of studies^(27-32,35-37) (Table 1).

Table 1.

The effects of ASA on different stem cell in vitro studies [adapted from Zafarmand et al.⁽³⁷⁾]

Differentiation	Cell type	Aspirin		In brief
		Dose (μM)	Duration (days)	
Osteogenesis	PDLSC	250–1000	Up to 21	Facilitation in osteogenesis.
	DPSC	≤560	Up to 21	
	MSC	≤800	Up to 21	
	SHED	≤280	3	
	BM-MSC	280- 800	14	

A study by Wang et al.⁽²⁷⁾ showed that ASA could promote tenogenesis of tendon stem cells (TSCs) via the SMMAD pathway. The study involved RNA sequencing, and the results showed that GDF6, GDF7, and GDF11 were upregulated in the induction medium with the ASA compared to the induction medium group. GDF7 increased tenogenesis and activated Smad1/5 signaling. This showed that ASA promotes TSC tenogenesis and tendinopathy healing through GDF7/Smad1/5 signaling.

Our previous study⁽²⁸⁾ showed that ASA at 1,000μM enhances the osteogenic potential of PDLSCs. Using a fold change (FC) of 2.0 as a threshold value, the gene expression analyses indicated that 19 genes were differentially expressed. FGF9, VEGF-A, VEGF-C, and FGF2 were markedly upregulated (FC range, 6 to 15), whereas pleiotropin, FGF5, brain-derived neurotrophic factor, and DKK1 were among those markedly downregulated (FC 32). This study showed that ASA modulates the expression of growth factor-associated genes and enhances osteogenic potential in PDLSCs. ASA upregulated the expression of genes that could activate biological functions and canonic pathways related to cell proliferation, human embryonic stem cell pluripotency, tissue regeneration and differentiation. ASA could enhance PDLSCs' function.⁽²⁹⁾ Thus, ASA enhances PDLSC function and may be useful in regenerative dentistry applications, particularly in the areas of periodontal health and regeneration.

We suggest that ASA acts as a regulator for FGF1 to bind with the FGFR1 receptor and activates several bone-related marker genes. FGFs are heparin-binding proteins and signal through binding to the tyrosine kinase in the intracellular region of the FGR receptor (FGFR). The FGFRs contain an extracellular ligand-binding domain, a transmembrane region, and an intracellular-divided tyrosine kinase domain. The binding of FGFs to FGFRs enables the autophosphorylation of tyrosine in the intracellular region of FGFR, leading to the activation of intracellular downstream signaling pathways, such as Ras/MAPK, protein kinase B, protein kinase C, phospholipase C, and also the p21 pathways.⁽³⁸⁾ In addition, Miraoui et al.⁽³⁹⁾ reported that FGFR2 acts as a novel regulatory

molecule that promotes osteogenic differentiation in murine MSCs. The effect of FGFR2 is mediated by PKC α and ERK1/2 pathways that have critical roles in FGFR2-induced osteogenic differentiation of murine MSCs.

A study by Xie et al.⁽³⁰⁾ showed that low-dose aspirin (<100 μ g/mL), which is widely recommended for the prevention of thrombosis, is very likely to be beneficial for maintaining bone mass and qualities by activation of osteoblastic bone formation and inhibition of osteoclast activities via the cyclooxygenase-independent pathway.

A study by Zhang et al.⁽³⁵⁾ demonstrated that the tetra-PEG hydrogel-based aspirin sustained release system exerts beneficial effects on PDL SC-mediated bone regeneration. The researchers developed a tetra-PEG hydrogen sealant with rapid gelation speed, strong tissue adhesion, and high mechanical strength to achieve a prognosis of the bone defect. After in situ encapsulation of aspirin, this drug-loaded tetra-PEG hydrogel possessed sustained release, anti-inflammation, and osteoinductive properties. In their study, the viability of PDLSCs was significantly improved in both PEG and PEG-ASA hydrogels at 24 hours, indicating that the hydrogels that were fabricated obtained superior biocompatibility to support PDLSCs. In their study, ASA-loaded PEG hydrogels displayed a slow-release profile that could promote osteogenic differentiation of PDLSC both in vivo and in vitro. This data suggests that ASA can promote the osteogenic differentiation capacity of PDLSCs.

ASA induces growth factors in promoting osteoblastic potential in stem cells

The effects of ASA on various growth factors have been shown to enhance osteoblastic potential of derived stem cells.^(28-30,40) ASA could promote the immunomodulatory function of BMMSCs by upregulation of regulatory T-cells and downregulation of Th17-cells via 15-deoxy-delta-12,14-prostaglandin J2/peroxisome proliferator-activated receptor- γ /transforming growth factor- β 1 pathway.⁽⁴⁰⁾

FGFs have been isolated from many sources, and although they have a pivotal role in cell proliferation, they also display many functions in the epithelial and mesenchymal cells.⁽⁴¹⁾ Structurally, FGFs contain 22 members, and their molecular weight ranges between 17 to 34kDa.⁽⁴²⁾ FGFs are divided into two classes: acidic (a-FGF) and basic (b-FGF), and these were originally isolated from the brain and pituitary glands as growth factors for fibroblasts.⁽⁴²⁾ b-FGFs have shown various biological functions, including development, differentiation, angiogenesis, and wound healing. The expressions of FGFs play major roles in bone development and are found in mesenchymal progenitor and osteoblast cells. In addition, a study has shown that b-FGF, especially FGF2, maintains the proliferation as well as trilineage differentiation capacity in MSCs through the early mitogenic cycles. However, eventually, all the MSCs differentiate into the chondrogenic line.⁽⁴³⁾ FGF2 was reported to markedly enhance the growth rates and the life spans of MSCs from rabbit, canine, and human bone marrows in monolayer cultures.⁽⁴⁴⁾ b-FGF could enhance the levels of cAMP, ALP, OCN, mineralization, and differentiation of osteogenic precursor cells of rat stromal bone marrow cells (rSBMC) isolated from young adult rats.

This suggests b-FGF is able to stimulate rSBMC growth and biochemical functions.⁽⁴⁵⁾ The exposure of b-FGF to rat BMMSCs enhances *in vitro* osteogenic development in the presence of dexamethasone as the inducer.⁽⁴⁶⁾ The treatment of rat BMMSC in combination with b-FGF and BMP2 was also shown to synergistically enhance the osteogenic potential of the stem cells, compared to BMP2 treatment alone.

FGF18 was reported to be expressed in mesenchymal tissues during the differentiation of osteoblasts in calvarial bone development and in the perichondrium of developing bones.⁽⁴⁷⁾ It appears to positively affect osteogenesis by regulating cell proliferation and differentiation while at the same time negatively regulating chondrogenesis.⁽⁴⁷⁾ The biological activities of FGFs are dependent on the presence of BMP proteins. FGF4 and FGFR signals play important roles during BMP2-induced bone formation, as observed in rats.⁽⁴⁸⁾

FGF signaling controls osteoblast gene expression

FGFs are heparin-binding proteins that function by binding to the following: tyrosine kinase in the intracellular region of FGR receptors (FGFRs), non-transducing heparan sulfate-containing proteoglycans, the cysteine-rich receptor, and binding proteins.⁽⁴¹⁾ FGFRs contain an extracellular ligand-binding domain, a transmembrane region, and an intracellular-divided tyrosine kinase domain.

There are four distinct types of FGFR tyrosine kinase receptors (FGFR1, FGFR2, FGFR3, and FGFR4) with different FGF-binding properties. FGFRs are expressed in many different cell types and regulate proliferation, differentiation, and survival. FGFR1 and FGFR2 are expressed in MSCs prior to deposition on the extracellular bone matrix during bone development, as shown in Figure 1. FGFR1 was recently shown to be an important transducer of FGF2 signals in proliferating osteoprogenitor cells and subsequent differentiation during short-term cultivation.⁽⁴⁹⁾

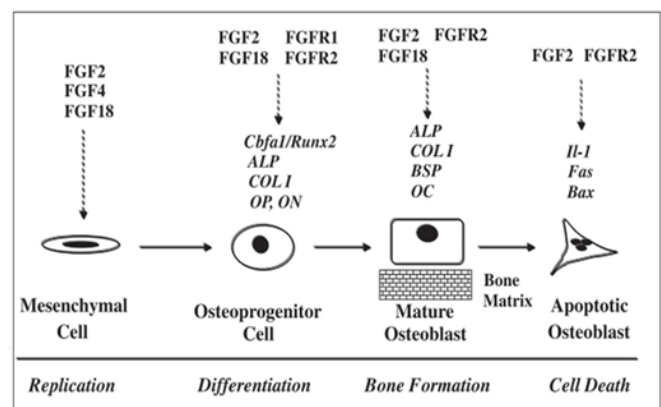


Fig. 1. Roles of FGF and FGR in osteoblast differentiation and fate. FGF acts through FGFR (dashed arrows) to control genes involved in osteoblast proliferation, differentiation, and apoptosis [adapted from P.J. Marie⁽³⁾].

ALP - alkaline phosphatase; Col1 - type I collagen; OP - osteopontin; ON - osteonectin; BSP - bone sialoprotein; OC - osteocalcin

The proliferation of osteoblasts at an early stage allows osteoblastic lineage commitment to take place. FGF signaling

regulates the expression of various genes that are involved in osteoblast differentiation. Its effect on osteoblast marker genes varies, depending on the cell types, either directly or indirectly. The direct effect of FGF signaling is mediated by transcription factors, while the indirect effect is mediated by soluble factors or cell-matrix interactions.⁽³⁾

The stimulation of Runx2/Cbfa1 expression by FGF2 provides an important molecular mechanism by which FGF/FGFR signaling directly activates the expression of osteoblast genes that are dependent on Runx2/Cbfa1. FGFs signaling could also have indirect effects on growth factors, matrix degradation, and cell proteins. In such cases, FGF signaling could affect osteoblast differentiation. Another example of the indirect effect of FGF signaling is to regulate osteogenic differentiation through the interaction of the growth factor signaling pathway.

In addition, FGF can also influence other growth factors that influence osteoblast formation, and this could enhance bone formation. For example, FGFs increase VEGF and hepatocyte growth factor (HGF) levels, both of which are mitogenic factors for osteoprogenitor cells.⁽⁵⁰⁾ FGFs also regulate the genes involved in matrix degradation (Table 2). FGF signaling regulates the expression of IL6, which is an important mediator of the effects of b-FGF on bone cells.^(51,52) FGF2 modulates the bone matrix by regulating the expression of collagenase activity. In fetal rat osteoblast-enriched (Ob) cells, it has been observed that b-FGF can stimulate collagenase-3 gene promoter activity through the AP-1 promoter binding site⁽⁵³⁾ and stromelysin-3 transcription.

Table 2.

Gene regulation by FGF/FGF signaling in bone [adapted from P.J. Marie⁽³⁾]

Transcription factors	Matrix and cell proteins	Growth factors	Matrix degradation	Apoptotic proteins
AP-1 Cbfa1/Runx2 Twist	Col I ON, OP BSP OC N-Cadherin Connexin-43 Noggin	TGFβ IGF1 IGFBP5 VEGF HGF	MMP1 Collagenase-3 TIMPs Stromelysin MMP1	Bax IL-1 Fas

Conclusion

ASA may potentially enhance periodontal regenerative processes by stimulating a selected number of growth factor-associated genes in PDLSCs or/and via its enhancement of osteogenic potential. These observations suggest that ASA could be supportive of regenerative processes and may help to improve periodontal health. However, further in-depth investigations, such as global proteome and transcriptome profiling studies, may provide additional insights on the impact of ASA on the regenerative activities of PDLSCs and how it could affect PDL functions in periodontal health and regeneration.

Competing Interests

The authors declare that they have no competing interests.

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Modified Chest X-Ray Radiography through Glass Window for Imaging COVID-19 Pneumonia: Techniques and Radiation Dose

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Abstract

The requirement for infection control during the COVID-19 pandemic led to modifying the exposure parameters in conventional radiography for performing chest X-ray radiography (CXR) through-the-glass (TTG) for imaging COVID-19 pneumonia. Herein, we reviewed and reported the current experiences with the TTG protocol, and summarized the current implementation strategies and modified technique factors. For implementing TTG techniques, measurements are required in a simulated environment using a patient equivalent phantom, and a certain number of investigations must be performed before the patient examination. However, the TTG technique requires modification due to the decrease in photon intensity caused by the attenuation in the glass barrier. This study discussed factors affecting CXR and some related radiation dose terminology required for implementing the TTG technique. Moreover, it summarized the exposure factors of CXR using the TTG technique compared with the standard CXR. Radiation exposure to the patient and the staff using the TTG technique remains within the recommended limits for safe practice. Image quality issues arose following the implementation of the TTG technique, mainly related to suboptimal positioning; image artifacts resulted due to glass attenuation, the increased source-to-image distance (SID), and patient movement. Overall, the reviewed results in this study could help formulate international guidelines and recommendations for the TTG technique for COVID-19 patient imaging, thereby minimizing the cost and time required for setting up the protocol. (**International Journal of Biomedicine. 2023;13(2):194-201.**)

Keywords: X-ray radiography • glass window • COVID-19 • radiation dose

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Abbreviations

AK, air kerma; **CXR**, chest X-ray radiography; **EI**, exposure index; **ED**, emergency department; **FSD**, focus to skin distance; **FFD**, focal film distance; **ICU**, intensive care unit; **pCXR**, portable CXR; **PA**, posteroanterior; **PPE**, personal protective equipment; **RT-PCR**, reverse transcription polymerase chain reaction; **SID**, source-to-image distance; **TTG**, through-the-glass.

Introduction

COVID-19 is an infectious respiratory disease caused by the SARS-CoV-2 virus that spread rapidly and became a global pandemic. It causes serious respiratory infections,

resulting in a significant number of mortalities.⁽¹⁻³⁾ Timely detection is essential for quick intervention through necessary control measures to prevent the further spread of the disease to reduce mortality rates and general life disruption. Reverse transcription polymerase chain reaction (RT-PCR)

is the standard technique for diagnosing COVID-19, with a sensitivity of 91%.⁽⁴⁾ X-ray computed tomography (CT) and chest X-ray radiography (CXR) are other options used to detect and follow up on COVID-19 pneumonia. Compared to CXR, CT has several disadvantages:⁽⁵⁻¹⁰⁾ (1) CT scans are not as easy to perform because of the limited availability and scanner disinfection requirements; (2) CT scans cause significant radiation exposure to examined individuals, compared to CXR; (3) during the peak period of the pandemic, the constant use of chest CT scans became difficult to sustain over time. In this regard, CXR is valuable in detecting lung involvement, assessing disease progression, confirming line and tube placement, and evaluating the onset of complications, such as volume overload.

During the pandemic, conventional radiography protocols had to be modified to align with infection control protocols. This led to the use of CXR through a glass window (TTG) to detect COVID-19 pneumonia.⁽¹¹⁾ TTG is carried out in an isolated room in the intensive care unit (ICU) or emergency department (ED) to minimize cross-infection. The use of glass barriers decreases the need to use personal protective equipment (PPE) and minimizes the risk of staff exposure. Moreover, the patients can remain in isolation and do not need transportation to other areas of the hospital, which would increase the infection risk. In general, it is useful in situations where infection control is important.⁽⁵⁾ In hospitals, CXR is used as a primary diagnostic tool for screening suspected COVID-19 cases; it provides results faster than RT-PCR, which minimizes the risk of cross-infection by reducing patient movement.

The TTG method involves imaging with pCXR outside the patient's room and taking pictures through a glass barrier.^(7,12,13) A technician outside the isolation room performs the pCXR. In our study, the image receptor was placed in two plastic bags and handed to the staff inside the isolation room, who cleaned the outer bag and placed it for exposure. After the instructions were provided, the technician outside the isolation room was exposed. The staff in the room removed the detector, cleaned it, and sent it for processing. Patients can be involved in this process by holding the detector during exposure. TTG reduces the need for PPE, cleaning supplies, and staff time for cleaning, thus preventing contamination.

Although radiation doses in CXR are low compared to other imaging procedures, the radiation's carcinogenic effect has no threshold, with probability increasing with radiation dose. Thus, the Commission on Radiological Protection (ICRP) has established a radiological protection system that governs the peaceful use of ionizing radiation, comprising justification of practice, optimization, and dose limit.⁽¹⁴⁾ For optimal protection, the radiation dose should be kept as low as reasonably achievable, which is known as the ALARA principle. A radiation shield, minimal time, and distance were combined to reduce radiation exposure when implementing the TTG technique for imaging COVID-19 pneumonia. This study aimed to review and report the technical factors and radiation doses used in TTG for imaging COVID-19 pneumonia. As an additional feature of this article, radiation safety practices will be discussed in comparison with conventional radiography using TTG.

Materials and Methods

In this study, we reviewed all published data concerning the use of CXR through a glass window for imaging COVID-19 pneumonia, up to 2022. We identified the data by searching the Web of Knowledge, Scopus, PubMed, and references from relevant articles, using search terms with suitable keywords. The authors reported their first experience at their hospitals regarding the steps taken to implement the TTG technique, including environment simulation, exposure factors used, and measurement of patient and staff doses. The authors then critically evaluated the articles to extract the relevant information. A search of a scientific literature database yielded 136 articles. After removing duplicates and evaluating titles and abstracts, 20 full-text articles were accessed for eligibility criteria analysis. We analyzed and presented data from 10 articles per predetermined eligibility criteria.

Inclusion and exclusion criteria

All studies on radiation exposure and technical parameters for implementing CXR using a glass window were included. The following were excluded: literature reviews and internal reports, articles that present only clinical data, and articles written in languages other than English.

Data extraction

Data extracted from the surveyed literature included information on glass attenuation factors, patient radiation dose, associated exposure factors used for chest PA examination, scattered radiation data, and the overall influence of the TTG technique on image quality. Two authors checked all the extracted data against the publications to ensure the completeness and accuracy of the collected data.

Results and Discussion

Table 1 summarizes the surveyed studies, their objectives, subjects, and major findings. The survey results have been analyzed in the subsections.

Requirements for the TTG technique

Setting for the TTG technique

When implementing the TTG technique, measurements are required in a simulated environment, such as in the ICU. The subjects needed for the experiment may include the ICU or ED room where TTG will be performed, an anthropomorphic chest phantom, an ionization chamber for patient dose measurements in diagnostic radiology, a survey meter for scattered radiation measurement, and a personal dosimeter. Although the techniques vary from hospital to hospital as they depend significantly on the clinical setting, they most commonly involve the three steps.^(7,12,13,15,16) These investigative measurements must be performed before the clinical examination of the patient.

The patient radiation dose can be determined by using a suitable patient equivalent phantom placed in a typical testing room, as well as by the CXR exposure factors: peak kilovoltage (kVp), tube current-exposure time product (mAs), and source-to-image distance (SID) needed to accommodate the TTG X-ray technique.

1. With the glass in place, measurements were made to estimate the glass transmission, which is important in

determining the amount of increase required in the technical parameters (kVp and mAs).

2. The scattered radiation in different positions inside and outside the isolated room was measured to determine the best staff locations to ensure that the TTG technique results in a minimal dose to staff.

3. The scattered radiation was measured at 1m from the glass window to mimic the location of the emergency department staff.

4. The dosimeter measured scattered radiation at 1 m from the phantom to mimic the location of the radiographer within the patient's room.

5. The maximum number of X-ray examinations per week was determined based on a dose constraint of 1mSv for the uncontrolled area.

6. The image quality parameters were assessed to ensure that the diagnostic image quality was preserved when using the TTG technique.

Glass Transmission

The anthropomorphic chest phantom was placed in an isolated room to simulate a real TTG technique. Measurements were taken with a glass in place to estimate the glass transmission. Glass transmission can be estimated from the measurement of the AK before and after glass placement.⁽¹⁵⁾ Glass transmission can also be estimated using the fitting parameters for plate glass published by the National Council on Radiation Protection and Measurements (NCRP) Report No 147.⁽²¹⁾ According to this report, the transmission factor of the X-ray beams passing through a glass sheet is determined as follows:

$$B = [(1 + \beta/\alpha)e^{\alpha\gamma x} - \beta/\alpha]^{1/\gamma}$$

where α , β , and γ are fitting parameters and x is the barrier thickness.

The measurement of transmission is important in determining the amount of increase required in the technique factors (kVp and mAs) due to the glass barrier.

Table 1.

Surveyed studies: objectives, subjects, and major findings

Authors	Task	Study Type Parameters Measured	Major Findings
Moirano et al. ⁽¹²⁾	To use TTG to minimize infections and keep working efficiency	<u>Phantom Study</u> : TTG portable chest radiography protocol Radiation dose	TTG technique is easy to implement and safe to use for imaging COVID-19 patients and can be used in conditions where infection control is required.
Chan et al. ⁽¹³⁾	To evaluate TTG implementation strategy and assess the image quality, radiation safety, and the amount of PPE saving	<u>Phantom Study</u> : Transmission Scattered radiation, Radiation dose, Phantom image quality	Rapid implementation of TTG is possible with the buy-in and training of staff; the resulting image quality is sufficient for COVID-19 detection.
Brady et al. ⁽¹⁵⁾	To evaluate the technical parameters used in the TTG, radiation safety needs, and image quality	<u>Clinical Study</u> : Glass attenuation Radiation dose Scattered radiation Image quality	Successfully implemented TTG for imaging COVID-19 patients; the time for imaging COVID-19 patients is greatly reduced by avoiding the need for disinfection of the X-ray unit.
Mckenney et al. ⁽¹⁶⁾	To provide comprehensive recommendations on TTG technique, including glass transmission, beam penetrability, and radiation	Phantom & clinical Study: Glass transmission Patient safety Staff safety	TTG techniques produced no apparent degradation of image quality, and the patient dose remained unchanged. Scattered radiation levels were low. The authors recommend the use of protective tools to reduce occupation exposure where feasible.
Rai et al. ⁽¹⁷⁾	To ensure radiation safety and acceptable image quality using TTG for imaging adult COVID-19 patients	<u>Phantom Study</u> : Image quality assessment Backscattered dose measurements	TTG technique using smart glass is achievable at acceptable image quality and patient dose.
Schelleman and Boyd ⁽¹⁸⁾	To study the effect of TTG on scattered radiation levels and image quality	<u>Phantom Study</u> : Radiation dose Image quality	Increased exposure factors used in TTG resulted in a good image quality but relatively higher patient doses. The increase in scattered radiation is negligible and can be further reduced by applying common radiation protection measures, including wearing lead aprons and keeping an appropriate distance.
England et al. ⁽¹⁹⁾	To evaluate the effect of TTG on image quality and radiation dose	<u>Phantom Study</u> : Radiation dose Image quality assessment	It is possible to TTG in certain conditions that cause an increase in radiation output and reduction in image quality; TTG implementation requires additional training.
Gange et al. ⁽²⁰⁾	To develop TTG for saving PPE and reduce the need for disinfectants without compromising much on image quality	<u>Phantom Study</u> : Radiation exposure measurements Image quality PPE use	Unchanged image quality and patient radiation exposure while minimizing PPE needs

Exposure factors and dose quantities in radiography practice

During imaging of COVID-19 patients, technique factors (kVp, mAs, and FFD) used in conventional CXR require modification because of the required increase in FFD for practical reasons and the decrease in photon intensity caused by attenuation in the glass barrier. Table 2 shows other factors affecting CXR and some related radiation dose terminology required to implement the TTG technique.

Table 2.

Factors affecting X-ray radiography and related radiation dose terminology.⁽²²⁻²⁹⁾

Source to Image Distance (SID)	Increasing film-focus distance (FFD), X-ray photon intensity will decrease according to the inverse square law hence the radiation dose. There is a requirement to increase the radiation dose to keep the same level of image quality.
Peak tube voltage (kVp)	In the TTG technique, glass attenuates about 40-60 % of the X-ray photons. There is the need to increase kVp, which leads to beam Hardening. The TTG technique inclines to reduce image contrast, which necessitates an increase in the exposure factors to preserve the image quality.
Tube current-exposure time (mAs)	mAs represents the quantity of the x-ray photons or the intensity interacting with the human body to form the image. In imaging, through the Glass, the mAs value decreases in two ways: first, by using longer
The exposure index (EI)	EI is a measure of the signal level produced by the image receptor/detector for a given incident air kerma corresponding to the required image quality.
Deviation Index (DI)	The purpose of DI is to give feedback to the technologist on whether the exposure factors used are appropriate for the target imaging quality.
Entrance Surface Air Kerma (ESAK)	ESAK is the air kerma at the entrance of the skin at the center of the beam and includes the backscattered radiation. Radiation dose to a patient undergoing radiographic X-ray is expressed in ESAK.
Effective dose	Effective dose is the tissue-weighted sum of the equivalent doses of all specified tissues and organs of the human body and represents the risk to the of the cancer whole body from partial body irradiations.

Typical technique factors and patient exposure in TTG

As the first step, glass transmission is measured to determine the amount of increase needed in the technique factors (kVp and mAs) due to X-ray beam attenuation in the glass. Brady et al.⁽¹⁵⁾ estimated the glass attenuation to be in the range of 41% at 90 kVp to 49% at 120 kVp for TTG performed using a glass door. McKenney et al.⁽¹⁶⁾ reported a mean transmission value of $39 \pm 3\%$, corresponding to an estimated increase in the exposure factors by a factor of 2.5, yielding the same EI. They reported a glass barrier attenuation of 61% with an effective dose reduction ranging from 50% to 80%. However, when the TTG technique was applied at the same SID, the patient's effective dose increased by

approximately 5%–10%. The authors concluded that when all other exposure factors are held constant, due to glass attenuation, the TTG technique requires an increase in the X-ray intensity by a factor of 2.5, which is normally achieved by increasing the mAs. In another study, Rai et al. estimated the linear attenuation coefficient of X-ray photons passing a smart glass in the range of 0.874–0.617 for 90–150 kVp.

Some clinics determine the modified exposure factors based on the experience of the radiographers and by using EI as a segregate for detector dose and image quality. In the TTG technique, the glass attenuates low-energy X-ray photons and depletes them, resulting in increased image noise. Therefore, exposure factors must be increased to preserve image quality.

Brady et al.⁽¹⁵⁾ determined the kVp and mAs values when using TTG according to the patient's size and FSD. Mostly, 105–110 kVp and 4.5–5.0 mAs were used. Rai et al.⁽¹⁷⁾ used an adult anthropomorphic thorax phantom to perform TTG. The technique parameters used for the standard CXR were 100–125 kVp at 1–3 mAs, compared with 125 kVp at 3.2 mAs when using the TTG technique. In a study by Moirano et al.,⁽¹²⁾ a technician determined the exposure factors for the TTG technique based on their experience and prior knowledge. They used 130 kVp, 6 mAs, and 180 cm of SID for TTG compared with 130 kVp, 6 mAs, and 180 cm of SID for standard pCXR. Schellman and Boyd⁽¹⁸⁾ investigated the TTG technique by using an anthropomorphic phantom. They used 100 kVp/1.6 mAs for a typical pCXR exposure, compared with 120 kVp/5 mAs for TTG. Finally, Gange et al.⁽²⁰⁾ retrospectively reviewed 100 radiographs obtained using the TTG technique, in addition to 50 radiographs obtained using the standard CXR. For standard-sized patients, exposure factors of 86 kVp, 2.2–3.6 mAs, and 183 cm of FFD were applied for TTG.

Brady et al.⁽¹⁵⁾ found the KA product value using the TTG to be $0.10 \text{ Gy} \cdot \text{cm}^2$, which is similar to the established UK diagnostic reference levels (DRLs) for chest AP ($0.1 \text{ Gy} \cdot \text{cm}^2$) and EC DRLs for chest PA ($0.12\text{--}1 \text{ Gy} \cdot \text{cm}^2$). TTG resulted in an effective dose of 0.02 mSv , similar to a standard CXR. McKenney et al.⁽¹⁶⁾ evaluated TTG techniques by estimating the patient radiation doses and scattered AK. Rai et al.⁽¹⁷⁾ reported adequate image quality when using the TTG technique at acceptable ESAK and showed no significant difference in radiation dose and image between the standard and TTG techniques. In Schellman and Boyd's study,⁽¹⁸⁾ the incident AK to the phantom under TTG ($67.82 \mu\text{Gy}$) was double that of the standard CXR ($29.47 \mu\text{Gy}$). The values remained below the established DRLs for PA-CXR ($150 \mu\text{Gy}$).⁽³⁰⁾ The image quality scores in TTG were comparable to those acquired using standard CXR. England et al.⁽¹⁹⁾ reported incident AK of $76.3 \mu\text{Gy}$ using the TTG technique, compared with $53.7 \pm 0.1 \mu\text{Gy}$ using the standard CXR. The authors used EI as a surrogate for detector dose and image quality to maintain control of zero DI. Gange et al.⁽²⁰⁾ used EI values as a surrogate for radiation dose and image quality.

The doses administered to the patients were compared with the DRLs in CXR. Table 3 shows the internationally established diagnostic reference levels for adult CXR. Consequently, patient doses in the TTG technique are compared with established international DRLs for adult chest radiography.

Table 3.**International established diagnostic reference levels for adult CXR.**

ESAK per radiograph (mGy)	KAP value per radiograph (mGy.cm ²)	Reference
0.15	100	30
0.30	**	31,32

Table 4.**Exposure factors for conventional radiography versus the modified TTG techniques for adult patients.**

Hospital	Protocol	Exposure factor	ESAK (ED)	Reference
H01	TTG	125-130 kVp; 6 mAs; 180 cm SID	Not reported	Moirano et al. ⁽¹²⁾
	Standard	100–103 kVp; 2–2.5 mAs; 180 cm SID	Not reported	
H02	TTG	110 kVp; SID 218 cm; 4 mAs	42.94 µGy	Chan et al. ⁽¹³⁾
	Standard	110 kVp; 168 cm SID; 0.9 mAs	37.07	
H03	TTG	105–110 kV; 4.5–5.0 mAs	(20 µSv)	Brady et al. ⁽¹⁵⁾
H04	TTG	100 kVp; 3.2 mAs	38.2 µGy (7.7 µSv)	McKenney et al. ⁽¹⁶⁾
	Standard	100 kVp; 1.6 mAs	28.4 µGy (7.2 µSv)	
H05	TTG	95 kV; 6.4 mAs	50.3 µGy (16.6 µSv)	McKenney et al. ⁽¹⁶⁾
	Standard	95 kVp; 4 mAs	59.5 µGy (21 µSv)	
H06	TTG	125 kVp; 6.3 mAs; 180 FDD	146.3 µGy	Rai et al. ⁽¹⁷⁾
	Standard	125 kVp; 3.2 mAs; 180 FDD	150.9 µGy	
H07	TTG	100 kVp; 20 mAs; 180 FDD	240 µGy	Rai et al. ⁽¹⁷⁾
	Standard	100 kVp; 2.0 mAs; 180 FDD	52.8 µGy	
H08	TTG	120 kVp; 5 mAs; SID 219 cm	91.6 µGy	Schelleman and Boyd ⁽¹⁸⁾
	Standard	100 kVp; 1.6 mAs; SID 219 cm	39.8 µGy	
H09	TTG	90 kV; SID 180 cm; 11.25 mAs	76.3±0.8 µGy	England et al. ⁽¹⁹⁾
	Standard	90 kV; SID 180 cm; 8 mAs	53.7±0.1 µGy	
H10	TTG	110 kVp; 5.9-10.0 mAs; 183 cm FFD	157-288 µGy	Gange et al. ⁽²⁰⁾
	Standard	86 kVp; 2.2-3.6 mAs; 183 cm FFD	132-217 µGy	

Value of incident air kerma (IAK) is converted to ESAK using ($ESAK = IAK \cdot BSF$; $BSF = 1.35$ for chest X-ray)

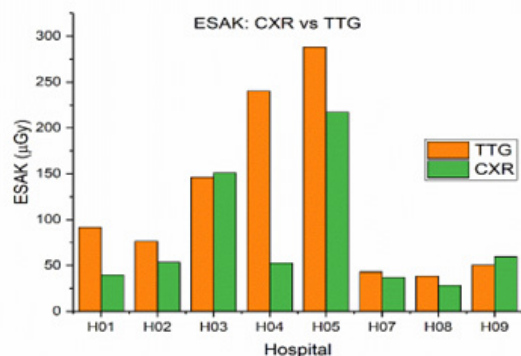
**Fig. 1.** Comparison of ESAK values of adult patients measured using conventional CXR and TTG

Table 4 shows the exposure factors for standard pCXR for an average-sized adult patient. Figure 1 shows the comparison of ESAK values of adult patients measured using conventional CXR and TTG. The data presented in Table 4 are meant to provide common directions for typical exposure factors that can be expected when applying TTG and should not be used as recommended values. Typical exposures usually depend on hospital conditions, which are determined as described previously.

Image Quality Issues with TTG

When using the TTG technique, Moirano et al.⁽¹²⁾ noticed that most image artifacts were motion-related, probably caused by increased exposure time, and are more noticeable in intubated hypersthene patients. Artifacts could also be caused by the glass barrier type and design; these can be mitigated by reducing the SID where possible. Furthermore, a respiratory therapist standing behind suitable shielding can ask patients to hold their breath, and using a ventilator, if necessary, could reduce the artifact. Moirano's findings are corroborated by McKenney et al.⁽¹⁶⁾

In the study by Brady et al.,⁽¹⁵⁾ radiologists and radiographers assessed the TTG technique. The radiologist determined the image quality, whereas the radiographer critically assessed the techniques affecting the diagnostic

quality. Both groups reported an overall good image quality when using the TTG technique. According to radiographers, under-collimation is the most problematic aspect of the through-glass technique. When the SID is too long, minor adjustments can significantly affect the collimation. According to Rai et al.,⁽¹⁷⁾ glass attenuation of low-energy X-ray photons results in increased image noise, necessitating an increase in exposure factors to preserve the image quality. The authors reported adequate image quality when using the TTG technique at an acceptable radiation dose; further, they found that the difference in the image quality was not significant between the standard and TTG techniques. In pCXR, where the patient suffers from an acute condition and does not cooperate, positioning and inspiratory effort may not be optimal. As a result, TTG images were highlighted for their suboptimal positioning issues.

Schellman and Boyd⁽¹⁸⁾ reported that ED patients presenting with suspected or confirmed COVID-19 could be imaged through glass with high image quality. England et al.⁽¹⁹⁾ measured the signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) as physical image quality parameters. The authors reported insignificant differences in SNR between the CXR and TTG techniques. In contrast, a small reduction in CNR was observed when TTG was used, which was not apparent in the perceptual analysis. According to the authors, CNR is more sensitive than visual inspections in revealing changes in image quality.

Staff Exposure, Radiation Protection, and Safety

One of the requirements for using the TTG technique is the measurement of scattered radiation at the radiographer's location and at different points.

The TTG technique could involve different exposure categories, including medical exposure of patients, occupational exposure of workers, and, to a lesser extent, public exposure to patients' relatives and caretakers. According to the International Commission on Radiological Protection, both the public and radiation workers are subject to the principle of dose limitation. The staff should adhere to the basic radiation protection points, which involves minimizing the time and distance of exposure while shielding as practically possible. Radiology technicians, nurses, and radiologists involved in TTG and other imaging procedures must wear lead-equivalent aprons and personal dosimeters. Hence, it is ideal for hospitals to establish radiation surveillance programs. According to NCRP, the level of radiation exposure in uncontrolled areas should not exceed the recommended dose limits.⁽²¹⁾

Brady et al.⁽¹⁵⁾ measured AK from scattered radiation using an anthropomorphic adult chest phantom. They simulated both a standard and a TTG X-ray environment using 110 kV, 5 mA, and an SID of 205 cm. The measured AK from the scattered radiation at the position of the radiographer 1m from the X-ray unit reached up to 0.06 $\mu\text{Gy}/\text{mAs}$, whereas the backscattered AK from the glass was as high as 0.3 μGy . Inside the room, the AK from the scattered radiation was 0.4 μGy at 1m and 0.1 μGy at 2 m. An operator received a total dose of 0.03–0.04 mSv over one month. According to their findings, unshielded personnel consistently positioned 1m from the patient were likely to remain within the occupational

dose limits. To provide maximum protection to the staff, they used a dose constraint of 1 mSv. A worker exposed to 0.5 mSv at 1m from the X-ray tube would need 2000 chest radiographs to reach 1 mSv in one year. Similarly, McKenney et al.⁽¹⁶⁾ measured AK from scattered radiation in a simulated TTG technique using an anthropomorphic phantom. They found that lead aprons, mobile shields, and increased distances, where possible, helped lower staff radiation doses.

Rai et al.⁽¹⁷⁾ measured backscattered radiation at 2 m from the patient. For a standard CXR, the backscatter was 0.02 μSv , compared with 0.04–0.22 μSv for the TTG CXR. At 100 kVp and 20 mAs, the maximum AK from the backscattered radiation from the glass was in the range of 0.14–0.22 μSv . In another study, Gange et al.⁽²⁰⁾ measured the AK rate of the technician standing 1.8 m (6 feet) from the pCXR and found it to be 7 μGy using the TTG technique, compared with 10 μGy during a typical CXR examination. However, the radiation entrance exposure to the technician standing 6 feet behind the radiographic unit was as high as 16 μGy . Considering the recommended yearly dose of 20 mSv for radiation workers, this limit is unlikely to be exceeded for moderate workloads per annum. According to Yeung et al.,⁽³³⁾ there was no statistically significant increase in the number of staff receiving doses exceeding 0.01 mSv per quarter when performing TTG. Therefore, the radiation risk to radiographers was not significant.

As shown in Table 5, using the modified imaging technique, the AK rates from scatter radiation were ensured to be within the acceptable limit for staff positions inside and outside the patient's room. On average, approximately 2000–3200 X-ray radiographs could be taken per year without exceeding the public dose limit of 1mSv for staff standing at least 2 m from the X-ray machine during exposure.

Table 5.

Scatter radiation measured during TTG technique.

Study	Inside the isolation room		Outside the isolation room at the operator
	Behind the patient*	Beside the patient**	
Brady et al. ⁽¹⁵⁾	0.4 μGy	**	0.3 μGy
McKenney et al. ⁽¹⁶⁾	0.16	0.212	0.26
McKenney et al. ⁽¹⁶⁾	0.008	0.15	0.02
Rai et al. ⁽¹⁷⁾	0.02(0.04-0.22)	**	0.05
Schelleman and Boyd ⁽¹⁸⁾	0.51 μGy	**	0.99 μGy
Gange et al. ⁽²⁰⁾	**	**	7-16 μGy

*Dosimeter measuring scatter radiation at 1 m from the phantom to mimic the location of the radiographer within the patient room.

**Dosimeter measuring scatter radiation at 1 m from the glass window to mimic the location of pCXR operator outside the isolation room

Conclusions and Future Directions

The current survey reviewed and reported the latest experience with TTG and summarized the implementation

strategies, technical factors, and radiation doses administered directly or indirectly to patients and staff. The TTG technique may be performed in the ED or ICU to minimize the requirements for disinfection of imaging equipment and reduce cross-infection. Initially, a feasibility study was required in a simulated environment using an anthropomorphic adult chest phantom to determine the appropriate technical factors pertaining to the TTG technique, measure the scatter radiations, and determine appropriate staff positions. Some hospitals determine the aforementioned factors by relying on their staff's experience and knowledge. When imaging through a glass barrier, the X-ray photon intensity decreases owing to the increased SID, according to the inverse square law and attenuation by the glass barrier.

Thus, the TTG technique requires an increase in both kV and mA to compensate for the photon loss. The DRL values in radiography are used to ascertain that the patient dose levels remain within the recommended criterion. The optimization of protection requires practicing the basic radiation safety principles of shielding, time, and distance. Hospitals ensure this by using a lead apron and scatter radiation measurements to ascertain that exposure levels remain within the recommended dose limits.

A noticeable effect of the TTG technique is a reduction in image quality owing to the increased SID and beam hardening due to glass attenuation, which affects the image contrast. During TTG, EI is used as a surrogate for detector dose and image quality, and it should be within the recommended value.

Overall, the results of the surveyed literature could help formulate international guidelines and recommendations for the use of TTG technique for imaging COVID-19 patients. Such guidelines can help minimize the cost and time required for outpatients and accelerate pCXR examinations in suspected and confirmed COVID-19 patients, helping reduce the disease's morbidity and mortality.

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Competing Interests

The authors declare that they have no competing interests.

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The Need for Increased HPV Vaccination Awareness and Access

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Abstract

Human papillomavirus (HPV) in the genital region is a frequently occurring sexually transmitted disease that can result in genital warts and various types of cancer. Most sexually active individuals acquire HPV at a certain point during their lifetime, but fortunately, several of the most harmful HPV types are preventable with vaccinations. All boys and girls aged 9 to 12 should get the HPV vaccine, although it can be given to individuals up to age 45. The HPV vaccine triggers an immune response that helps the body recognize and fight off the virus. There are currently two different HPV vaccines available: Gardasil and Cervarix. Gardasil guards against the two HPV strains that most commonly result in cervical cancer (CC) and various additional strains that can result in genital warts or other types of cancer. Cervarix only offers protection against the two forms of HPV that trigger CC. Mild side effects may occur, but more severe side effects are rare. Despite the availability of HPV vaccination, vaccination rates remain suboptimal in many countries. Raising awareness and expanding access to HPV vaccination are critical steps toward reducing HPV-related diseases. This article explores the basics of HPV and the role of vaccination in preventing its spread. (**International Journal of Biomedicine. 2023;13(2):202-204.**)

Keywords: HPV • genital infection • cancer • vaccination

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Introduction

Genital HPV is a transmittable disease that affects both women and men and is associated with various health conditions, including cervical, anal, and oropharyngeal cancer, as well as genital warts.⁽¹⁾ It is among the most prevalent sexually transmitted infections globally, with an expected 570,000 new cases of CC and 311,000 deaths annually worldwide.⁽²⁾

Fortunately, vaccination against HPV has been available since 2006, with the release of the first HPV vaccine. Currently, there are two types of HPV vaccines available: two quadrivalent vaccines that guard against HPV strains 6, 11, 16, and 18, and one bivalent vaccine that protects against HPV strains 16 and 18.^(3,4)

Despite the availability of HPV vaccination, vaccination rates remain suboptimal in many countries.⁽⁵⁾ Increasing awareness about the significance of HPV vaccination and improving access to vaccination is critical in reducing the

burden of HPV-related diseases. In this review article, we will discuss genital HPV, its associated health consequences, and the significance of HPV vaccination in preventing HPV-related diseases.

Genital HPV and Associated Health Consequences

There are about 150 forms of HPV, more than 40 of which can invade the genital region. Most people who contract HPV do not experience any symptoms, and their immune system clears the infection within 1-2 years. However, the virus persists in some cases and can lead to various health consequences.⁽¹⁾

The most significant health consequence associated with genital HPV infection is the development of malignancy. HPV infection is the leading reason of cancer cervix, with HPV strains 16 and 18 being responsible for approximately 90% of all occurrences. Additionally, HPV infection is associated with other types of cancer, including oropharyngeal, anal, vulvar, and vaginal cancer.^(1,6)

In addition to cancer, HPV infection can also cause genital warts, noncancerous lesions on the genitals or anus. Genital warts are often embarrassing and uncomfortable and can be challenging to treat.⁽⁷⁾

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HPV Life Cycle, Immunogenicity, and Receptors

The life cycle of HPV begins when the virus attacks the basal epithelial cells. The virus enters the cell by binding to cell surface receptors, such as heparan sulfate proteoglycans, $\alpha 6\beta 4$ integrins, and possibly others. Once inside the cell, the viral genome is delivered to the nucleus, where it is replicated and transcribed. The virus employs the host cell's machinery to create viral proteins, which are then assembled into new virus particles. The newly released virus particles infect other cells after being liberated from the host cell.⁽⁸⁾

The immune system is crucial in preventing HPV infection. HPV infection stimulates both innate and adaptive immune responses. Innate immune responses include the activation of natural killer cells, dendritic cells, and macrophages, while adaptive immune responses involve the production of antibodies and the activation of T cells. HPV-specific T cells have a significant function in HPV infection by recognizing and killing infected cells.⁽⁹⁾

HPV infects epithelial cells that express specific cell surface receptors, including heparan sulfate proteoglycans, $\alpha 6\beta 4$ integrins, and possibly others. The viral capsid protein, L1, contains neutralizing epitopes that can induce the production of neutralizing antibodies. These antibodies can bind to the virus and prevent infection of susceptible cells.⁽¹⁰⁾

The Fate of HPV Infection

The fate of HPV infection is determined by several factors, including the kind of HPV virus involved, the individual's immune system, and whether therapy is received.⁽⁹⁾

In many situations, HPV infections resolve independently, with no long-term health consequences. This is particularly true for younger people with strong immune systems. Most HPV-infected individuals will recover within two years and sometimes considerably sooner. Over 90% of HPV infections resolve without treatment within two years.⁽⁷⁾

However, certain HPV infections might persist and evolve into chronic ones. These ongoing infections may cause aberrant cell proliferation, which may ultimately result in cancer. This is why it is critical to get frequent screenings for CC and other HPV-related malignancies, particularly for those with persistent HPV infections (Table 1).⁽¹¹⁾

Table 1.

Cancer sites, the associated HPV types, and the percentage of association.⁽¹⁴⁻¹⁷⁾

Cancer Site	Associated HPV types	Percentage of association
Cervical Cancer	16, 18, 31, 33, 35, 45, 52, and 58	>90%
Anal Cancer	16, 18, 31, 33, 35, 45, 52, and 58	>90%
Oropharyngeal Cancer	16 and 18	60-70%
Vulvar Cancer	16, 18, 31, and 33	50-70%
Vaginal Cancer	16 and 18	>80%
Penile Cancer	16, 18, 31, 33, 35, 45, 52, and 58	30-50%

There are also various therapies available for HPV infections and associated health issues. Medication to cure genital warts, surgery to remove aberrant tissue, and different medicines to treat cancer caused by HPV are presented in detail in the Advisory Committee on Immunization Practices (ACIP) Recommendations.⁽¹²⁾

In addition to these therapies, vaccinations are available to prevent specific forms of HPV infection. The HPV vaccination is extremely successful in preventing infections caused by the most prevalent strains of HPV that cause cancer and genital warts. Vaccination is advised for both boys and girls, and it is usually administered during early adolescence before sexual activity begins.⁽¹³⁾

HPV Vaccination in Preventing HPV-Related Diseases

HPV vaccination is an effective method of avoiding HPV infection and its associated health consequences. There are now two types of HPV vaccinations available: a bivalent vaccine against HPV types 16 and 18 and a quadrivalent vaccine against HPV strains 6, 11, 16, and 18. Both vaccinations have been proven safe and effective in preventing HPV infection and lowering the prevalence of HPV-related diseases.⁽¹³⁾

Numerous studies have shown that HPV vaccination could lower the risk of CC and other HPV-related malignancies. For example, a systematic review of real-world data found that the quadrivalent HPV vaccination was beneficial in lowering the prevalence of HPV-related diseases such as genital warts, CC, and other HPV-related malignancies. Additionally, a long-term follow-up study of a random clinical trial discovered that the bivalent HPV vaccination was highly efficient in limiting HPV type 16 and 18 infections and reducing CC incidence (Table 2).⁽²⁰⁾

Table 2.

Types of HPV vaccines and their side effects.^(13,19,20)

HPV Vaccine	Route Administration	Age Administration	Side Effects (potential)
Cervarix	Intramuscular injection	Recommended at age 9–25	Pain, swelling, and redness at the injection site; headache, fatigue, muscle pain, and gastrointestinal symptoms
Gardasil	Intramuscular injection	Recommended at age 9-45	Pain, swelling, and redness at the injection site; fever, headache, nausea, dizziness, and fainting

Furthermore, HPV vaccination can provide herd immunity when a significant proportion of the population is vaccinated. This reduces the overall prevalence of the disease, protecting unvaccinated individuals from HPV infection.⁽⁵⁾

In conclusion, genital HPV is a prevalent infection that can lead to various health consequences, including cancer and genital warts. The HPV vaccine is a safe and efficient way to limit HPV infection and reduce the incidence of HPV-related

diseases. Increasing awareness about the value of the HPV vaccine and improving access to vaccination is critical in lowering the incidence of HPV-associated disorders.

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Competing Interests

The authors declare that they have no competing interests.

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Vitamin Deficiencies and Their Impact on Skin Function

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Abstract

Vitamins are essential dietary items because of their functions as enzyme cofactors and catalysts in the body. Vitamin deficiency can significantly impact the skin, leading to a variety of dermatological problems, such as dryness, premature aging, hyperpigmentation, palmoplantar keratoderma, and even nail disorders. Alcoholism, rigid diets, inadequate parental nourishment, and intestinal absorption issues may all be factors in vitamin deficits. The most commonly deficient vitamins in relation to skin health are vitamin A, biotin, vitamin C, and vitamin D. Vitamin A helps to maintain the skin's barrier function and promote skin cell growth, while biotin helps to support the skin's underlying structure. Vitamin C is essential for collagen synthesis and skin brightening, and vitamin D is a crucial nutrient for overall health, including the skin. A balanced and varied diet and proper skin care can help prevent and address vitamin deficiencies, keeping the skin healthy and radiant. This review examines the most common vitamin deficiencies and their impact on the skin. (International Journal of Biomedicine. 2023;13(2):205-209.)

Keywords: skin • malnutrition • vitamin deficiency • dermatological signs

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Introduction

Vitamin deficiency can significantly impact the health and appearance of the skin. Vitamins are crucial in maintaining skin health, as they support various cellular processes that keep skin youthful, radiant, and healthy.⁽¹⁾ Some of the most common skin-related issues associated with vitamin deficiencies include wrinkles, dullness, uneven skin tone, dark circles, and even skin diseases like psoriasis and eczema.⁽²⁾ It is important to understand the role vitamins play in skin health so that any potential deficiencies in the diet can be identified and addressed. By consuming a balanced diet rich in vitamins and antioxidants, people can help maintain healthy skin and prevent the negative impacts of vitamin deficiency on the skin. This study demonstrates the prevalence of vitamin deficiency and its dermatological consequences.

Fat-Soluble Vitamins

Vitamin D

Vitamin D is known to be essential for bone health as well as being a hormone that regulates a number of physiological processes. It can be produced by food sources or, when ultraviolet (UV) light is present, via the skin's cholesterol

progenitors. This hormonally inactive version needs two further hydroxylation processes to become active. The initial conversion to 25-hydroxyvitamin D, the most active and abundant version of vitamin D within the body, happens in the liver and numerous other organs.⁽³⁾ Vitamin D's physiologically active, hormonal form, 1,25-dihydroxyvitamin D, is produced by the kidneys and is necessary for bone health, calcium and phosphorus metabolism, and other bodily functions. Many cell types, beyond the skeletal structure, have vitamin D sensors and can transform blood 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D for regional uses, such as immunological control and cell proliferation and differentiation. Vitamin D nutritional status may be determined by measuring blood levels of 25-hydroxyvitamin D. The ideal levels are in the 75–125 nmol/L range.⁽⁴⁾

Deficiency

According to the United States Endocrinal Association, a vitamin D level of 21 to 29 ng/mL is inadequate, and a level of 20 ng/mL is deficient. Vitamin D status is influenced by UV radiation exposure, skin color, sex, body mass index, physical activity, vitamin D receptor mutations, and alcohol consumption.^(3,4) Vitamin D is a crucial nutrient for overall health, including the skin. A deficiency of vitamin D may result in various skin problems and affect the appearance and function of the skin. Here are some ways in which vitamin D deficiency can impact the skin:

1. Atopic dermatitis has been connected to vitamin D receptor polymorphisms and low vitamin D levels. People with atopic dermatitis were found to have a higher incidence of vitamin D receptor gene variations than do healthy individuals, highlighting the importance of vitamin D in the development of the condition.⁽⁵⁾ A recent study revealed that low levels of vitamin D in the blood were correlated with more severe eczema symptoms.⁽¹⁾

2. Psoriasis: IFN- γ , IL-2, IL-6, and IL-8 are among the inflammatory mediators that are inhibited by 1,25-dihydroxyvitamin D, while IL-10, an anti-inflammatory cytokine, is increased. These actions help to limit the inflammatory process in the pathogenesis of psoriasis.⁽³⁾ Furthermore, vitamin D's immune regulatory ability aids in modulating and controlling the levels of antimicrobial peptides involved in the pathogenesis of psoriasis. A recently published study demonstrated that inadequate vitamin D levels had been linked to an increased chance of developing psoriasis and worsening symptoms in people with the condition.⁽⁶⁾

3. Vitiligo: At present, the pathogenic mechanism of vitiligo is not completely clear. There are studies suggesting that vitamin D may increase the melanogenesis and tyrosinase content of human melanocytes through its anti-apoptotic effect, thus preventing the loss of skin pigment.⁽⁷⁾ A meta-analysis conducted by Zhang et al.⁽⁸⁾ in 2018 indicated that patients with vitiligo had a lower level of 25(OH)D compared with controls. A meta-analysis performed by Varikasuvu et al.⁽⁹⁾ showed significantly decreased Vitamin D levels in vitiligo, and its association with indoor/outdoor type of work of vitiligo patients. However, a meta-analysis performed by Song et al.⁽¹⁰⁾ provided no convincing evidence for a causal effect of 25(OH)D or 25(OH)D3 levels on the risk of vitiligo. According to recent observational studies that have produced conflicting findings, it is unclear if insufficient vitamin D contributes to the development of vitiligo.⁽⁸⁾

4. Connective tissue disorders are also associated with a vitamin D deficit. With rates reaching 90%, vitamin D insufficiency is particularly frequent in those with systemic sclerosis. Lower vitamin D levels have also been linked to cardiovascular problems and lupus nephritis in people with systemic lupus erythematosus. Also, there is some association between Sjogren syndrome and decreased vitamin D blood levels.⁽¹¹⁾

5. Alopecia areata: In keeping with earlier research demonstrating that vitamin D deficiency may be related to autoimmune illnesses, it has been proposed that vitamin D's immune modulatory activity contributes to alopecia areata. Most observational studies have found a significant correlation between vitamin D deficiency and autoimmune reactions in alopecia areata, with disease severity rising.⁽¹²⁾

6. Autoimmune bullous diseases: Vitamin D insufficiency has also been connected to autoimmune bullous diseases. Insufficiency has been shown to be more prevalent in pemphigus and pemphigoid patients, although this connection cannot be related to the onset of autoimmune bullous diseases.⁽¹³⁾

7. Acne: According to research, vitamin D may play a part in the development of acne. Based on one study, low vitamin D levels were linked to more severe acne.⁽¹⁴⁾

8. Wrinkles: Vitamin D is important for skin health and skin aging. According to one study, insufficient vitamin D levels were associated with increased skin aging and wrinkles.⁽¹⁵⁾

9. Pigmentation Disorders: Vitamin D is also involved in regulating skin pigmentation. One published study suggested that low vitamin D levels relate to an increased incidence of pigmentation disorders such as melasma.⁽¹⁶⁾

10. Wound healing: Vitamin D is essential for wound healing because it promotes the production of antimicrobial peptides and regulates inflammation. Low levels of vitamin D have been linked to impaired wound healing.⁽¹⁷⁾

11. Skin infections: Vitamin D has antimicrobial properties and helps the immune system respond to skin infections. Low vitamin D levels have been linked to an increased risk of skin infections.⁽¹⁸⁾

12. Skin neoplasms: Vitamin D levels have been studied in numerous tumor subtypes, and most of these neoplastic disorders have a substantial correlation with vitamin D concentrations. Recent research has revealed that cutaneous T-cell lymphoma cells express the vitamin D receptor and can undergo apoptosis in response to a significant blood vitamin D level.⁽¹⁹⁾ Numerous studies have indicated a link between melanoma and vitamin D levels. Melanoma has been linked to both the receptor's polymorphisms and expression. Vitamin D prevents tumor angiogenesis, invasion, and metastasis. Patients with stage IV melanoma were found to have lower serum vitamin D levels than those with stage I melanoma. Additionally, those with insufficient vitamin D levels have higher risks of melanoma recurrence.⁽²⁰⁾ Vitamin D insufficiency has also been linked to basal cell cancer. By inhibiting Hedgehog signaling, vitamin D produced by UV radiation protects the skin from the carcinogenic effects of UV radiation. However, such a response was not observed with vitamin D taken orally. Mutations in the vitamin D receptors are also related to the development of basal cell cancer. Keeping vitamin D values over 25 ng/mL could greatly prevent basal cell carcinoma recurrence.⁽²¹⁾

Vitamin A

Vitamin A, or retinoid, is necessary for healthy skin and barrier functions, as well as for a proper immune system, growth and development, eyesight, and reproduction. The human diet contains two sources of vitamin A: preformed vitamin A (retinol and retinyl esters) and provitamin A carotenoids.^(22,23) Retinol is a type of vitamin A obtained from animals that serves as a precursor to the body's most active form (retinoic acid). The plant forms of vitamin A, alpha-carotene, beta-carotene, and beta-cryptoxanthin, can all be transformed into retinol in the body. The liver stores vitamin A, and the total body quantity is 300 to 700g/day for children and 700 to 900 g/day for adults.⁽²⁴⁾

Deficiency

Vitamin A is an essential nutrient for maintaining healthy skin. A deficiency of vitamin A can lead to several skin problems. Vitamin A deficiency is thought to affect around 30% of children worldwide. The most common cause of vitamin A deficiency is inadequate nutritional intake due to malnutrition. In affluent countries, people with hepatic cirrhosis, small bowel syndrome, cystic fibrosis, and

alcoholism frequently have vitamin A deficiency.⁽²⁴⁾ The risk of clinical manifestation is related to serum retinol levels of 0.35mol/L. One of the main effects of a vitamin A deficiency is xerosis, or dry skin. This is because vitamin A is necessary for sebum production, an oil produced by the skin that helps keep it hydrated. Without enough vitamin A, the skin can become dry, flaky, and itchy.⁽²⁵⁾ Another impact of vitamin A deficiency on the skin is a decreased ability to fight infections. Vitamin A is required for the immune system, and a deficiency can lead to an increased risk of infections, such as boils, impetigo, and folliculitis. In addition, vitamin A is essential for cell development and repair, and a deficiency can lead to a reduction in the rate of cell turnover. This can result in a buildup of dead skin cells, leading to a dull, rough, and uneven skin texture. Finally, vitamin A deficiency can also lead to a loss of skin elasticity, causing wrinkles and fine lines to form. This is because vitamin A helps to maintain the skin's collagen levels, which are important for skin elasticity and firmness. Vitamin A palmitate oil is used to treat vitamin A deficiency. The recommended dosages are 60,000 IU taken orally for two days, followed by 4,500 IU each day.⁽²⁶⁾

Water-Soluble Vitamins

Vitamin B7

Vitamin B7, also known as biotin, is a water-soluble vitamin that plays a critical role in several metabolic processes in the body. This vitamin serves as a coenzyme for five carboxylases dependent on vitamin B7 and is involved in essential processes such as gluconeogenesis, lipogenesis, and amino acid breakdown. In addition, biotin is crucial for protein synthesis, including the production of keratin, which is necessary for the proper development of hair and nails. Biotin can be obtained from various dietary sources, including meats, eggs, salmon, sunflower seeds, and sweet potatoes.⁽²⁷⁾

Deficiency

Biotin deficiency can be either inherited or acquired. The consumption of raw eggs is a common cause of acquired biotin insufficiency, as a protein called avidin, which is present in raw egg whites, binds strongly to biotin, preventing its use as an important cofactor. Other factors that can lead to acquired biotin deficiency include alcoholism, pregnancy, the use of certain medications such as valproic acid and isotretinoin, reduced absorption, or prolonged use of antibiotics, which can disrupt the natural gut flora.⁽²⁸⁾ Congenital or hereditary biotin deficiency, on the other hand, can occur due to a lack of enzymes such as biotinidase or holocarboxylase synthase, which are inherited as an autosomal recessive trait. The neonatal form, due to a deficiency in the holocarboxylase synthase enzyme, which manifests within the first six weeks of life, is a potentially fatal condition. Extensive dermatitis and severe alopecia are possible skin lesions. The infantile variant appears after three months of age and is caused by a deficiency of the enzyme biotinidase, which is important in carboxylase breakdown and subsequent free biotin absorption. It is possible to lose hair on the scalp, brows, and eyelids. In advanced instances, hair loss can be complete.⁽²⁹⁾ Alopecia, seborrhea, conjunctivitis, eczema, and numerous neurologic

abnormalities, such as seizures, lethargy, and hypotonia, are common signs of biotin insufficiency. Biotin insufficiency is uncommon as it is available in a variety of foods and is also generated by organisms in the gut flora. One way to detect biotin deficiency is to measure the levels of biotinylated propionyl-CoA carboxylase and methylcrotonyl-CoA carboxylase in white blood cells. Additionally, the levels of biotin in urine and serum can also be measured as an indicator of biotin deficiency. Biotin plasma concentrations typically range from 400 to 1,200 ng/L. A plasma reading of less than 200 ng/L is considered deficient. For adults, the daily nutritional requirement for biotin is 5 mg. A varied diet containing enough biotin is usually sufficient.⁽³⁰⁾

Vitamin C

Vitamin C, also called ascorbic acid, is an antioxidant and plays a crucial role in the hydroxylation of specific amino acids such as tyrosine and tryptophan. As these amino acids are integral components of collagen, vitamin C supports collagen synthesis, thereby aiding in the formation of the skin barrier and dermal collagen. It has anti-aging qualities as well since it guards the skin against oxidation. Vitamin C must be obtained from food; humans are unable to manufacture it due to a lack of the L-gluconolactone oxidase enzyme. The most significant food sources are fresh fruits and vegetables. Because vitamin C is heat-sensitive, cooking or boiling reduces its nutritional value. The entire body store of ascorbic acid is 1,500 mg, and symptoms of insufficiency appear when that amount falls below 350 mg.⁽³¹⁾

Deficiency

Alcoholism, anorexia, cigarette usage, poor nutrition, male gender, fever, viral infections, and antibiotic use are all risk factors for ascorbic acid deficiency. Scurvy is the most common symptom of insufficiency. It is induced by vitamin C's function in collagen production and the maintenance of the triple helix's collagen stability. Although scurvy is uncommon, it remains a condition that occurs, particularly in children who are receiving pasteurized milk.⁽³²⁾ Fatigue, mood swings, despair, irritability, and anorexia are the first indications of a lack of ascorbic acid for eight to twelve weeks. Specific dermatologic findings following these nonspecific symptoms may include enlargement of the gingiva with tooth loss, poor wound healing, hyperkeratosis, and ecchymosis. Hair irregularities, such as swan-neck and corkscrew hair, are common in scurvy due to a disturbance in the production of the disulfide bond. Perifollicular hemorrhages on the lower limbs, forearms, and belly are noticed in the early stages of the illness. When these features occur on the legs, they may cause woody edema, which is characterized by ecchymosis, discomfort, and restricted mobility. Koilonychia and splinter hemorrhages are two diseases of the nails that have been linked to ascorbic acid deficiency. Although the gums initially appear red, puffy, and glossy, they eventually turn black and necrotic in the later stages. Tooth loss is common, as are ocular manifestations such as retrobulbar hemorrhage into the optic nerves, which causes papilledema and atrophy.⁽³³⁾ Up to 80% of patients with this deficiency experience musculoskeletal symptoms such as muscle hematomas, arthralgia, and

myalgia. Risk factor assessment, clinical assessment, and lab testing all precede the diagnosis. The radiologic evidence will be pathognomonic. Low-plasma ascorbic acid (0.2 mg/dL) or leukocyte ascorbic acid (7 mg/dL) levels are much more accurate in diagnosis.⁽³⁴⁾ The typical therapy is vitamin C replenishment. Scurvy patients should take one to two grams of ascorbic acid daily for two to three days, followed by a daily intake of 500 mg for a week, and then a daily intake of 100 mg for one to three months. Treatment should continue until all clinical symptoms have resolved. Hair begins to respond after one month, whereas cutaneous signs take two weeks. A full recovery is anticipated after three months of ascorbic acid administration. When there is no shortage, daily needs for males, females, and breastfeeding mothers range from 90 mg to 120 mg for men, up to 45 mg for youngsters, and 75 mg for women.⁽³⁵⁾

In conclusion, vitamins are essential for proper bodily functions, and their deficiency can result from inadequate intake, poor absorption, poor nutrient utilization, or increased breakdown. The development of mucocutaneous changes, such as skin lesions, can aid in identifying underlying vitamin deficiencies, although they may not be specific for all vitamin deficits. Scurvy is a disease caused by vitamin deficiency and associated with skin lesions that can be diagnostic. Other skin lesions commonly associated with vitamin deficiencies include cutaneous and mucosal pigmentation, palmoplantar keratoderma, and nail layering. Clinical observations and laboratory measurements of blood or urine vitamin levels can be used to diagnose deficiencies.

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Competing Interests

The authors declare that they have no competing interests.

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Association of *AGT* (T704C) and *NOS3* (G894T) Gene Polymorphisms with Treatment-Resistant Hypertension in the Uzbek Population

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Abstract

The aim of our study was to assess the effect of polymorphic markers of the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs on the risk of the development of treatment-resistant hypertension (TRH).

Methods and Results: The study included 178 patients (mean age of 56.67±11.12 years) with AH Grades 1-3 (ESC/ESH, 2018), who were on outpatient treatment at the Republican Specialized Scientific and Practical Medical Center for Cardiology. The effectiveness of therapy was assessed by achieving the target BP level according to 2018 ESH/ESH Guidelines for the management of AH. The primary target level for SBP and DBP was <140 mmHg and <90 mmHg, respectively.

Genomic DNA samples were isolated from the peripheral blood leukocytes by using the Diatom™ DNA Prep 200 Kit (Isogen Laboratory LLC, Moscow) according to manufacturer's protocol. A multiplex RT-PCR assay was used to detect the *AGT* T704C (M235T) rs699 SNP and *NOS3* G894T (Glu298Asp) rs1799983 SNP.

We studied the distribution of the *AGT* T704C (M235T) rs699 SNP in 61 Uzbek patients with TRH (cases) and 117 Uzbek patients with non-TRH (controls) (Group 1) and the distribution of the *NOS3* G894T (Glu298Asp) rs1799983 SNP in 61 Uzbek patients with TRH (cases) and 115 Uzbek patients with non-TRH (controls) (Group 2).

Our results indicate a significantly greater accumulation of the C allele and CC genotype of the *AGT* T704C (M235T) rs699 SNP among TRH patients than among patients with non-TRH. We found a significant association between the *AGT* T704C (M235T) rs699 SNP and the risk of TRH under the multiplicative genetic model (C vs. T: OR=1.85, 95% CI: 1.17-2.92, $P=0.008$), additive model (CC vs. TT vs. TC; OR=3.00, 95% CI: 1.56-5.75, $P=0.009$), and recessive model (CC vs. TC+TT; OR=3.00, 95% CI: 1.56-5.75, $P=0.0008$). For the *NOS3* G894T (Glu298Asp) rs1799983 SNP, the multiplicative model showed a significant risk of TRH with the carriage of the T allele (OR=1.99, 95% CI: 1.20-3.28, $P=0.007$), and the additive model showed a significant risk of TRH with the carriage of the heterozygous GT genotype (OR=2.25, 95% CI: 1.17-4.33, $P=0.01$). At the same time, the carriage of the G allele (OR=0.5, 95% CI: 0.30-0.83, $P=0.007$) and GG genotype (OR=0.40, 95% CI: 0.21-0.76, $P=0.01$) may be protective against the development of TRH.

Conclusion: Further genetic studies of TRH may help achieve better individual outcomes by optimizing drug therapy based on genetic variation. (International Journal of Biomedicine. 2023;13(2):210-216.)

Keywords: treatment-resistant hypertension • angiotensinogen • nitric oxide synthase • single nucleotide polymorphism

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Abbreviations

AH, arterial hypertension; **AGT**, angiotensinogen; **BP**, blood pressure; **BMI**, body mass index; **DBP**, diastolic BP; **CIMT**, carotid intima-media thickness; **HWE**, Hardy–Weinberg equilibrium; **LVH**, left ventricular hypertrophy; **LVMI**, left ventricular mass index; **NOS**, nitric oxide synthase; **SBP**, systolic BP; **SNP**, single nucleotide polymorphism; **TRH**, treatment-resistant hypertension.

Introduction

Treatment-resistant hypertension (TRH) is defined as uncontrolled hypertension on ≥ 3 antihypertensive medication classes or requiring ≥ 4 antihypertensive medications to reach their BP goals.^(1,2) Among US adults taking antihypertensive medication, the prevalence of apparent TRH was 17.7% (9.2 million persons) when applying the definition in the 2008 Scientific Statement, whereas it was 19.7% (10.3 million persons) using the 2018 Scientific Statement definition.⁽³⁾ The etiology of TRH appears to be multifactorial. Risk factors for TRH include older age, obesity, impaired renal function, diabetes mellitus, African American race, and other factors, including genetic ones.⁽⁴⁻⁸⁾

The question of whether there are specific genetic risk factors for TRH is of great interest, especially considering race and ethnicity. Although previous studies have identified numerous genetic variants associated with hypertension and blood pressure,⁽⁹⁻¹²⁾ there is little evidence regarding the molecular genetic factors of TRH. To date, the available evidence surrounding pharmacogenomics in TRH is limited and primarily focused on candidate genes.⁽¹³⁻¹⁵⁾ In recent years, several studies with an integrated genetic approach, genome-wide association studies (GWASs), have identified some significant susceptibility loci for TRH in the US population.⁽¹⁶⁻¹⁹⁾

A published paper entitled “Genetic and adverse health outcome associations with TRH in GenHAT” by Lynch et al.⁽²⁰⁾ evaluated the association between 78 candidate gene polymorphisms and TRH. The main finding was the association of two genetic variants in the *AGT* gene, the M allele of rs699 and the G allele of rs5051, and TRH in white but not in African American subjects.

The M235T molecular variant (T704C, rs699) of the *AGT* gene, encoding a threonine instead of a methionine at residue 235 of the mature protein, has been associated with a higher plasma AGT level and higher BP in patients homozygous for the T allele and occurs among various ethnic populations.⁽²¹⁻¹³⁾ In a meta-analysis, the TT genotype was associated with a 32% increase in the risk of hypertension in white people but not in non-white people, when compared with the MM genotype.⁽²⁴⁾

The most examined rs1799983 polymorphism (also known as G894T or Glu298Asp) is located in exon 7 of the *NOS3* gene and formed by a transversion from guanine (G) to thymine (T), resulting in the replacement of glutamic acid (Glu) residue with aspartic acid (Asp) residue in the NOS3 polypeptide.⁽²⁵⁾ This genetic mutation reduces the production of NO and subsequently affects the development of AH.⁽²⁶⁾ In some studies, the T allele of the rs1799983 polymorphism was reported to be associated with a decreased level of NO.⁽²⁷⁻²⁹⁾

Unfortunately, the literature data on genetic studies of resistant arterial hypertension, especially in the Asian population, are limited. A better understanding of genetic risk may improve clinical care for TRH and prevent associated cardiovascular disease morbidity and mortality.

The aim of our study was to assess the effect of polymorphic markers of the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs on the risk of TRH development.

Materials and Methods

The study included 178 patients (mean age of 56.67 ± 11.12 years) with AH Grades 1-3 (ESC/ESH, 2018), who were on outpatient treatment at the Republican Specialized Scientific and Practical Medical Center for Cardiology. The effectiveness of therapy was assessed by achieving the target BP level according to 2018 ESH/ESH Guidelines for the management of AH. The primary target level for SBP and DBP was <140 mmHg and <90 mmHg, respectively.

Exclusion criteria were symptomatic hypertension, valvular heart disease, acute coronary syndrome, chronic heart failure (NYHA FC>III), cardiac arrhythmia, history of stroke and myocardial infarction, diabetes, occlusive peripheral arterial disease, renal impairment, severe co-morbidities, orthostatic hypotension.

All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, and echocardiography. Office BP was measured using a mercury sphygmomanometer, according to Korotkov's method. BP was measured 3 times, and the means of these measurements were used in the analyses. Echocardiography was carried out according to the recommendations of the American Society of Echocardiography in M- and B-modes using Philips EnVisor C Ultrasound Machine (the Netherlands). LVM was calculated using the formula R. Devereux (1994). Left ventricular hypertrophy (LVH) was defined as LVMI of >95 g/m² (for women) and >115 g/m² (for men).⁽³⁰⁾ Carotid intima-media thickness (CIMT) was assessed for both left and right carotid arteries using a 7.5 MHz linear array transducer (Sonoline Versa Pro ultrasound system, Siemens, Germany).

Blood levels of TC, TG, HDL-C, LDL-C, and VLDL-C were determined in the venous blood using automatic biochemical analyzer Daytona (RANDOX, United Kingdom) and RANDOX test systems by the enzymatic colorimetric method. The content of LDL-C was calculated according to Fridvald's formula.

Genomic DNA samples were isolated from the peripheral blood leukocytes by using the Diatom™ DNA Prep 200 Kit (Isogen Laboratory LLC, Moscow, Russia) according to manufacturer's protocol. The quantity and quality of DNA were determined on a NanoDrop 2000 spectrophotometer (Thermo Scientific™ Wilmington, DE, USA). A multiplex RT-PCR assay was used to detect the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs.

Statistical analysis was performed using the statistical software «Statistica» (v10.0, StatSoft, USA). For descriptive analysis, results are presented as mean \pm standard deviation (SD). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. The Mann-Whitney U Test was used to compare the differences between the two independent groups (for nonparametric data). Group comparisons with respect to categorical variables were performed using chi-square test. Genetic markers for HWE were tested. Differences in the allele and genotype distribution between the groups were assessed by χ^2 -test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

Four genetic models were analyzed: the dominant model, the recessive model, the multiplicative model, and the additive model (the Cochran-Armitage trend test). A probability value of $P < 0.05$ was considered statistically significant.

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided the written informed consent.

Results and Discussion

We studied the distribution of the *AGT* T704C (M235T) rs699 polymorphism in 61 Uzbek patients with TRH (cases) and 117 Uzbek patients with non-TRH (controls) (Group 1). We also studied the distribution of the *NOS3* G894T (Glu298Asp) rs1799983 polymorphism in 61 Uzbek patients with TRH (cases) and 115 Uzbek patients with non-TRH (controls) (Group 2). The clinical characteristics of AH patients are presented in Table 1.

In Group 1, the mean age of the 178 AH patients was 56.67 ± 11.12 years, the mean duration of AH was 9.42 ± 5.49 years, and the average SBP and DBP were 166.53 ± 17.68 mmHg and 98.55 ± 12.02 mmHg, respectively. Obesity and overweight were found in 52.8% and 32.0% of cases, respectively. About 87.6% and 74.7% of patients were diagnosed with LVH and increased CIMT, respectively. Dyslipidemia was detected in 77.0% of patients. In Group 2, the mean age of the 176 AH patients was 56.71 ± 11.14 years, the mean duration of AH was 9.56 ± 5.83 years, and the average SBP and DBP were 166.55 ± 17.79 mmHg and 98.89 ± 12.17 mmHg, respectively.

Obesity and overweight were found in 52.8% and 31.8% of cases, respectively. About 88.1% and 75.0% of patients were diagnosed with LVH and increased CIMT, respectively. Dyslipidemia was detected in 77.3% of patients. Thus, given the previous data, our AH patients had a high and high-to-very-high cardiovascular risk. In both groups, TRH patients were older than non-TRH patients and had a longer course of AH, higher SBP and DBP, and LVH frequency.

Results of the genotyping of the *AGT* T704C (M235T) rs699 and *NOS3* G894T (Glu298Asp) rs1799983 SNPs are presented in Table 2.

The distribution of polymorphic markers of the *AGT* T704C (M235T) rs699 SNP in TRH patients and non-TRH patients was in HWE. In TRH patients and non-TRH patients, the genotype distribution was as follows: CC=50.8%, CT=32.8%, TT=16.4% and CC=25.6%, CT=53.8%, TT=20.5%, respectively. An analysis of the frequency distribution of alleles of the *AGT* T704C (M235T) rs699 SNP showed that the carriage of the C allele was dominant in TRH patients (67.2% vs. 32.8% for the T allele; $P=0.000$), compared to non-TRH patients (52.6% vs. 47.4% for the T allele; $P>0.05$).

Analysis of the multiplicative model for the *AGT* T704C (M235T) rs699 SNP showed a significant risk of TRH with the carriage of the C allele (OR=1.85, 95% CI: 1.17-2.92, $P=0.008$). The additive and recessive models for the *AGT* T704C (M235T) rs699 SNP showed a significant risk of TRH with the carriage of the homozygous CC genotype (OR=3.00, 95% CI: 1.56-5.75, $P=0.009$) (Table 3).

Table 1.

Clinical characteristics of AH patients in the study groups.

Variable	Group 1 <i>AGT</i> T704C (M235T) rs699 SNP				Group 2 <i>NOS3</i> G894T (Glu298Asp) rs1799983 SNP			
	Total (n=178)	TRH (cases) n=61	non-TRH (controls) n=117	<i>P</i>	Total n=176	TRH (cases) n=61	non-TRH (controls) n=115	<i>P</i>
Age, years	56.67 ± 11.12	61.52 ± 9.43	54.06 ± 11.17	0.000	56.71 ± 11.14	61.52 ± 9.43	54.09 ± 11.16	0.000
AH duration, years	9.42 ± 5.49	10.60 ± 5.69	8.77 ± 5.32	0.035	9.56 ± 5.83	10.60 ± 5.69	8.69 ± 5.84	0.039
SBP, mmHg	166.53 ± 17.68	172.42 ± 20.62	162.73 ± 16.31	0.001	166.55 ± 17.79	172.42 ± 20.62	162.91 ± 16.17	0.001
DBP, mmHg	98.55 ± 12.02	101.13 ± 9.60	97.17 ± 13.02	0.038	98.89 ± 12.17	101.13 ± 9.60	97.75 ± 11.23	0.048
BPmean, mmHg	120.92 ± 12.18	124.89 ± 12.35	118.78 ± 11.65	0.001	121.67 ± 13.85	124.89 ± 12.35	119.46 ± 12.70	0.007
BMI, kg/m ²	32.17 ± 5.68	33.53 ± 5.92	31.79 ± 5.61	0.056	32.21 ± 5.72	33.53 ± 5.92	31.86 ± 5.63	0.068
BMI>30 (kg/m ²), %	94 (52.8%)	36 (59.0%)	58 (49.6%)	0.234	93 (52.8%)	36 (59.0%)	57 (49.6%)	0.236
BMI>25<30 (kg/m ²), %	57 (32.0%)	21 (34.4%)	36 (30.8%)	0.626	56 (31.8%)	21 (34.4%)	35 (30.4%)	0.589
LVH, %	156 (87.6%)	59 (96.7%)	97 (82.9%)	0.008	155 (88.1%)	59 (96.7%)	96 (83.5%)	0.01
CIMT ≥ 0.9 mm, %	133 (74.7%)	48 (78.7%)	85 (72.6%)	0.376	132 (75.0%)	48 (78.7%)	84 (73.0%)	0.407
Dyslipidemia, %	137 (77.0%)	51 (83.6%)	86 (73.5%)	0.130	136 (77.3%)	51 (83.6%)	85 (73.9%)	0.145

P - between cases and controls in Groups 1 and 2.

Table 2.

The distribution of polymorphic markers of the *AGT* T704C (M235T) rs699 SNP and *NOS3* G894T (Glu298Asp) rs1799983 SNP in *TRH* patients and non-*TRH* patients (controls).

Gene	SNP	Genotype	TRH	HWE	χ^2	<i>P</i>	Control	HWE	χ^2	<i>P</i>	Allele	Frequency of alleles	
												TRH	Control
<i>AGT</i>	rs699 T704C	TT	0.164	0.107	1.72	0.19	0.205	0.225	0.35	0.56	T	0.328	0.474
		CT	0.328	0.441			0.538	0.499			C	0.672	0.526
		CC	0.508	0.452			0.256	0.276					
<i>NOS3</i>	rs1799983 G894T	GG	0.459	0.463	0.00	1	0.678	0.654	1.42	0.23	G	0.680	0.809
		GT	0.443	0.435			0.261	0.309			T	0.320	0.191
		TT	0.098	0.102			0.061	0.037					

Table 3.

Genetic predisposition to *TRH*.

Genetic model	Allele, Genotype	Cases	Controls	χ^2	<i>P</i>	OR (95%CI)	
		n=61	n=117			OR	95%CI
AGT T704C (M235T) rs699 SNP							
Multiplicative model (χ^2 test, df=1)	T	0.328	0.474	7.05	0.008	0.54	0.34-0.85
	C	0.672	0.526			1.85	1.17-2.92
Additive model ([CATT], xi=[0,1,2], df=1)	TT	0.164	0.205	6.74	0.009	0.76	0.34-1.71
	TC	0.328	0.538			0.42	0.22-0.80
	CC	0.508	0.256			3.00	1.56-5.75
Dominant model (χ^2 test, df=1)	TT	0.164	0.205	0.44	0.51	0.76	0.34-1.71
	TC + CC	0.836	0.795			1.32	0.58-2.97
Recessive model (χ^2 test, df=1)	TT + TC	0.492	0.744	11.28	0.0008	0.33	0.17-0.64
	CC	0.508	0.256			3.00	1.56-5.75
NOS3 G894T (Glu298Asp) rs1799983 SNP							
Genetic model	Allele, Genotype	Cases	Controls	χ^2	<i>P</i>	OR (95%CI)	
		n=61	n=115			OR	95%CI
Multiplicative model (χ^2 test, df=1)	G	0.680	0.809	7.29	0.007	0.50	0.30-0.83
	T	0.320	0.191			1.99	1.20-3.28
Additive model ([CATT], xi=[0,1,2], df=1)	GG	0.459	0.678	6.62	0.01	0.40	0.21-0.76
	GT	0.443	0.261			2.25	1.17-4.33
	TT	0.098	0.061			1.68	0.54-5.25
Dominant model (χ^2 test, df=1)	GG	0.459	0.678	8.00	0.005	0.40	0.21-0.76
	GT + TT	0.541	0.322			2.48	1.31-4.70
Recessive model (χ^2 test, df=1)	GG + GT	0.902	0.939	0.82	0.37	0.59	0.19-1.85
	TT	0.098	0.061			1.68	0.54-5.25

The distribution of polymorphic markers of the *NOS3* G894T (Glu298Asp) rs1799983 SNP in *TRH* patients and non-*TRH* patients were in HWE. In *TRH* patients and non-*TRH* patients, the genotype distribution was as follows: GG=45.9%, GT=44.3%, TT=9.8% and GG=67.8%,

GT=26.1%, TT=6.1%, respectively, thus GG genotype prevailed in non-*TRH* patients, compared to *TRH* patients ($\chi^2=8.005$, $P=0.018$). An analysis of the frequency distribution of alleles of the *NOS3* G894T (Glu298Asp) rs1799983 SNP showed that the carriage of the G allele was dominant in *TRH*

patients (68.0% vs. 32.0% for the T allele) and non-TRH patients (80.9% vs. 19.1% for the T allele) with the highest degree of dominance in non-TRH patients vs. TRH patients ($\chi^2=7.29$, $P=0.007$).

Analysis of the multiplicative model for the *NOS3* G894T (Glu298Asp) rs1799983 SNP showed a significant risk of TRH with the carriage of the T allele (OR=1.99, 95% CI: 1.20-3.28, $P=0.007$). Analysis of the additive model for the *NOS3* G894T (Glu298Asp) rs1799983 SNP showed a significant risk of TRH with the carriage of the heterozygous GT genotype (OR=2.25, 95% CI: 1.17-4.33, $P=0.01$). At the same time, the carriage of the G allele (OR=0.5, 95% CI: 0.30-0.83, $P=0.007$) and GG genotype (OR=0.40, 95% CI: 0.21-0.76, $P=0.01$) may be protective against the development of TRH.

It should be noted that data on the study of molecular genetic markers of resistant hypertension are limited, especially in the Asian population. To identify novel genetic loci associated with resistant hypertension in the Japanese population, Takahashi et al.⁽³²⁾ conducted a genome-wide association study with 2705 resistant hypertension cases and 21,296 mild hypertension controls, all from BioBank Japan. The authors identified one novel susceptibility candidate locus, rs1442386 on chromosome 18p11.3 (DLGAP1), achieving genome-wide significance (OR=0.85, 95% CI: 0.81-0.90, $P=3.75 \times 10^{-8}$), and 18 loci showing suggestive association, including rs62525059 of 8q24.3 (CYP11B2) and rs3774427 of 3p21.1 (CACNA1D).⁽³²⁾ Yugar-Toledo et al.⁽³³⁾ examined 70 resistant, 80 well-controlled hypertensive patients, and 70 normotensive controls. All subjects were genotyped for *ACE* insertion/deletion (rs1799752), *AGT* M235T (rs699), and *NOS3* Glu298Asp (rs 1799983), and the multifactor dimensionality reduction analyses showed that carriers of the *AGT* 235T allele were at increased risk for resistant hypertension, especially if they were older than 50 years.

The vasodilator effect of NO that eNOS produces is very important for maintaining the vascular function,⁽³¹⁾ and the G894T polymorphism, which is associated with reduced eNOS expression and activity, and subsequently reduced NO production, could be a potential candidate marker for hypertension development.^(34,35) In a study by Shi et al.,⁽³⁶⁾ a total of 60 eligible articles involving 14,185 cases and 13,407 controls were finally selected. The authors found a significant association between eNOS rs1799983 polymorphism and hypertension under any genetic model (T vs G: OR=1.44, 95% CI 1.26–1.63; GT vs GG: OR=1.34, 95% CI 1.18–1.52; TT vs GG: OR=1.80, 95% CI 1.41–2.31; GT+TT vs GG: OR=1.42, 95% CI 1.25–1.63; TT vs GG+GT: OR=1.68, 95% CI 1.35–2.08; GT vs GG+TT: OR=1.24, 95% CI 1.11–1.40). Jáchymová et al.⁽³⁷⁾ showed that the T allele of the *NOS* G894T (Glu298Asp) rs1799983 SNP may be a factor in the resistance to conventional antihypertensive therapy.

Despite known advances in genetic research technology, TRH has not yet fully taken advantage of more complex genetic approaches, such as GWAS, genome sequencing, and others used in pharmacogenomics research. Gaining a complete understanding of the genetic background of TRH is critical to predicting individual TRH risk and improving

individual outcomes by optimizing drug therapy based on clinical features and genetic risk factors.

Our results indicate a significantly greater accumulation of the C allele and CC genotype of the *AGT* T704C (M235T) rs699 SNP among TRH patients than among patients with non-TRH. We found a significant association between the *AGT* T704C (M235T) rs699 SNP and the risk of TRH under the multiplicative genetic model (C vs. T: OR=1.85, 95% CI: 1.17-2.92, $P=0.008$), additive model (CC vs. TT vs. TC; OR=3.00, 95% CI: 1.56-5.75, $P=0.009$), and recessive model (CC vs. TC+TT; OR=3.00, 95% CI: 1.56-5.75, $P=0.0008$). For the *NOS3* G894T (Glu298Asp) rs1799983 SNP, the multiplicative model showed a significant risk of TRH with the carriage of the T allele (OR=1.99, 95% CI: 1.20-3.28, $P=0.007$), and the additive model showed a significant risk of TRH with the carriage of the heterozygous GT genotype (OR=2.25, 95% CI: 1.17-4.33, $P=0.01$). At the same time, the carriage of the G allele (OR=0.5, 95% CI: 0.30-0.83, $P=0.007$) and GG genotype (OR=0.40, 95% CI: 0.21-0.76, $P=0.01$) may be protective against the development of TRH. Further genetic studies of TRH may help achieve better individual outcomes by optimizing drug therapy based on genetic variation.

Competing Interests

The authors declare that they have no competing interests.

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Effect of Nitrendipine and Amlodipine on Cognitive Functions of Patients with Arterial Hypertension

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Abstract

Background: Arterial hypertension (AH) is one of the main factors causing a high risk of cardiovascular complications and mortality. The existence of a relationship between high blood pressure (BP) and the risk of developing central nervous system pathology, primarily stroke, and cognitive impairment, has been shown. The purpose of this study was a comparative assessment of the effect of 12-month antihypertensive therapy (AHT) with the inclusion of nitrendipine (NIT) or amlodipine (AML) on cognitive functions (CF) in hypertensive patients.

Methods and Results: The study included 111 patients of both genders aged 30-75 years with AH Grades 1-3 (ESC/ESH, 2018). All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, pulse contour analysis, and 24-hour ambulatory blood pressure monitoring, neuropsychological tests (Mini-Cog test, Montreal Cognitive Assessment (MoCA) test, Hospital Anxiety and Depression Scale (HADS), and self-assessment questionnaire for memory, attention, thinking, ability to cope with one's affairs, and ability to make decisions). After the screening stage, all patients were discontinued from previous therapy and assigned to the 2 regimes of AHT. Group 1 included 58 AH patients who received NIT as monotherapy or as part of combination AHT; Group 2 included 53 patients who received AML as monotherapy or as part of combination AHT. Correlation analysis between the parameters of diurnal blood pressure profile and the MoCA test revealed a weak but statistically significant negative correlation between the total MoCA score and the average 24-h systolic BP ($r_s = -0.33$, $P = 0.015$). In addition, there was a weak but statistically significant negative correlation between the total MoCA score and the daytime systolic BP variability and daytime diastolic BP variability ($r_s = -0.40$ and $r_s = -0.35$, respectively, $P = 0.000$ in both cases). A weak but statistically significant negative correlation was found between the total Mini-Cog score and pulse wave velocity and central pulse pressure ($r_s = -0.24$, $P = 0.01$ and $r_s = -0.27$, $P = 0.007$, respectively). Analysis of the office BP indicators showed high antihypertensive efficacy of 12-month therapy in both groups, regardless of the therapy regimens.

A comparative analysis of the effect of AHT with the inclusion of NIT or AML on CF in AH patients showed the advantages of combined AHT with the inclusion of NIT. Amlodipine treatment did not significantly affect any test score. Thus, in Group 1, after 12 months of therapy, there was an increase in the total Mini-Cog score from 3.8 ± 1.08 points to 4.55 ± 0.75 points ($P < 0.001$), while in Group 2, there was a non-significant decrease in this score from 4.26 ± 0.98 points to 3.92 ± 0.95 points ($P > 0.05$). There was also an increase in the total MoCA score in Group 1 from 23.3 ± 2.8 points to 25.08 ± 2.6 points ($P < 0.001$), while in Group 2, there was a non-significant decrease in this score from 24.06 ± 2.73 points to 23.07 ± 2.7 points ($P > 0.05$). It should be noted that only in Group 1 did we find a significant improvement in CF, such as abstraction, delayed recall, memory, and attention, as well as a significant improvement in work-coping and decision-making. In Group 1, the HADS Depression score decreased from 4.6 ± 3.7 points to 3.32 ± 2.95 points ($P < 0.05$), HADS Anxiety score decreased from 7.01 ± 5.37 points to 4.95 ± 3.75 points ($P < 0.02$). At the same time, in Group 2, in contrast, the HADS Depression score and the HADS Anxiety score did not significantly change.

Conclusion: A weak but statistically significant negative correlation was found between the total MoCA score and the daytime SBP/DBP variability in AH patients. A weak but statistically significant negative correlation was found between the total Mini-Cog score and pulse wave velocity and central pulse pressure. A pronounced antihypertensive efficacy of 12-month combination therapy was noted, with the inclusion of both NIT and AML. The NIT-based treatment contributed to a significant increase in the total Mini-Cog score and the total MoCA score and a substantial improvement in CF. Abstraction, delayed recall, memory, attention, work-coping, and decision-making significantly improved, compared to AML-based treatment. The presented data allow a differentiated approach to tactics for treating AH patients with severe cognitive impairment. (International Journal of Biomedicine. 2023;13(2):217-223.)

Keywords: arterial hypertension • cognitive impairment • amlodipine • nitrendipine

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Abbreviations

AH, arterial hypertension; **AHM**, antihypertensive medication; **AHT**, antihypertensive therapy; **ACEI**, angiotensin-converting enzyme inhibitor; **ARB**, angiotensin receptor blocker; **AP**, augmentation pressure; **AIx**, augmentation index; **BP**, blood pressure; **BMI**, body mass index; **CCBs**, calcium channel blockers; **CF**, cognitive functions; **CIMT**, carotid intima-media thickness; **DBP**, diastolic BP; **DBPP**, diurnal blood pressure profile; **HADS**, Hospital Anxiety and Depression Scale; **LVH**, left ventricular hypertrophy; **MoCA**, Montreal Cognitive Assessment; **PPc**, central pulse pressure; **PP**, pulse pressure; **PWV**, pulse wave velocity; **SBP**, systolic BP; **TD**, thiazide diuretic.

Introduction

Arterial hypertension (AH) is one of the main factors causing a high risk of cardiovascular complications and mortality. Overall, according to ESH/ESC data (2018), the prevalence of hypertension is in the range of 30%-45% of the general population, with a sharp increase with age. In people over 65, AH is detected in 30%-50% of cases. According to ESH/ESC (2018), 76% of patients with hypertension are at risk of dying within 10 years.⁽¹⁾ The existence of a relationship between high blood pressure (BP) and the risk of developing central nervous system pathology, primarily stroke, and cognitive impairment, has been shown.⁽²⁾ Cerebral complications of hypertension occur the earliest, dominate, and make the greatest contribution to the structure of mortality associated with hypertension.

Numerous studies have found a relationship between taking antihypertensive medication (AHM) and the state of cognitive function, which confirms the positive effect of antihypertensive therapy (AHT) on slowing the progression of cognitive impairment.⁽³⁻⁶⁾

An analysis of data from a number of published studies has demonstrated the relationship between treatment with calcium channel blockers (CCBs) and less cognitive impairment in patients, compared with the use of other antihypertensive medications (AHMs).⁽⁶⁻⁸⁾ However, when analyzing the results of most observational studies, the type of CCB prescribed was not taken into account, although some of the studies show the benefits of dihydropyridine derivatives.⁽⁹⁾ Animal studies have demonstrated that dihydropyridine CCBs can influence the production and clearance of amyloid in the brain (the pathological substrate of Alzheimer's disease). Another possible mechanism for the effect of CCB therapy on cognitive function is the protection of neurons from the influx of excess calcium ions, which can trigger apoptosis.^(6,10,11) In a number of experimental studies, the above positive effects were observed with the use of nitrendipine, nicardipine, lercanidipine, nimodipine, and some other rarely used CCBs.⁽¹⁰⁻¹²⁾

A special place among the drugs of this class is occupied by nitrendipine, which, to a greater extent than other CCBs (nilvadipine, nimodipine, nicardipine and lercanidipine), has the ability to block the formation of beta-amyloid, and also

increases its clearance, unlike felodipine and amlodipine.⁽¹³⁾ The clinical efficacy of nitrendipine has been demonstrated in randomized placebo-controlled trials Syst-Eur and Syst-China.^(14,15)

It is well known that the most commonly prescribed and most studied dihydropyridine CCB is amlodipine. Its high antihypertensive and organ-protective efficacy has long been known. However, nitrendipine is a CCB with neuroprotective activity. The purpose of this study was a comparative assessment of the effect of 12-month AHT with the inclusion of nitrendipine (NIT) or amlodipine (AML) on cognitive functions (CF) in hypertensive patients.

Materials and Methods

The study included 111 patients of both genders aged 30-75 years with AH Grades 1-3 (ESC/ESH, 2018), who were on outpatient treatment at the Republican Specialized Scientific and Practical Medical Center for Cardiology. Exclusion criteria were symptomatic hypertension, valvular heart disease, acute coronary syndrome, chronic heart failure (NYHA FC>III), cardiac arrhythmia, history of stroke and myocardial infarction, diabetes, occlusive peripheral arterial disease, renal impairment, severe co-morbidities, orthostatic hypotension.

Office BP was measured using a mercury sphygmomanometer, according to Korotkov's method. BP was measured 3 times, and the means of these measurements were used in the analyses. The 24-hour ABPM was performed using a BR-102 plus (SCHILLER, Switzerland).

The pulse contour analysis was carried out using the SphygmoCor device (AtCor Medical, Australia), which obtains peripheral arterial pressure waveforms by applying an arterial applanation tonometer to the wrist. Such indicators as the central SBP (SBPc), central DBP (DBPc), central PP (PPc), augmentation pressure (AP), augmentation index (AIx), and pulse wave velocity (PWV) were analyzed.

CF were assessed using neuropsychological tests: Mini-Cog test (drawing a clock, reproducing words), Montreal Cognitive Assessment (MoCA) test, and self-assessment questionnaire for memory, attention, thinking, ability to cope with one's affairs, and ability to make decisions. The Hospital Anxiety and Depression Scale (HADS) was used to assess levels of anxiety and depression.

After the screening stage, all patients were discontinued from previous therapy and assigned to the 2 regimes of AHT. Group 1 included 58 AH patients who received NIT (Nitresan, Pro.Med.CS, Czech Republic) as monotherapy or as part of combination AHT; Group 2 included 53 patients who received AML (Normodipin, "Gedeon Richter", Hungary) as monotherapy or as part of combination AHT.

The average daily dose of NIT and AML was 13.6±7.05mg and 6.13±2.11mg, respectively.

In Group 1, 37.9% of patients received monotherapy, 43.1% - dual therapy (NIT+TD or NIT+ACEI/ARB), and 18.9% - triple therapy (NIT+ACEI/ARB+TD).

In Group 2, 32.0% of patients received monotherapy, 43.4% - dual therapy (AML+TD or AML+ACEI/ARB), and

22.4% - triple therapy (AML+ACEI/ARB+TD) (Table 1). Dosing of AHM, taking into account the maximum doses, was titrated at 2-week intervals to achieve a target blood pressure. The effectiveness of the prescribed therapy was evaluated after 12 months of treatment.

The effectiveness of therapy was assessed by achieving the target BP level according to 2018 ESH/ESH Guidelines for the management of AH. The primary target level for SBP and DBP was <140 mmHg and <90 mmHg, respectively.

Table 1.

Characteristics of antihypertensive therapy

Therapy	Group 1 n=58	Group 2 n=53
Monotherapy	22 (37.9%)	17 (32.0%)
Dual therapy	25 (43.10%)	23 (43.4%)
CCB + TD BKK+ ACEI/ARB	15 (25.9%) 10 (17.2%)	14 (26.4%) 9 (16.9%)
Triple therapy		
ACEI/ARB +CCB + TD	11 (18.9 %)	13 (22.4%)
BB	40 (69.0%)	38 (71.7%)

Statistical analysis was performed using the statistical software «Statistica» (v10.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean± standard deviation (SD) for continuous variables. The Mann-Whitney U Test was used to compare the differences between the two independent groups (for nonparametric data). The Wilcoxon criterion was used to compare the differences between the paired samples. Group comparisons with respect to categorical variables were performed using chi-square test. Spearman's rank correlation coefficient (r_s) was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided written informed consent.

Results

In Groups 1 and 2, the average age of the patients was 58.6 ± 11.6 years and 53.7 ± 12.6 years, respectively, and the average duration of AH was 11.2 ± 7.07 years and 8.12 ± 5.84 years, respectively (Table 2).

Correlation analysis between the parameters of DBPP and the MoCA test revealed a weak but statistically significant negative correlation between the total MoCA score and the average 24-h SBP ($r_s=-0.33$, $P=0.015$) (Table 3). In addition, there was a weak but statistically significant negative correlation between the total MoCA score and the daytime SBP

variability and daytime DBP variability ($r_s=-0.40$ and $r_s=-0.35$, respectively, $P=0.000$ in both cases). A weak but statistically significant negative correlation was found between the total Mini-Cog score and PWV and PPc ($r_s=-0.24$, $P=0.01$ and $r_s=-0.27$, $P=0.007$, respectively) (Table 4).

Table 2.

Clinical characteristics of AH patients in the study groups

Parameter	Group 1 n=58	Group 2 n=53	P
Age, yrs	58.6 ± 11.6	53.7 ± 12.6	0.06
AH duration, yrs	11.2 ± 7.07	8.12 ± 5.84	0.01
SBP, mmHg	157.8 ± 16.1	161.7 ± 16.2	0.1
DBP, mmHg	95.9 ± 8.85	98.2 ± 7.68	0.09
BPmean, mmHg	116.5 ± 10.1	119.3 ± 9.36	0.8
BMI, kg/m ²	30.6 ± 4.85	30.9 ± 4.78	0.2
BMI >30 kg/m ² , %	28 (48.2%)	30 (56.6%)	0.378
BMI >25<30 kg/m ² , %	19 (32.7%)	19 (35.8%)	0.7321
LVH, %	49 (84.4%)	41 (77.3%)	0.343
PE/PA< 1.0, %	43 (74.0%)	35 (66.0%)	0.360
PWV >10 m/sec, %	35 (60.0%)	29 (54.7%)	0.574
CIMT ≥0.9 mm, %	36 (62.0%)	27 (50.9%)	0.241
Dyslipidemia, %	41(70.6%)	30 (56.6%)	0.127

Table 3.

Correlation analysis between the parameters of DBPP and the MoCA test.

	r_s	P
Average 24-h SBP and MoCA total score	-0.33	0.015
Daytime SBP variability and MoCA total score	-0.40	0.000
Daytime DBP variability and MoCA total score	-0.35	0.000

Table 4.

Correlation analysis between the total Mini-Cog score and PWV and PPc.

	r_s	P
PWV and Mini-Cog total score	-0.24	0.01
cPP and Mini-Cog total score	-0.27	0.007

Analysis of the office BP indicators showed high antihypertensive efficacy of 12-month therapy in both groups, regardless of the therapy regimens (Table 5): SBP, DBP, and BPmean significantly decreased.

Table 5.**Antihypertensive efficacy of 12-month therapy in the study groups**

Parameter		Group 1 n=58	Group 2 n=53	P
SBP, mmHg		157.7±16.1 125.1±10.7*	161.1±16.2 125.5±11.5*	0.1
DBP, mmHg		95.9±8.85 79.5±5.94*	98.2±7.68 78.7±7.69*	0.09
BPmean, mmHg		116.5±10.1 94.7±7.12*	119.3±9.36 94.4±8.17*	0.8
Δ% SBP		-20.26±7.09	-21.7±8.97	0.4
Δ% DBP		-16.53±8.73	-19.1±9.29	0.2
Δ% BPmean		-18.35±6.99	-20.4±8.01	0.5
Achieving the target level of BP	SBP	49(84.4%)	45(84.9%)	0.942
	DBP	50(86.2%)	44(83.0%)	0.642
	SBP&DBP	48(82.7%)	43(81.1%)	0.828

The numerator represents the results before treatment and the denominator - after treatment. P-value - between Groups 1 and 2 before treatment. * - $P < 0.001$ before treatment and after 12-month therapy within the group.

In general, in both groups, against the background of therapy, a positive dynamic of the DBPP indicators was noted; however, the advantages of therapy with the inclusion of AML were revealed. In Group 2, the nighttime SBP load decreased more significantly (from 73.4±28.7% to 42.5±30.7%, $P < 0.05$), as well as the nighttime DBP load (from 59.4±32.7% to 27.6±31.7%, $P < 0.02$) vs. Group 1 (from 64.83±36.6% to 48.5±35.9%, $P < 0.05$, and from 51.4±38.6% to 30.5±36.6%, respectively) (Table 6).

The effectiveness of both therapy regimens was found for parameters of central hemodynamics (SBPc, PPc) and arterial stiffness, with the advantages of therapy with the inclusion of NIT (Table 7). In particular, the indicators of AP and PWV decreased significantly only in Group 1.

A comparative analysis of the effect of AHT with the inclusion of NIT or AML on CF in AH patients showed the advantages of combined AHT with the inclusion of NIT. AML treatment did not significantly affect any test score (Table 8). Thus, in Group 1, after 12 months of therapy, there was an increase in the total Mini-Cog score from 3.8±1.08 points to 4.55±0.75 points ($P < 0.001$), while in Group 2, there was a non-significant decrease in this score from 4.26±0.98 points to 3.92±0.95 points ($P > 0.05$). There was also an increase in the total MoCA score in Group 1 from 23.3±2.8 points to 25.08±2.6 points ($P < 0.001$), while in Group 2, there was a non-significant decrease in this score from 24.06±2.73 points to 23.07±2.7 points ($P > 0.05$). It should be noted that only in Group 1 did we find a significant improvement in CF, such as abstraction, delayed recall, memory, and attention, as well as a significant improvement in work-coping and decision-making. In Group 1, the HADS Depression score decreased from 4.6±3.7 points to 3.32±2.95 points ($P < 0.05$), HADS Anxiety score decreased from 7.01±5.37 points to 4.95±3.75 points ($P < 0.02$) (Table 8). At the same time, in Group 2, in contrast, the HADS Depression score and the HADS Anxiety score did not significantly change.

Table 6.**Dynamics of ABPM indicators against the background of 12-month therapy in the study groups.**

Parameter	Group 1 n=58	Group 2 n=53	Mann-Whitney U Test	
			U	P
Average 24-h SBP, mmHg	144.5±23.3 125.6±12.4*	144.08±19.9 123.7±8.01*	484	0.11
Average 24-h DBP, mmHg	88.5±16.2 76.3±8.11*	88.5±13.5 75.2±7.85*	482	0.88
Average daytime SBP, mmHg	147.18±22.9 125.9±11.7	145.9±20.42 125.05±8.97	2158	0.9
Average daytime DBP, mmHg	90.3±14.96 77.5±9.7*	89.8±13.5 76.07±8.6*	543	0.6
Average nighttime SBP, mmHg	137.7±24.5 120.5±14.47*	136.1±17.8 118.5±9.62*	532	0.7
Average nighttime DBP, mmHg	81.7±15.2 72.04±10.75°	82.5±12.09 70.1±8.69*	514	0.5
Daytime SBP variability, mmHg	15.8±5.62 14.05±3.54	13.6±4.43 12.9±2.35	1564	0.9
Daytime DBP variability, mmHg	14.1±6.2 11.3±2.65	15.3±4.93 12.2±4.43	1534	0.2
Nighttime SBP variability, mmHg	15.9±19.3 11.5±3.92	14.7±5.13 11.6±4.81	2000	0.2
Nighttime DBP variability, mmHg	11.7±11.3 9.2±3.02	12.27±4.8 9.6±3.43	2196	0.4
Daytime SBP load, %	48.02±36.6 24.3±27.4	51.6±34.9 18.02±19.3	2245	0.6
Daytime DBP load, %	43.97±35.3 23.7±29.05	46.2±31.3 18.3±23.1	2213	0.7
Nighttime SBP load	64.83±36.6 48.5±35.9	73.4±28.7 42.5±30.7^	674	0.6
Nighttime DBP load, %	51.4±38.6 30.5±36.6	59.4±32.7 27.6±31.7°	707	0.7

The numerator represents the results before treatment and the denominator - after treatment. P-value - between Groups 1 and 2 before treatment. * - $P < 0.001$, ° - $P < 0.02$, and ^ - $P < 0.05$ before treatment and after 12-month therapy within the group.

Table 7.**Dynamics of parameters of central hemodynamics and vascular stiffness against the background of 12-month therapy**

Parameter	Group 1 n=58	Group 2 n=53	Mann-Whitney U Test	
			U	P
SBPc, mmHg	156.2±21.8 142.8±18.7°	155.1±26.5 138.9±18.5*	1438	0.7
DBPc, mmHg	79.8±15.01 81.9±9.47	82.8±17.6 82.4±10.1	1252	0.4
PPc, mmHg	75.1±18.8 59.9±14.9*	71.1±20.01 61.09±14.83°	1155	0.1
AP, mmHg	18.6±7.94 16.05±6.6*	14.8±7.54 14.6±5.7	1114	0.06
AI, %	32.5±10.3 32.2±8.09*	29.02±11.8 31.8±9.47*	4951	0.5
PWV, m/sec	11.3±2.67 9.85±2.41^	10.6±2.65 9.17±2.05	4229	0.8

The numerator represents the results before treatment and the denominator - after treatment. P-value - between Groups 1 and 2 before treatment. ^ - $P < 0.001$, ° - $P < 0.02$, and * - $P < 0.05$ before treatment and after 12-month therapy within the group.

Table 8.

Dynamics of cognitive functions against the background of 12-month therapy in the study groups.

Parameter	Group 1 n=58	Group 2 n=53	Mann-Whitney U Test	
			U	P
<u>Mini-Cog test</u>				
Total score	$\frac{3.8 \pm 1.08}{4.55 \pm 0.75^*}$	$\frac{4.26 \pm 0.98}{3.92 \pm 0.95}$	1162	0.02
Word recall	$\frac{2.17 \pm 0.86}{2.72 \pm 0.58^*}$	$\frac{2.52 \pm 0.63}{2.33 \pm 0.61}$	1186	0.06
Clock draw	$\frac{1.67 \pm 0.63}{1.82 \pm 0.42}$	$\frac{1.73 \pm 0.59}{1.66 \pm 0.55}$	1436	0.5
HADS Anxiety score	$\frac{7.01 \pm 5.37}{4.95 \pm 3.75^o}$	$\frac{5.88 \pm 3.39}{5.96 \pm 2.57}$	1469	0.6
HADS Depression score	$\frac{4.6 \pm 3.7}{3.32 \pm 2.95^\wedge}$	$\frac{4.73 \pm 3.29}{5.88 \pm 2.47}$	1473	0.7
<u>MoCA test</u>				
Total score	$\frac{23.3 \pm 2.8}{25.08 \pm 2.6^*}$	$\frac{24.06 \pm 2.73}{23.07 \pm 2.7}$	1197	0.04
Visuospatial abilities	$\frac{3.67 \pm 1.26}{4.08 \pm 0.97^\wedge}$	$\frac{4.13 \pm 1.01}{3.84 \pm 1.06}$	1216	0.05
Naming	$\frac{2.72 \pm 0.45}{2.87 \pm 0.37}$	$\frac{2.96 \pm 0.3}{2.81 \pm 0.48}$	1400	0.1
Attention	$\frac{4.84 \pm 1.18}{5.27 \pm 1.12^*}$	$\frac{5.32 \pm 0.75}{5.03 \pm 1.03}$	1197	0.04
Language	$\frac{2.20 \pm 0.61}{1.98 \pm 0.54}$	$\frac{1.83 \pm 0.91}{1.75 \pm 0.64}$	11293	0.04
Abstraction	$\frac{1.3 \pm 0.68}{1.82 \pm 0.5^*}$	$\frac{1.67 \pm 0.56}{1.67 \pm 0.51}$	1078	0.006
Delayed recall	$\frac{2.8 \pm 1.22}{3.29 \pm 1.02^\wedge}$	$\frac{2.86 \pm 1.24}{2.64 \pm 1.22}$	1533	0.9
Orientation	$\frac{5.89 \pm 0.3}{5.87 \pm 0.32}$	$\frac{5.88 \pm 0.31}{5.77 \pm 0.5}$	1522	0.9
<u>Self-assessment questionnaire</u>				
Memory	$\frac{7.04 \pm 2.36}{8.24 \pm 1.24^*}$	$\frac{7.26 \pm 1.77}{6.89 \pm 1.73}$	1509	0.5
Attention	$\frac{8.75 \pm 1.72}{9.34 \pm 1.17^\wedge}$	$\frac{8.26 \pm 1.89}{7.85 \pm 1.57}$	1518	0.2
Thinking	$\frac{9.09 \pm 1.58}{9.36 \pm 0.38}$	$\frac{9.08 \pm 1.31}{8.26 \pm 1.38}$	1684	0.9
Work-coping	$\frac{8.03 \pm 1.18}{9.41 \pm 1.17^*}$	$\frac{8.72 \pm 1.8}{8.2 \pm 1.63}$	1788	0.9
Decision-making	$\frac{7.84 \pm 2.3}{9.02 \pm 1.36^*}$	$\frac{8.4 \pm 2.19}{7.97 \pm 1.71}$	1551	0.03

The numerator represents the results before treatment and the denominator - after treatment. P-value - between Groups 1 and 2 before treatment. * - $P < 0.001$, ° - $P < 0.02$, ^ - $P < 0.05$ before treatment and after 12-month therapy within the group.

Discussion

To date, numerous studies have shown that parameters of the circadian rhythm of BP are more closely correlated with target organ damage than with BP measured by the traditional method, and, in addition, the assessment of DBPP provides additional information on indicators such as the increased variability and impaired BP circadian rhythm, and the increased rate of morning rise in BP.⁽¹⁶⁻¹⁸⁾ In particular, “non-dipper” AH patients with insufficient nighttime BP reduction and increased BP variability in the morning and early morning hours are at high risk for developing cerebrovascular and cardiovascular complications, as well as LVH.⁽¹⁹⁾ It is important to note that BP variability is an independent predictor of the development of dementia and its subtypes. In particular, in a study by Jung Eun Yoo et al.⁽²⁰⁾ a relationship was noted between higher BP variability and the incidence of dementia. Our study showed a negative correlation between the total MoCA score and the average 24-h SBP, Daytime SBP variability, and Daytime DBP variability, consistent with the literature data.

PPc and PWV are known to be independent predictors of cardiovascular disease, including stroke,⁽²¹⁻²⁴⁾ and are considered markers of preclinical cardiovascular disease.⁽²⁵⁾ Both high and low PP predict the onset of Alzheimer’s disease.⁽²⁶⁾ In addition, higher PP has been associated with lower levels of cognition among people without dementia.⁽²⁷⁾ In cross-sectional studies, PWV has been found to be higher in patients with vascular dementia, Alzheimer’s disease, or mild cognitive impairment than in people with normal cognitive function.⁽²⁸⁾ Higher PWV has also been associated with lower levels of cognition when screened with the Mini-Mental State Examination (MMSE) test.^(29,30) The data obtained in the presented study are consonant with the literature data. In particular, a negative correlation was found between the PWV and CPP index and the total Mini-Cog score.

It is well known that AHM should not only have a prolonged antihypertensive effect during the day, helping to improve DBPP with regression of target organ damage, but also have a positive effect on impaired CF. Modern classes of AHM have approximately the same antihypertensive efficacy; however, not all classes of AHM can improve CF in AH patients. It should be noted that CCBs have a fairly convincing evidence base in improving the prognosis of AH patients (ASCOT, TOMHS, PREVENT, ALLHAT). A unique feature of CCBs is their ability to penetrate the blood-brain barrier and reduce the metabolism of monoamine mediators, which become deficient in degenerative dementia. This property underlies their preventive action against cognitive impairment in AH patients. One of the new representatives of the CCB class is NIT, which belongs to the group of dihydropyridine derivatives and has a convincing evidence base.⁽¹³⁻¹⁵⁾ The randomized, double-blind, placebo-controlled, multicenter the Systolic Hypertension in Europe (Syst-Eur) trial⁽¹⁵⁾ examined the effect of NIT on preventing cardiovascular complications in patients over 60 years with isolated systolic hypertension, as well as on quality of life and the incidence of post-stroke dementia. There was a 27% reduction in cardiovascular mortality, a 56% reduction in myocardial infarction, a 42%

reduction in strokes, and a 31% reduction in the combined rate of all fatal and non-fatal cardiovascular endpoints. At the same time, NIT had a pronounced cerebroprotective effect, reducing the risk of developing dementia by 55%. In the Syst-China study, an NIT-based regimen significantly reduced the risk of stroke by 38%, all-cause mortality by 39%, cardiovascular mortality by 39%, stroke mortality by 58%, and all cardiovascular events by 37%.⁽³¹⁾

Our clinical experience with using NIT in combination with other AHM has shown its high efficiency, comparable with the therapy regimen based on AML, in organ protection in AH patients with a high risk of CVD. However, in the NIT-based group, compared with the AML-based group, a significant improvement in CF was demonstrated by the end of 12 months of therapy. It should be noted that the NIT-based treatment contributed to a significant increase in the total Mini-Cog score, in contrast to the decrease in this indicator under AML-based therapy. We also noted an increase in the total MoCA score during the NIT-based therapy, in contrast to AML-based therapy, which did not significantly affect this score.

It should be noted that only in Group 1 was there a significant improvement in CF, such as abstraction, delayed recall, memory, and attention, as well as a significant improvement in work-coping and decision-making. Testing on the HADS scale showed that the severity of anxiety and depression significantly decreased in Group 1; on the contrary, an increase in depression was noted in Group 2. The presented data allow a differentiated approach to tactics for treating AH patients with severe cognitive impairment.

Conclusions:

A weak but statistically significant negative correlation was found between the total MoCA score and the daytime SBP/DBP variability in AH patients.

A weak but statistically significant negative correlation was found between the total Mini-Cog score and PWV and PPc.

A pronounced antihypertensive efficacy of 12-month combination therapy was noted, with the inclusion of both NIT and AML.

The NIT-based treatment contributed to a significant increase in the total Mini-Cog score and the total MoCA score and a substantial improvement in CF. Abstraction, delayed recall, memory, attention, work-coping, and decision-making significantly improved, compared to AML-based treatment.

Competing Interests

The authors declare that they have no competing interests.

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Comparative Effectiveness of Aksaritmin and Propafenone in the Prevention of Atrial Fibrillation

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Abstract

Background: The purpose of this study was to evaluate the efficacy of Aksaritmin (Aks) in comparison with Propafenone (Pr) for the prevention of atrial fibrillation (AF) in patients with none or minimal signs of structural heart disease.

Methods and Results: The study included 60 patients aged 18-70 years (mean age of 58.1 ± 7.7 years) with paroxysmal (frequency of more than 2 paroxysms/3 months episodes of AF) or persistent AF with no or minimal signs of structural heart disease. The patients were divided into two groups: Group 1 included 30 patients taking Aks, and Group 2 included 30 patients taking Pr. According to the study protocol, the starting dose of Aks was 75 mg/day, with a possible dose increasing to 112.5 mg/day. The starting dose of Pr was 450 mg/day, with a possible dose increase to 600 mg/day.

In Group 1, preventive efficacy of Aks was observed in 29(96.7%), 26(86.7%), and 24(80%) patients by 1-, 3- and 6-month follow-up, respectively. Of these, 26(86.7%), 22(73.9%), and 16(53.3%) patients showed absolute preventive efficacy of the drug. In Group 2, preventive efficacy of Pr was observed in 28(93.3%), 26(86.7%), and 23(76.7%) patients at 1-, 3- and 6-month follow-up, respectively. Of these, 25(83.3%), 21(70%), and 14(46.7%) patients showed absolute AAE of the Pr.

The initial recurrence rate of AF was 4.5 ± 1.4 and 4.2 ± 1.3 in Groups 1 and 2, respectively ($P=0.3933$ between groups); but after 3 and 6 months of therapy, the recurrence rate decreased to 0.7 ± 1.1 ($P<0.0001$) and 0.8 ± 1.3 ($P<0.0001$), and 0.8 ± 1.0 ($P<0.0001$) and 1.1 ± 1.0 ($P<0.0001$), respectively, which was statistically significant in both groups.

Conclusion: The preventive efficacy of Aks (including in combination with BB) at a dose of 75-112.5 mg/day in recurrent forms of AF is comparable to the "reference" drug Pr at a dose of 450-600 mg/day. (International Journal of Biomedicine. 2023;13(2):224-228.)

Keywords: atrial fibrillation • antiarrhythmic efficacy • propafenone • aksaritmin

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Abbreviations

AAE, antiarrhythmic efficacy; AF, atrial fibrillation; AADs, antiarrhythmic drugs; AAE, antiarrhythmic efficacy; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BB, beta-blockers; CCB, calcium channel blockers; LA, left atrial; LVEF, left ventricular ejection fraction.

Introduction

Atrial fibrillation (AF) is one of the most common forms of heart rhythm disturbances with substantial medical and social significance. The current prevalence of AF in adults is between 2% and 4%. It is expected to increase 2.3-fold due

to increased life expectancy in the general population and an increased search for undiagnosed AF.

Propafenone (Pr) is a Class Ic antiarrhythmic agent, which is highly efficient in restoring sinus rhythm in patients with paroxysms of AF and is a reasonably fast action. A series of controlled trials in patients with recent-onset AF without heart failure who were hospitalized with enforced bed rest has shown that orally taken propafenone (450 to 600 mg as a single dose) exerts a relatively quick effect (within 3 to 4 hours) and a high rate of efficacy (72% to 78% within 8 hours). At the

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same time, Pr is a highly effective “rhythm control” drug in AF. In a recent update of the 2006, 2012, and 2015 reviews (the mean follow-up period was 10.2 months), moderate- to high-certainty evidence showed that propafenone reduced the recurrence of AF (RR=0.67, 95% CI: 0.61 to 0.74).

In Uzbekistan, besides Pr, AF is treated with such antiarrhythmic drugs (AADs) as ethacizine hydrochloride (ethacyzine), lappaconitine hydrobromide (allapinin), and amiodarone. Flecainide is not registered. Among these drugs, allapinin is distinguished by its plant origin. After *per os* administration of allapinin in patients with AF, the frequency of atrial impulses naturally decreases up to transformation into atrial flutter (in 14% of cases), after which, in 71% of cases, sinus rhythm is restored.

Allapinin was included in National and Eurasian guidelines for preventing AF and restoring sinus rhythm. However, in some cases (18%–65%), there are side effects on the central nervous system (dizziness, headache, diplopia), which have limited the scope of its administration and have resulted in patients refusing to take the drug in up to 10% of cases. In this regard, because of scientific and practical interest, a new AAD – aksaritmin (Aks) – was developed at the Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. It contains nine alkaloids, similar in chemical structure to allapinin. Aks is obtained from the roots and rhizomes of the *Aconitum septentrionale*. The technology for its production is much simpler than that for allapinin, and the economic cost of obtaining raw materials is two times lower.

It is recognized that the composition of allapinin mainly contains lappaconitine (up to 80%). The content of N-Deacetylappaconitine monochlorhydrate—the main metabolite of lappaconitine hydrobromide, which is not inferior to lappaconitine hydrobromide in activity, but is less toxic—in the composition of allapinin does not exceed 5%. In contrast to allapinin, in the composition of Aks the proportion of lappaconitine hydrobromide is less, about 40%-60%, while N-Deacetylappaconitine monochlorhydrate is greater, about 10%-20%. According to the developers, due to this ratio of components, in various experimental models of cardiac arrhythmias, Aks showed less toxicity and greater therapeutic latitude.

The purpose of this study was to evaluate the efficacy of Aks in comparison with Pr for the prevention of AF in patients with none or minimal signs of structural heart disease.

Materials and Methods

The study included 60 patients aged 18-70 years (mean age of 58.1±7.7 years) with paroxysmal (frequency of more than 2 paroxysms/3 months episodes of AF) or persistent AF with no or minimal signs of structural heart disease. Exclusion criteria were valvular heart disease, acute coronary syndrome, chronic heart failure (NYHA FC I-IV), sinus node dysfunction, second-third-degree AV block, LV wall hypertrophy over 14 mm, taking other AADs within the last 5 days (2-4 weeks if taking amiodarone), pregnancy and lactation, thyroid diseases, and other conditions with the need to correct the hormonal status.

The patients were divided into two groups: Group 1 included 30 patients taking Aks, and Group 2 included 30 patients taking Pr. Patients in both groups were comparable in baseline characteristics (Table 1). According to the study protocol, the starting dose of Aks was 75 mg/day (one pill every 8 hours), with a possible dose increasing to 112.5 mg/day. The starting dose of Pr was 450 mg/day, with a possible dose increase to 600 mg/day. Using 24-hour Holter ECG monitoring (HMECG), the AAE of drugs was assessed on Days 4-5 (for safety assessment) of the start of therapy as well as at the stages of 1, 3, and 6 months of follow-up against the background of standard therapy for the underlying disease. According to the study protocol the AAE of the drugs was recorded with a decrease in the number of paroxysms by 70% or more (moderate-high positive AAE) from the initial level and with the elimination of episodes of AF by 100% (absolutely positive AAE).

Table 1.

Baseline characteristics of study patients.

Variable	Group 1 (n=30)	Group 2 (n=30)
Age, years	57.±7.9	58.7±8.5
Male, n (%)	17 (56.7)	19 (63.3)
Paroxysmal AF, n (%)	26 (86.7)	25 (83.3)
History of AF, months	16.3±3.4	15.2±3.8
CHA2DS2-VASc, score	1.8	1.9
LA, mm	36.5±4.8	35.8±4.5
LVEF, %	62.8±3.5	63.1±4.1
Mitral regurgitation		
Grade I, n (%)	14 (46.7)	13 (43.3)
Grade II, n (%)	0	0
Grade III-IV, n (%)	0	0
Medication		
Anticoagulants, n (%)	25 (83.3)	24 (80)
ACEI/ARB, n (%)	24 (80)	25 (83.3)
Beta-blockers, n (%)	19 (63.3)	12 (40)
CCB, n (%)	2 (6.7)	3 (10)

All patients at the beginning of the study underwent electrocardiography (ECG), including an acute drug test, echocardiography (Echo), HMECG, ultrasound of the liver, kidneys, and thyroid gland, and a biochemical blood test to exclude concomitant conditions potentially generating AF. Given that Aks belongs conditionally to the Class Ic of AADs, special attention was paid to Echo parameters. In addition to Aks and Pr, patients were prescribed standard therapy in accordance with the underlying disease.

Statistical analysis was performed using the statistical software «Statistica» (v13.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation (SD) for continuous variables. Inter-group comparisons were performed using Student's t-test. Group comparisons with respect to categorical variables were performed using the chi-square test. A probability value of P<0.05 was considered statistically significant.

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided written informed consent.

Results

The main disease was essential hypertension, observed in 24(80%) patients in Group 1 and 22(73.3%) patients in Group 2. Six (20%) patients in Group 1 and 8 (26.7%) patients in Group 2 suffered from chronic persistent myocarditis.

In Group 1, preventive efficacy of Aks was observed in 29(96.7%), 26(86.7%), and 24(80%) patients by 1-, 3- and 6-month follow-up, respectively. Of these, 26(86.7%), 22(73.9%), and 16(53.3%) patients showed absolute preventive efficacy of the drug (Figure 1).

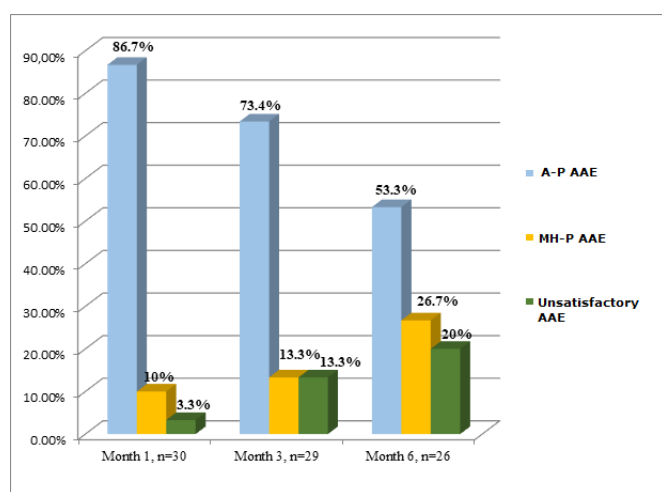


Fig. 1. Preventive efficacy of aksaritmin in AF.

In Group 2, preventive efficacy of Pr was observed in 28(93.3%), 26(86.7%), and 23(76.7%) patients at 1-, 3- and 6-month follow-up, respectively. Of these, 25(83.3%), 21(70%), and 14(46.7%) patients showed absolute AAE of the Pr (Figure 2).

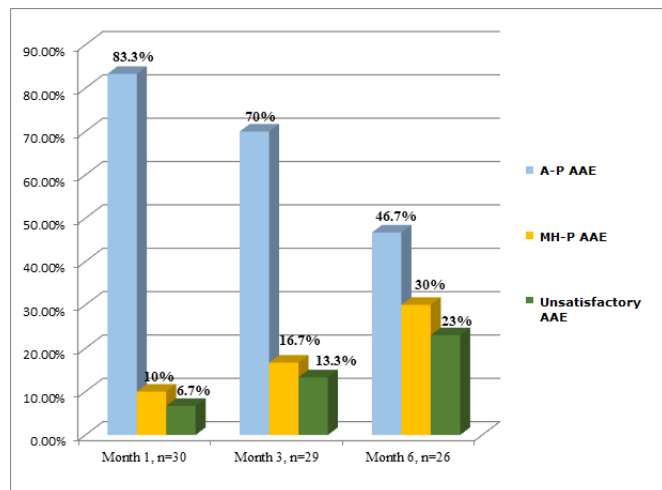


Fig. 2. Preventive efficacy of propafenone in AF.

In patients with an unsatisfactory AAE of the drugs, the dose of Aks was increased to 112.5 mg/day and Pr to 600 mg/day.

The initial recurrence rate of AF was 4.5 ± 1.4 and 4.2 ± 1.3 in Groups 1 and 2, respectively ($P=0.3933$ between groups); but after 3 and 6 months of therapy, the recurrence rate decreased to 0.7 ± 1.1 ($P<0.0001$) and 0.8 ± 1.3 ($P<0.0001$), and 0.8 ± 1.0 ($P<0.0001$) and 1.1 ± 1.0 ($P<0.0001$), respectively, which was statistically significant in both groups (Figure 3).

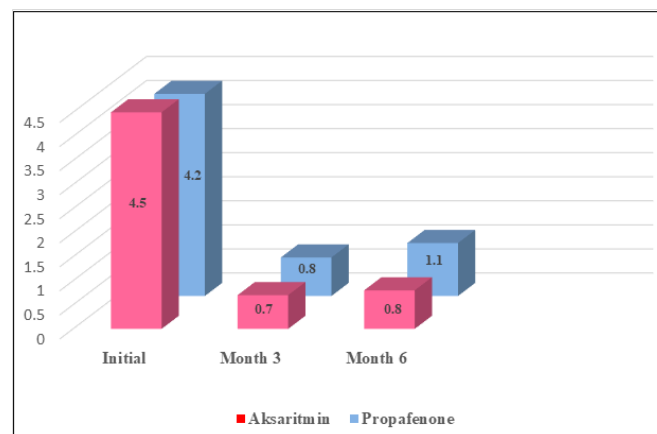


Fig. 3. Dynamics of the frequency of AF recurrence during treatment.

Aks and Pr were used either as a single AAD to prevent AF recurrence or in combination with beta-blockers (BB). The combination with BB in the Aks group was observed in 63.3% of cases, and in the Pr group in 40% of cases. A greater percentage of the combination of Aks with BB, compared to Pr, is explained by the fact that with Aks, the heart rate tends to increase, and with Pr it slows down (due to the presence of beta-blocking properties in Pr). Given this circumstance, in our opinion, it was advisable to evaluate the AAE of Aks without and in combination with BB. Aks in combination with BB had AAE in 100% and without BB in 90.9% of cases after one month of therapy ($\chi^2=1.729$; $P=0.1885$). After 3 and 6 months of therapy, AAE of Aks with BB and without BB was found in 89.5% and 81.8% ($\chi^2=0.346$; $P=0.5565$), and 84.2% and 72.7%, respectively ($\chi^2=0.556$; $P=0.4558$) (Figure 4).

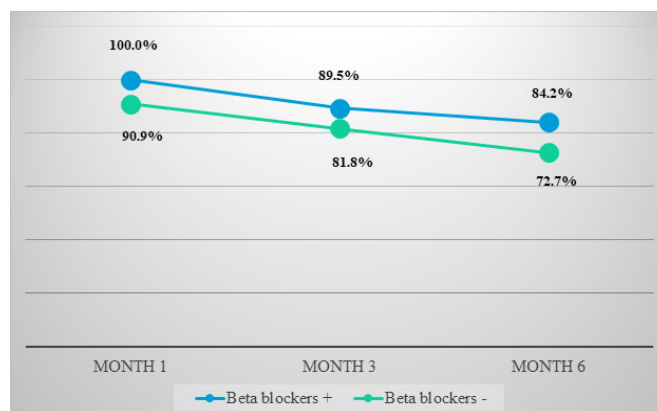


Fig. 4. AAE of Aks in combination with beta-blockers.

Discussion

In 2020, within the framework of the European Congress of Cardiology (ESC), a new edition of the recommendations for the management of patients with AF was presented. Since 2000, the ESC has revised recommendations for the management of patients with AF six times. This circumstance is due to the social-economic significance of this pathology, on the one hand, and on the other hand, the accumulation of an extensive scientific and practical database based on the principles of evidence-based medicine, which requires systematization and analysis.

Despite the existing limitations in the recommendations for the use of Class Ic AADs in patients with severe organic myocardial changes, the drugs of this group are used with great efficiency in a large group of patients with no or minimal manifestations of organic myocardial disease.

It should be noted that, according to several experts, allapinin is conditionally assigned to the Class Ic of AADs. The conditionality of this classification is because the drug, in addition to a pronounced suppression of Na⁺ channels, also exhibits electrophysiological properties characteristic of AADs Classes III and IV. This feature of plant-derived AADs—the simultaneous detection (manifestation) of electrophysiological properties characteristic of different classes—has been described in recent publications. In this connection, it is logical to assume that Aks, as well as allapinin, being an herbal drug, may be devoid of side effects characteristic of the classic representatives of Class Ic, which are of inorganic origin, due to which Class Ic is generally contraindicated in organic heart diseases.

Herbal medicines, including AADs, have several advantages:

1. Most patients are ready to take natural medicines for a long time without fear. Standardized science-based natural substances cause fewer adverse effects and allergic reactions.

2. Allapin and Aks do not have a negative effect on the generation of an impulse in the sinus node and myocardial contractility, which is typical for artificial AADs. Allapinin and its derivatives are the drugs of choice in a number of clinical situations, in particular, arterial hypotension and bradycardia.

4. According to some scientists, the effect of a decrease in antiarrhythmic efficacy in long-term use of classical AADs is not very specific for AADs prepared from plant substances. At the same time, obtaining good results is possible only with standardization and strict quality control of phytodrugs.

Conclusion

The preventive efficacy of Aks (including in combination with BB) at a dose of 75-112.5mg/day in recurrent forms of AF is comparable to the “reference” drug Pr at a dose of 450-600mg/day. A decrease in the preventive efficacy of Aks is noted (86.7% and 80% after 1 month and 6 months of treatment, respectively), to a greater extent due to a decrease in the proportion of patients with absolute preventive efficacy.

Competing Interests

The authors declare that they have no competing interests.

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Assessment of Red Cell Distribution Width among Sudanese Patients with Subclinical Hypothyroidism

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Abstract

Background: Thyroid dysfunction has a strong association with anemia. Red blood cell distribution width (RDW) was traditionally regarded as a part of the routine evaluation of anemia. Several studies have indicated that elevated RDW level is significantly associated with subclinical hypothyroidism and hypothyroidism. The present study aimed to assess the impact of subclinical hypothyroidism (SHT) on RDW in Sudanese patients.

Methods and Results: The study was designed as a case-control, laboratory-based study carried out at the National Cancer Institute – University of Gezira (NCI-UG) (Wad Medani, Gezira State, Sudan) from January to October 2020. The samples were collected randomly from 100 subjects: 50 patients (mean age 38.50 ± 10.46 years; 36% males and 64% females) with SHT (case group) and 50 apparently healthy individuals (mean age 35.52 ± 11.64 years; 46% males and 54% females) (control group). The case group was divided into 2 subgroups: Sub1 included 43(86%) patients with SHT grade 1 (TSH of 6-10 μ IU/mL), and Sub2 included 7(14%) patients with SHT grade 2 (TSH > 10 μ IU/mL). A 3ml venous blood sample was collected in an EDTA container from each participant. The parameters of the RBCs (RBC count, mean corpuscular volume [MCV], RDW-CV, and RDW-SD) were measured using the Sysmex XP-300 Automated Hematology Analyzer.

In the case group, the average levels of RDW-SD, RDW-CV, and MCV were higher than in the control group ($P=0.000$ in all cases). There was a significant difference in RDW-CV between Sub1 and Sub2 ($P=0.040$). We found no significant differences in RDW-SD and RDW-CV between different age groups. There was a significant difference in RBC count between different age groups ($P=0.022$), and significant differences in RBC count and MCV between males and females. RDW-SD and RDW-CV had a significant positive correlation within TSH and a significant negative correlation within T3 and T4.

Conclusion: RDW-CV may be used as a marker of subclinical hypothyroidism grade 2. (International Journal of Biomedicine. 2023;13(2):229-233.)

Keywords: red blood cell distribution width • hypothyroidism • Sudan

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Abbreviations

RBC, red blood cell; **RDW**, RBC distribution width; **MCV**, mean corpuscular volume; **RDW-CV**, RDW coefficient of variation; **RDW-SD**, RDW, standard deviation; **SHT**, subclinical hypothyroidism; **TSH**, thyroid-stimulating hormone; **T3**, triiodothyronine; **T4**, thyroxine.

Introduction

Thyroid hormones play a vital role in normal development, differentiation, metabolic balance, physiological functioning of tissues in the human body, and regulating the production of RBCs.⁽¹⁾ Many disorders can arise from the thyroid producing excessive amounts of hormone (hyperthyroidism) or insufficient amounts (hypothyroidism). Hypothyroidism is caused by insufficient secretion of thyroid hormones by the thyroid gland or by the complete loss of its function due to congenital thyroid abnormalities (thyroid deficiency at birth) or iodine deficiency. The most frequent cause of hypothyroidism and endemic goiter worldwide is iodine deficiency. In areas with sufficient dietary iodine, hypothyroidism is most often caused by Hashimoto's thyroiditis (chronic autoimmune thyroiditis).⁽²⁾ This condition is characterized by infiltration of the thyroid gland with T-lymphocytes and autoantibodies against specific thyroid antigens, such as thyroid peroxidase, thyroglobulin, and the TSH receptor.⁽³⁾ Many previous studies have postulated a relationship between thyroid hormones and hematological abnormalities.⁽⁴⁾ Hypothyroidism is usually progressive and associated with anemia, hyperlipidemia, and a reversible increase in creatinine.⁽⁵⁾

More than one billion persons are at risk of iodine deficiency worldwide, and 200 million have a goiter. In Sudan, endemic iodine deficiency disorders are serious health problems in many areas. The prevalence among school children was estimated to be 85% in the Darfur region in western Sudan, 74% in the Kosti area in the center of Sudan, 13.5% in Port-Sudan in eastern Sudan, 17% in Khartoum, 22.3% in the southern Blue Nile area of Sudan.⁽⁶⁾

Thyroid hormones have a significant effect on erythropoiesis through hyper-proliferation of immature erythroid progenitors,^(7,8) increased secretion of erythropoietin by inducing erythropoietin gene expression,⁽⁹⁾ and stimulation of bone marrow erythropoiesis.^(10,11) Therefore, thyroid dysfunction has a strong association with anemia.^(7,12,13)

Red blood cell distribution width (RDW), a component of the standard complete blood count, is an indicator of the heterogeneity of erythrocyte size, and elevated RDW implicates homeostatic imbalance of erythrocytes. RDW was traditionally regarded as a part of the routine evaluation of anemia.⁽⁷⁾

RDW is an independent risk factor for many chronic inflammatory disorders characterized by inflammation, such as cardiovascular diseases,⁽¹⁴⁾ celiac disease,⁽¹⁵⁾ cancer,⁽¹⁶⁾ and chronic obstructive pulmonary disease.⁽¹⁷⁾ Several studies have indicated that elevated RDW level is significantly associated with subclinical hypothyroidism,⁽¹⁸⁾ overt hypothyroidism,⁽¹⁹⁾ and Hashimoto's thyroiditis.⁽²⁰⁾

The present study aimed to assess the impact of subclinical hypothyroidism (SHT) on RDW in Sudanese patients.

Materials and Methods

The study was designed as a case-control, laboratory-based study carried out at the National Cancer Institute – University of Gezira (NCI-UG) (Wad Medani, Gezira State, Sudan) from January to October 2020. The samples were collected randomly from 100 subjects: 50 patients (mean age 38.50 ± 10.46 years; 36% males and 64% females) with SHT (case group) and 50 apparently healthy individuals (mean age 35.52 ± 11.64 years; 46% males and 54% females) (control group). The case group was divided into 2 subgroups: Sub1 included 43(86%) patients with SHT grade 1 (TSH of 6-10 $\mu\text{IU/mL}$), and Sub2 included 7(14%) patients with SHT grade 2 (TSH >10 $\mu\text{IU/mL}$).

A 3 ml venous blood sample was collected in an EDTA container from each participant. The parameters of the RBCs (RBC count, MCV, RDW-CV, and RDW-SD) were measured using the Sysmex XP-300 Automated Hematology Analyzer.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp) and Statistica version 10.0 (StatSoft Inc., USA). Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare differences between 2 and 3 or more independent groups. The frequencies of categorical variables were compared using Pearson's chi-squared test or Fisher's exact test (2-tail), when appropriate. A probability value of $P < 0.05$ was considered statistically significant.

Results

Among 50 patients in the case group, 15(30%) had a family history of hypothyroidism (Table 1). In the case group, the average levels of RDW-SD, RDW-CV, and MCV were higher than in the control group ($P = 0.000$ in all cases) (Table 2).

Table 1.

Demographic characteristics of study participants.

Factors	Cases (n=50)	Control (n=50)
Age (years)	38.50 ± 10.46	35.52 ± 11.64
Age group (years)		
Under the age of 30 years	18 (36%)	20 (40%)
30–50 years	25 (50%)	25 (50%)
Over the age of 50 years	7 (14%)	5 (10%)
Gender		
Male	18 (36%)	23 (46%)
Female	32 (64%)	27 (56%)
Family history		
Yes	15 (30%)	-
No	35 (70%)	-
Severity		
SHT grade 1	43 (86%)	-
SHT grade 2	7 (14%)	-

Table 2.

Comparison of RBCs parameters (RDW-SD, RDW-CV, RBCs count, and MCV) between study groups.

Parameters	Case group (mean \pm SD)	Control group (mean \pm SD)	P-value
RDW-SD, fl	47.65 \pm 4.29	42.10 \pm 4.45	0.000
RDW-CV, %	16.19 \pm 2.41	14.13 \pm 1.29	0.000
RBC count	4.12 \pm 0.67	4.27 \pm 0.64	0.255
MCV, fl	79.13 \pm 2.95	83.60 \pm 4.59	0.000

There was a significant difference in RDW-CV between Sub1 and Sub2 ($P=0.040$) (Table 3). We found no significant differences in RDW-SD and RDW-CV between different age groups. There was a significant difference in RBC count between different age groups ($P=0.022$) (Table 4), and significant differences in RBC count and MCV between males and females (Table 5).

Table 3.

Comparison of RBCs parameters (RDW-SD, RDW-CV, RBCs count, and MCV) between SHT subgroups

Parameters	Sub1 (n=43) (mean \pm SD)	Sub2 (n=7) (mean \pm SD)	P-value
RDW-SD, fl	47.24 \pm 4.14	50.19 \pm 4.65	0.093
RDW-CV, %	15.91 \pm 2.23	17.91 \pm 2.92	0.040
RBC count	4.15 \pm 0.66	3.93 \pm 0.79	0.420
MCV, fl	79.34 \pm 2.88	77.86 \pm 3.35	0.223

Table 4.

Comparison of RBCs parameters (RDW-SD, RDW-CV, RBC count, and MCV) between age groups.

Parameters	> 30 years (n=12)	30 – 50 years (n=25)	< 50 years (n=7)	P-value
RDW-SD, fl	47.53 \pm 5.46	47.82 \pm 4.13	46.87 \pm 3.97	0.904
RDW-CV, %	15.69 \pm 2.21	16.42 \pm 2.82	16.04 \pm 1.92	0.498
RBC count	4.20 \pm 0.65	4.18 \pm 0.74	3.69 \pm 0.33	0.022
MCV, fl	79.44 \pm 3.47	79.03 \pm 2.97	79.23 \pm 2.36	0.418

Table 5.

Comparison of RBCs parameters (RDW-SD, RDW-CV, RBC count, and MCV) between gender.

Parameters	Males (n=9) (mean \pm SD)	Females (n=41) (mean \pm SD)	P-value
RDW-SD, fl	48.51 \pm 2.84	47.46 \pm 4.56	0.387
RDW-CV, %	16.48 \pm 1.67	16.13 \pm 2.56	0.628
RBC count	4.92 \pm 0.85	3.94 \pm 0.48	0.000
MCV, fl	82.09 \pm 2.74	78.48 \pm 2.61	0.001

RDW-SD and RDW-CV had a significant positive correlation within TSH and a significant negative correlation within T3 and T4 (Table 6).

Table 6.

Correlation between RBCs parameters (RDW-SD, RDW-CV, RBC count, and MCV) and thyroid hormones.

Parameters		TSH	T3	T4
RDW-SD, fl	r	0.361	- 0.419	- 0.507
	P-value	0.000	0.000	0.000
RDW-CV, %	r	0.407	- 0.406	- 0.506
	P-value	0.000	0.000	0.000
RBC count	r	- 0.211	0.142	0.153
	P-value	0.035	0.157	0.128
MCV, fl	r	- 0.359	0.419	0.507
	P-value	0.087	0.891	0.000

Discussion

There are 200 million people worldwide who suffer from thyroid diseases. Erythrocyte abnormalities are usually linked to thyroid disorders. Although it has been reported that thyroid dysfunction might be associated with some forms of anemia, especially in childhood, the prevalence of this association in adults varies widely.⁽²¹⁾

Our data agree with different studies showing that hypothyroidism is more common in females than males.⁽²²⁻²⁷⁾ Elevated RDW-SD and RDW-CV in hypothyroid patients found in our study align with several previous studies.^(1,9,20,22,24,25,26-29) The cause of the increase in the RBC size and the minor degree of anisocytosis and poikilocytosis in hypothyroidism is unknown. However, it is possible that a corresponding change in the amount or distribution of lipids in the erythrocyte membrane may be responsible for the shift in erythrocyte volume and anisopoikilocytosis. In contrast to Saba et al.,⁽³⁰⁾ our study found that subclinical hypothyroidism severity was associated with RDW-CV but not RDW-SD. Also, our result showed no significant difference in the RDW-SD and RDW-CV according to age and gender. In our study, RDW-SD and RDW-CV in hypothyroid patients positively correlated with TSH and had inverse correlations with T3 and T4. Many studies also found RDW significantly correlated with TSH^(7,18,27) and T3.⁽⁷⁾ The absence of significant correlations between RBC count and MCV with TSH in our study agrees with some other studies⁽²²⁾ and contrasts with studies performed in India⁽³¹⁾ and Korea.⁽¹⁸⁾

A significant decrease in MCV in hypothyroid patients suggests the risk of microcytic anemia, which agrees with several studies.^(21,30) According to this study, there was no significant difference in MCV according to age, family history, and the severity of SHT; but MCV, like RBC count, was

significantly different according to gender due to differences in normal physiology between males and females.

Conclusion

RDW is significantly higher in hypothyroid patients and has a significant positive correlation with TSH and a significant negative correlation with T3 and T4. RDW-CV may be used as a marker of subclinical hypothyroidism grade 2.

Competing Interests

The authors declare that they have no competing interests.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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Detecting the Prevalence of Hepatitis C Virus among Iraqi People

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Abstract

Background: Infection with the hepatitis C virus (HCV) is a major public health issue worldwide and remains a vital etiology of long-term hepatitis. This study aimed to detect the prevalence of HCV infection among Iraqi people. This research was proposed to detect the frequency of HCV infection in renal failure patients, thalassemia patients, blood donors, and Iraqi medical staff. Such prevalence potentially could assist in the development of a preventive program for this infection and orient future studies.

Methods and Results: Cross-sectional research was conducted in Thi-Qar Province (Iraq). The enrolled 1650 individuals (1180 males and 470 females, age range of 1-85 years) were classified into four study groups. Group 1 included 120 patients with renal failure, Group 2 included 220 patients suffering from thalassemia, Group 3 included 1259 blood donor subjects, and Group 4 included 51 subjects from the medical staff. Serum anti-HCV-IgG-Abs were detected qualitatively by a human HCV-IgG-ELISA Kit (MyBioSource, USA). The findings revealed that out of 1650 subjects, only 53(3.2%) were infected with HCV. The highest prevalence was reported among thalassemia patients 34/220(15.45%), followed by renal failure patients 8/120(6.66%) and then the medical staff group 3/51(5.88%), whereas the lowest prevalence was reported among the blood donor group 8/1259(0.64%). The total infection rate of HCV was higher among males [33/53(62.3%)] than females [20/53(37.7%)], with significant differences ($P<0.05$). We found a significant difference in HCV infection rate according to the age range of the study subjects ($P<0.05$). The higher infection percentages of 29/53(54.7%) and 15/53(28.3%) were found in age groups of 1-20 years and 21-40 years, respectively, followed by the age group of 41-60 years, which had infection percentages of 7/53(13.2%), while the lowest infection rate was reported in the age group of more than 60 years, which was 2/53(3.8%).

Conclusion: The frequency rate of HCV infection among Iraqi people is similar to those in most Asian and non-Asian studied populations, and the infection rate was higher in males and inversely correlated with the age of the subjects. The main routes of HCV infection were blood transfusions, renal dialysis, and HCWs. Thalassemic and hemodialysis patients were potentially vulnerable to HCV infection. Effective screening methods and blood donor screening protocols are likely required to prevent the spread of HCV infection.(*International Journal of Biomedicine*. 2023;13(2):234-240.)

Keywords: hepatitis C virus • blood transfusions • thalassemia • renal dialysis

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Abbreviations

Abs, antibodies; **Ags**, antigens; **HCC**, hepatocellular cancer; **HCV**, hepatitis C virus; **HCWs**, health care workers; **HRP**, horseradish peroxidase.

Introduction

The hepatitis C virus (HCV) is a diminutive enveloped RNA virus from the *Hepacivirus* genus and the *Flaviviridae* family. HCV has positively polarized, single-stranded genomic RNA; it forms the virion after being assembled by the core protein and enclosed by lipid bilayers

that have the E1 and E2 viral glycoproteins. All have recently been defined as HCV genotypes that are hepatotropic and lethal, despite the differences in nucleotide sequences between them.⁽¹⁾ HCV infection causes an asymptomatic acute stage, but there is a high chance of having chronic HCV infection in about 75% of people who are acutely infected, in which 27% of patients develop liver cirrhosis, and 25% have HCC during

the first two decades following infection. One percent of the world's population is thought to have chronic HCV infection. In contrast, it has been estimated that about 14 million individuals in the European Union/European Economic Area have had chronic HCV infection, indicating a considerably high incidence of 1.5% in this area.⁽²⁾ Previous research from the region's nations revealed that the prevalence of HCV was 1.1% in Yemen, <1% in Iran, 1.8% in Saudi Arabia's youth, 4% in Pakistan's blood volunteers, and 0.2% among Iraqi people.⁽³⁾

To detect HCV infection, molecular and serological techniques are used. The serological tests are used to detect anti-HCV-Abs and HCV-Ags in the serum and/or plasma that are quickly obtained from venipuncture. Consequently, the serological assays are appropriate for mass screening of the general population for HCV. Additionally, the use of serological assays for monitoring treatment and confirming virologic clearance is extremely appropriate. Enzyme immunoassays are typically utilized in HCV screening settings to identify anti-HCV-Abs and HCV-Ags.⁽⁴⁾ For patient screening, a third-generation anti-HCV ELISA with great sensitivity is frequently used. Most third-generation ELISA tests currently available for the detection of HCV-Abs either depend solely on synthetic peptide Ags, recombinant protein Ags, or a combination of these types of HCV-Ags.⁽⁵⁾

The incidence of HCV infection is dramatically elevated in patients on maintenance hemodialysis, and this virus has been implicated in serious consequences, ranging from persistent hepatitis to severe cirrhosis and HCC.⁽⁶⁾ In regard to the usual course of HCV disease in renal failure subjects, controversial findings persist even in subjects with normal renal findings. Since HCV infection has a long duration, is generally asymptomatic, and has a disease onset that is difficult to define, defining the disease's natural history of infection remains challenging.⁽⁷⁾ In many nations, post-transfusion hepatitis and end-stage liver disease are the diseases most frequently caused by HCV. The overall survival of patients with hereditary hemolytic anemia, especially thalassemia, has been improved with regular blood transfusions, but there is a substantial threat that they will acquire a blood-borne viral infection, particularly the hepatitis virus.⁽⁸⁾ In general, body piercing, tattoos, intravenous drug use, iatrogenic exposures, vertical transmission, and highly risky sexual intercourse are all involved in the transmission of HCV, which occurs when an individual meets contaminated blood.⁽⁹⁾ Blood transfusions contribute to the increasing spreading pool of viral infections that can also be transmitted from asymptomatic infected people. Screening and evaluating the donor not only reduces the risk of transmission through contaminated blood products but also gives details on the rates of infection prevalence in the neighborhood. It is crucial to evaluate and track the prevalence and trend of HCV in blood donors to evaluate the quality and efficacy of donor screening, public awareness campaigns, blood screening assays, and the possible threat of HCV infection conveyed through transfusions.⁽¹⁰⁾ Blood safety has been enhanced by the regulation of screening assays and the invention and introduction of HCV nucleic acid technology. Transfusion-transmitted HCV infection still exists, despite highly sensitive diagnostic technology.⁽¹¹⁾ Infectious blood-

borne infections, such as HCV, pose a serious threat to HCWs, wherever they are working. The use of contaminated sharp tools, improper injection methods, mishandling of biological materials, and a lack of education are the main origins of the infection. The total number of people with HCV infection has a significant impact on the number of infected HCWs. This rate is frequently high among HCWs working in less developed nations.⁽¹²⁾ There is no vaccination to prevent HCV infection, and HCV therapy is expensive. Consequently, it is crucial to prevent primary HCV infection. Any HCV infection prevention program must be supported by precise data, including statistics on its prevalence. For the prevalence of HCV infections among Iraqis in some provinces, a small number of studies were conducted in past years. However, they are few and insufficient. This research was proposed to detect the frequency of HCV infection in renal failure patients, thalassemia patients, blood donors, and Iraqi medical staff. Such prevalence potentially could assist in the development of a preventive program for this infection and orient future studies.

Materials and Methods

Study design and subjects

Cross-sectional research was conducted in Thi-Qar Province (Iraq). The enrolled patients suffered from clinical manifestations of renal failure and/or thalassemia, whereas the other enrolled subjects were blood donors and medical staff individuals. The current study was carried out from August 2019 to October 2020.

A total of 1650 individuals (1180 males and 470 females, age range of 1-85 years) attending the Public Health Laboratory, Hereditary Blood Diseases Center, Al-Hussein Teaching Hospital's Renal Dialysis Unit, and Central Blood Bank were enrolled in this study, and they were classified into four study groups. Group 1 included 120 patients with renal failure, Group 2 included 220 patients suffering from thalassemia, Group 3 included 1259 blood donor subjects, and Group 4 included 51 subjects from the medical staff.

Eligibility criteria

Group 1: All patients with frequent renal dialysis who attended the Renal Dialysis Unit of Al-Hussein Teaching Hospital during the period of the current study were enrolled after giving their approval. Group 2 included patients with a completely confirmed diagnosis of thalassemia disease who attended the Hereditary Blood Diseases Center during the recent study time after giving their approval. Group 3 included the blood donor volunteers who attended the Central Blood Bank at the time of the current study after giving their approval to take a sample. Group 4 included the medical staff subjects from different medical centers in Thi-Qar Province who were approved to give a sample; the subjects had randomly selected specializations, like physician, dentist, laboratory assistant, radiologist, etc.

Samples collection

From each subject, 3-4 mL of peripheral blood was collected by vein puncture. Blood samples were allowed to complete the clotting process at ambient temperature before being centrifuged at 1500 rounds per minute for 10 minutes to

obtain the serum, which was then stored at -20°C till required for the serological test.

Serological test

The serological test was executed in the Public Health Laboratory at the Health Office of Thi-Qar. Anti-HCV-IgG-Abs were detected qualitatively by a human HCV-IgG-ELISA Kit (MyBioSource, USA). This kit was based on indirect ELISA techniques. Ninety-six well plates were pre-coated with recombinant HCV-Ags. Test samples were loaded into the wells, and a wash buffer was used to remove any unbound conjugates. If there were any HCV-IgG-Abs in the samples, they would then form an immunological complex when HRP-conjugated anti-human-IgG-Abs were added. Substrates containing tetra-methyl-benzidine were utilized to observe the HRP enzymatic process. Horseradish peroxidase accelerated the reaction to create a blue-colored product, which turned yellow once the acidic stop solution was added. A microtiter plate reader (Biokit, Germany) was used to determine the optical density of the formed color, and the results were regarded as negative and/or positive according to their absorbance values.

Statistical analysis was performed using the statistical software package SPSS version 24.0 (SPSS Inc, Armonk, NY: IBM Corp). Frequencies and percentages were used as descriptive statistics. The relationships between the variables were assessed using the chi-square test. The findings were regarded as statistically significant at a P -value lower than 0.05.

The study was approved by the Ethics Committee of the Thi-Qar Health Department, Ministry of Health, Iraq (Agreement No: 155). All participants provided written informed consent.

Results

A total of 1650 subjects (age range of 1-85 years, 1180 males and 470 females) were enrolled in the current study after strict application of the eligibility criteria mentioned in the materials and methods.

Figure 1 shows the results of anti-HCV-(IgG)-Abs in the total sum of the study groups. The findings revealed that out of 1650 subjects, only 53(3.2%) were infected with HCV, whereas 1597/1650(96.8%) were negative for HCV.

Figure 2 shows the prevalence of HCV according to study groups. The highest prevalence was reported among thalassemia patients 34/220(15.45%), followed by renal failure patients 8/120(6.66%) and then the medical staff group 3/51(5.88%), whereas the lowest prevalence was reported among the blood donor group 8/1259(0.64%). The total infection rate of HCV was higher among males [33/53(62.3%)] than females [20/53(37.7%)], with significant differences ($P<0.05$) (Table 1). We found a significant difference in HCV infection rate according to the age range of the study subjects ($P<0.05$) (Table 2). The higher infection percentages of 29/53(54.7%) and 15/53(28.3%) were found in age groups of 1-20 years and 21-40 years, respectively, followed by the age group of 41-60 years, which had infection percentages of 7/53(13.2%), while the lowest infection rate was reported in the age group of more than 60 years, which was 2/53(3.8%).

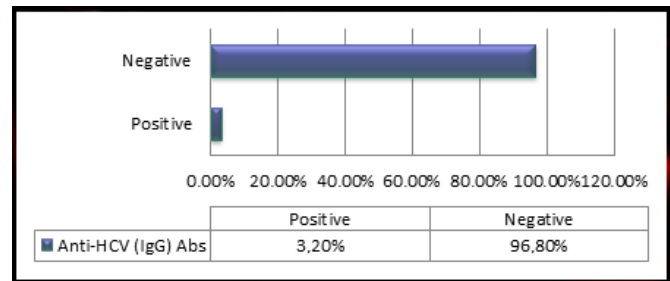


Fig. 1. The HCV prevalence in the study population

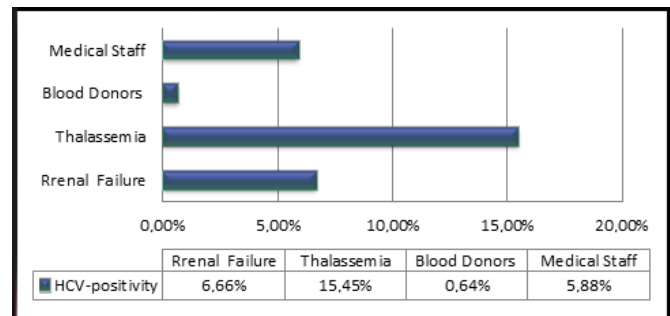


Fig. 2. HCV prevalence within each study group

Table 1.

HCV prevalence according to gender

Study group	Anti-HCV-IgG-Abs positivity		
	Males	Females	Statistics
	n (%)	n (%)	
Renal Failure (n=8)	5 (62.5)	3 (37.5)	$*P=0.9156$
Thalassemia (n=34)	20 (58.8)	14 (41.2)	
Blood Donors (n=8)	6 (75)	2 (25)	
Medical Staff (n=3)	2 (66.7)	1 (33.3)	
Total (n=53)	33 (62.3)	20 (37.7)	$P<0.05$

*Yates' P -value

Table 2.

HCV prevalence according to the age range of the study subjects

Age Subgroup (year)	Positive subjects for HCV				
	Renal Failure (n=8)	Thalassemia (n=34)	Blood Donors (n=8)	Medical Staff (n=3)	Total (n=53)
	n (%)	n (%)	n (%)	n (%)	n (%)
1-20	0 (0)	29 (85.3)	0 (0)	0 (0)	29 (54.7)
21-40	4 (50)	5 (14.7)	4 (50)	2 (66.7)	15 (28.3)
41-60	3 (37.5)	0 (0)	3 (37.5)	1 (33.3)	7 (13.2)
>60	1 (12.5)	0 (0)	1 (12.5)	0 (0)	2 (3.8)
Total	8 (100)	34 (100)	8 (100)	3 (100)	53 (100)
Statistics	$P<0.05$				

Discussion

HCV is one of the most common contagious diseases in humans, which is characterized by a wide number of clinical manifestations, including acute and/or fulminant hepatitis. HCV infection also may be clinical without symptoms or may be developed into chronic hepatitis and cirrhosis of the liver. Various behavioral, environmental, and host elements affect the prevalence of HCV infection, which fluctuates from one country to another.⁽¹³⁾ In contrast to developing nations like Iraq, where researchers are still working to control the infection, the incidence rate of HCV infection was decreased in developed countries.⁽¹⁴⁾ Due to Iraq's turbulent social and political circumstances throughout the past few decades, data on the frequency of HCV infection in the general population are not available. The only information on the prevalence of viral hepatitis in Iraq that has been published, as far as we are aware, was from studies on blood donors.

Research from nearby nations revealed a fluctuation in the incidence levels of HCV infection, which had a range of 0.4%-19.2%.⁽¹⁵⁾ In this study, the total prevalence of HCV infection was 3.2% (Figure 1). Consistent with our findings, another previous study at Duhok City found that the prevalence of HCV was 2.8%.⁽³⁾ On the other hand, the results of the current study were less than those in a previous study conducted in Iraq by Hamied et al.,⁽¹⁶⁾ which reported the HCV prevalence in Baghdad province (8.3%), and more than the findings obtained by Tarky et al.⁽¹³⁾ in all Iraqi governorates (0.4%) and Abdul-Kareem et al.⁽¹⁷⁾ in Al-Najaf province (0.34%). In comparison with findings of the previous research in other countries, the findings of the current study were in agreement with those in Turkey (2.4%),⁽¹⁸⁾ Thailand (2.8%), and Vietnam (2%-2.9%), less than those in Taiwan (4.4%), Pakistan (4.7%) and Egypt (14.9%), and more than those in Iran (0.5%), USA (0.01%), Australia (1.3%), China (1%-1.9%), Saudi Arabia (1%-1.9%), and Syria (1-1.9%).⁽¹⁹⁻²²⁾ The variations in the study population, sample-collecting methods, and diagnostic techniques may partially explain these variations in the prevalence rates. To investigate this, further population-based studies are required.

Patients with thalassemia and hemoglobinopathies need frequent blood transfusions, which are important for the improvement of their survival and reduce dangerous complications that are produced by severe anemia. On the other hand, these frequent blood transfusions will increase the probability of infection with different microbes, especially, human immunodeficiency virus, HCV, and hepatitis B virus.⁽²³⁾ Consistent with these findings, the prevalence ratio of HCV in the current study was 15.45% from 220 patients suffering from thalassemia. Previous studies in Duhok City (Iraq)⁽²⁴⁾ and Mosul City (Iraq)⁽²⁵⁾ reported that patients with thalassemia had HCV prevalence of 11.05% and 17%, respectively, which is in line with current data. The findings of the present study were lower than other previous studies in other Iraqi cities, including Diyala (26.4%)⁽²⁶⁾ and Karbala (37%),⁽²⁷⁾ and higher than Babylon city (7.5%).⁽²⁸⁾ This variation in the prevalence of HCV among thalassemia patients may belong to variations in hygienic surveillance, especially tests of blood. In addition, it reflects the variation in health awareness of the citizens in

these cities. HCV is characterized by low viral load, a long incubation period that may be extended to six months, and being asymptomatic in acute and chronic periods, all these reasons will delay the seroconversion and, finally, delay diagnosis of HCV in blood donors, leading to an increase the probability of HCV infection among patients with thalassemia and hemoglobinopathies through hemolysis, and this explains the elevated HCV infection rates among thalassemia patients in the current study. To limit HCV infection, many nations have integrated molecular biology technologies into standard protocol testing that can detect the depleted levels of virus nucleic acid.⁽²⁹⁾

HCV infection was a critical concern in hemodialysis centers. Both nosocomial infection and blood transfusion are considered major factors in the dissemination of HCV infection.⁽³⁰⁾ Hemodialysis patients in the province of Thi-Qar (Iraq) had an overall percentage of HCV infection of 6.66%, according to the findings of the present study. This finding was closer to that reported in Baghdad province (Iraq) (7.1%),⁽³¹⁾ Al-Anbar province (Iraq) (11.7%),⁽³²⁾ and Mexico (6.7%).⁽³³⁾ This prevalence was low when compared with reports from Sulaimani (Iraq) (26.7%)⁽³⁴⁾ and other developing countries (24% in Iran, 30% in India, and 26% in Oman),⁽³⁵⁾ but it is high when compared to reports from Western countries such as the United Kingdom (0.4%).⁽³⁶⁾

As previously illustrated above, HCV is a parenterally transmitted blood-borne virus. Before the onset of symptomatic liver diseases, infection typically results in a chronic, asymptomatic carrier status for many years. Healthcare workers infected with HCV may not be aware of their condition or the possibility of transmitting the disease to patients. The possible acquisition of blood-borne viral infection is increased by HCWs who perform exposure-prone operations, where an injury to the worker may expose the patient's open tissues to the HCW's blood and vice versa.⁽³⁷⁾ In the current study, we found that the prevalence of HCV infection among HCWs was 5.88%. It is closer to the results of ALHaj et al.,⁽³⁸⁾ and Ansari and Dixit⁽³⁹⁾ studies that were conducted in Yemen (4.17%) and India (4%) among HCWs, respectively. However, it was higher than the rate of HCV among HCWs in Dhaka-Bangladesh (1%), Poland (1.9%), and India (3%).⁽⁴⁰⁾ These differences were due to the HCWs' use of various preventive measures at different healthcare facilities. Overall, the current research may be useful for comprehending the prevalence of HCV among HCWs in the province of Thi-Qar (Iraq).

For the prevalence of HCV among blood donors, a recent study revealed that 0.64% of people had HCV-Abs. In agreement with these findings, previous studies in Baghdad⁽⁴¹⁾ and Babylon⁽⁴²⁾ governorates showed a closer prevalence rate of HCV infection (0.7% and 0.5%, respectively). As compared to other nations, the incidence of HCV among Kuwaiti nationals and non-Kuwaiti Arab first-time blood donors was reported to be 0.8% and 5.4%, respectively.⁽⁴³⁾ According to a hospital-based study conducted in Jordan, 0.9% of blood donors had HCV infection.⁽⁴⁴⁾ Overall, the variations in the prevalence of HCV infection may have been for different reasons, such as sample size, type of technique used (ELISA, Minividas,

Immunofluorescences, or Chemiluminescence), variations in kit types and their trademark, time of incubation during the test procedure and the differences in blood test procedures between countries, cities, and societies. In addition to that, the variations in customs and social customs in each society, such as tattoos, body piercing, and taking drugs by injection. The level of hygienic surveillance, as well as hygienic awareness of people, may interpret all these variations in results.

The results of the present study exhibited a significantly higher frequency percent of HCV positivity among males (62.3%) than females (37.7%). The current study findings were consistent with previous findings reported by other authors in Iraq⁽⁴⁵⁾ and Iran.⁽⁴⁶⁾ Data of blood donors from Pakistan and America, as well as outpatient clinic visitors from India⁽⁴⁷⁾ and American blood donors,⁽⁴⁸⁾ were also similar to our findings, and males had a higher prevalence than females. In Egypt, villagers and male blood donors were found to have a higher prevalence rate of HCV than females.^(49,50) Although males in Pakistan had slightly greater probability trends than females among all age categories, these differences were not statistically significant.⁽⁵¹⁾ In Poland, a large-scale survey found no differences in HCV prevalence based on the gender of subjects.⁽⁵²⁾ For the age groups distribution, the prevalence of HCV infection was significantly highest among persons in the age group of 1-20 years, followed by the age group of 21-40 years, and these results agreed with Amin et al.,⁽⁵³⁾ who reported that the 20 - 24-year-old age group had the highest HCV prevalence, with a strong majority of the infected population below the age of 50 years. The trend of seroprevalence through ages in Australasia's Western Pacific region showed a significant increase in HCV prevalence, which reaches its peak at 20 to 24 years of age. Additionally, Central Europe exhibited an early peak in children between the ages of 1-4 years.⁽⁵⁴⁾ In the current study, the prevalence of HCV infection decreased with age beyond 60 years, which may be related to the deterioration in functional ability and economic strength that causes a decline in the number of elder tests.

The first limitation of the current study was a small sample size because only one province of Iraq (Thi-Qar) was included. Another limitation was the time of the sample collection, because the serological test may have revealed negative results when the sample was collected during the window period of HCV infection. Most of the information available about the incidence of HCV infections in Iraq emerged from laboratory findings and personalized research projects at some academic institutions. Therefore, to reduce the infection with HCV, we suggested continuing monitoring of blood banks, HCWs, as well as patients, and introducing a highly sensitive molecular technology (PCR) for detecting HCV biomarkers within the window period.

Conclusion

In Iraq, 3.2% of the population had HCV infection, which was similar to those in most Asian and non-Asian studied populations, and the infection rate was higher in males and inversely correlated with the age of the subjects.

The main routes of HCV infection were blood transfusions, renal dialysis, and HCWs. Thalassaemic and hemodialysis patients were potentially vulnerable to HCV infection. As a result, effective screening methods and blood donor screening protocols are likely required to prevent the spread of HCV infection. The risk of HCV infection is higher among HCWs; hence, HCWs should take proper precautions while handling blood and other biological fluid and samples. Aseptic procedures should be carried out to prevent needle stick injury.

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Competing Interests

The authors declare that they have no competing interests.

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Association of Serum Procalcitonin Level with Severity of COVID-19 among Patients in Ajman, United Arab Emirates

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Abstract

The aim of the current study was to assess the association between the mean level of procalcitonin (PCT), demographic characteristics, and the symptoms, duration, and severity of COVID-19.

Methods and Results: This cross-sectional study included patients with a confirmed COVID-19 infection who visited the Thumbay Hospital in Ajman (UAE) between March and June 2022. A total of 231 COVID-19-positive patient records (170[73.6%] males and 61[26.4%] females) were included in the study. PCT levels were measured upon admission using the Beckman Coulter – UniCel DxI 800 Access Immunoassay System.

The mean patients' age was 47.44 ± 13.460 years, and the length of stay in the hospital was 11.21 ± 8.145 days. The PCT mean level was 0.545 ± 1.739 ng/ml with minimum and maximum values of 0.010 ng/ml and 16.667 ng/ml, respectively. In terms of COVID-19 severity, patients were categorized into mild (121[52.4%]), moderate (59[25.5%]), and severe 51(22.1%). We found no association between age categories and COVID-19 severity. There was a statistically significant difference in the mean PCT level among the severity groups. The mean PCT level increased with increasing severity of COVID-19: 0.0569 ± 0.0324 , 0.1736 ± 0.0594 , and 2.134 ± 3.254 ng/ml for mild, moderate, and severe COVID-19, respectively ($P=0.0000$). There was a statistically significant, moderate positive correlation between PCT level and disease severity ($r=0.433$, $P=0.001$). The linear regression results revealed that PCT level is a significant factor in COVID-19 severity.

Conclusion: The current study demonstrates that the serum PCT level may be a marker of disease severity in patients infected with SARS-CoV-2. (International Journal of Biomedicine. 2023;13(2):241-244.)

Keywords: SARS-CoV-2 • COVID-19 • procalcitonin • disease severity

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Introduction

The COVID-19 pandemic has led to 1,049,828 confirmed cases, including 2,348 deaths, in the UAE as of February 06, 2023.⁽¹⁾ From the start of the COVID-19 pandemic, it was clear that some people who were infected with the coronavirus were experiencing more severe illness than others, which increased their chances of being hospitalized.^(2,3)

According to the WHO, COVID-19 can lead to a range of symptoms, including fever, cough, fatigue, body aches, shortness of breath, and loss of taste or smell. Severe cases can progress to pneumonia, acute respiratory distress syndrome, septic shock, and multiple organ failure.⁽⁴⁾

The severity of COVID-19 symptoms can vary widely, with some patients experiencing mild symptoms, while others may become critically ill and require hospitalization (CDC, 2021).⁽⁵⁾ The severity and mortality of COVID-19 disease have been linked to higher levels of inflammatory markers.⁽⁶⁾ Specific inflammatory, biochemical, and immunological indicators have been shown in prior research to have

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predictive significance in individuals infected with SARS-CoV.⁽⁷⁾ The use of biomarkers to predict disease severity has proven essential for resource allocation, particularly for respiratory support needs.^(8,9)

The aim of the current study was to assess the association between the mean level of procalcitonin (PCT), demographic characteristics, and the symptoms, duration, and severity of COVID-19.

Materials and Methods

This cross-sectional study included patients with a confirmed COVID-19 infection who visited the Thumbay Hospital in Ajman (UAE) between March and June 2022. Using clinical data collected from the medical record, we compared the mean procalcitonin (PCT) level, demographic characteristics, and the symptoms, duration, and severity of the disease.

A total of 231 COVID-19-positive patient records (170[73.6%] males and 61[26.4%] females) were included in the study. PCT levels were measured upon admission using the Beckman Coulter – UniCel DxI 800 Access Immunoassay System.

Statistical analysis was performed using statistical software package SPSS version 25.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean (M) \pm standard deviation (SD). Inter-group comparisons were performed using Student's t-test. Multiple comparisons were performed with one-way ANOVA and Tukey's HSD Post-hoc Test. Correlation coefficients were calculated by linear regression analysis. The frequencies of categorical variables were compared using Pearson's chi-squared test with Yates's correction. A probability value of $P < 0.05$ was considered statistically significant.

The study was approved by the Institutional Review Board of Gulf Medical University (Ajman, United Arab Emirates).

Results

All patients were classified into five age groups: 20-29 years ($n=18$ [7.8%]), 30-39 years ($n=47$ [20.3%]), 40-49 years ($n=73$ [31.6%]), 50-59 years ($n=48$ [20.8%]), and >60 years ($n=45$ [19.5%]). The mean patients' age was 47.44 ± 13.460 years, and the length of stay in the hospital was 11.21 ± 8.145 days. The PCT mean level was 0.545 ± 1.739 ng/ml with minimum and maximum values of 0.010 ng/ml and 16.667 ng/ml, respectively (Table 1).

In terms of COVID-19 severity, patients were categorized into mild (121[52.4%]), moderate (59[25.5%]), and severe 51(22.1%) according to WHO recommendations. We found no association between age categories and COVID-19 severity (Table 2).

The most common symptom was fever (93.3%), followed by headache (64.9%), fatigue (61.5%), and abdominal pain (57.1%) (Table 3).

There was no association between COVID-19 severity and headache, fatigue, and fever. In contrast, cough, shortness

of breath, and pneumonia symptoms presented statistically significant associations with COVID-19 severity (Table 4).

Table 1.

Descriptive statistics of participant's ($n=231$).

Variable	Mean \pm SD	Minimum	Maximum
Procalcitonin (ng/ml)	0.545 ± 1.739	0.010	16.667
Age (years)	47.44 ± 13.460	15	82
Length of stay (days)	11.21 ± 8.145	1	76

Table 2.

Association between age categories and severity.

Variable	Age group	COVID-19 severity			P-value
		Mild	Moderate	Severe	
Age groups	20-29 ($n=18$)	13 (72.2%)	4 (22.2%)	1 (5.6%)	0.504
	30-39 ($n=47$)	27 (57.4%)	9 (19.1%)	11 (23.4%)	
	40-49 ($n=73$)	39 (53.4%)	18 (24.6%)	16 (21.9%)	
	50-59 ($n=48$)	21 (43.75%)	15 (31.25%)	12 (25.0%)	
	> 60 ($n=45$)	20 (44.4%)	13 (28.9%)	12 (26.7%)	

Table 3.

COVID-19 symptoms in study patients ($n=231$).

Variable	Group	n	%
Headache	No	81	35.1 %
	Yes	150	64.9 %
Fever	No	217	93.9 %
	Yes	14	6.1 %
Cough	No	126	54.5 %
	Yes	105	45.5 %
Fatigue	No	89	38.5 %
	Yes	142	61.5 %
Abdominal pain	No	99	42.9 %
	Yes	132	57.1%
Nausea and vomiting	No	125	54.1 %
	Yes	106	45.9 %
Pneumonia symptoms	No	173	74.9 %
	Yes	58	25.1 %
Shortness of breath	No	143	61.9 %
	Yes	88	38.1 %

Table 4.**Association between symptoms and COVID-19 severity (n=231)**

Symptoms		Mild	Moderate	Severe	Total	P-value
Fatigue	No	46 (51.7%)	24 (27.0%)	19 (21.3%)	89	0.922
	Yes	75 (52.8%)	35 (24.6%)	32 (22.5%)	142	
Fever	No	115 (53.0%)	57 (26.3%)	45 (20.7%)	217	0.295*
	Yes	6 (42.9%)	2 (14.3%)	6 (42.9%)	14	
Headache	No	42(51.9%)	19(23.5%)	20 (24.7%)	81	0.739
	Yes	79(52.7%)	40(26.7%)	31(20.6%)	150	
Cough	No	113 (89.7%)	1(0.8%)	12 (9.5%)	126	0.000
	Yes	8(7.6%)	58(55.2%)	39(37.1%)	105	
Shortness of breath	No	113 (79%)	23(16.1%)	7 (4.9%)	143	0.000
	Yes	8 (9.1%)	36 (40.9%)	44(50.0%)	88	
Pneumonia symptoms	No	114 (65.9%)	58(33.5%)	1(0.6%)	173	0.000
	Yes	7 (12.1%)	1 (1.7%)	50 (86.2%)	58	
Total		121(52.4%)	59(25.5%)	51(22.1%)	231	

*- Yates' P-value

In our study, the death rate for the total number of patients was 15(6%). We found a significant association between COVID-19 severity and patients' outcome ($P=0.01$), with a death rate of 60% for severe COVID-19 (Table 5).

Table 5.**Association between patients' outcome and COVID-19 severity.**

Variable	Group	Mild	Moderate	Severe	P-value
Patients' outcome	Alive	117(54.2%)	57 (26.4%)	42 (19.4%)	0.005*
	Dead	4 (26.7%)	2 (13.3%)	9 (60.0%)	

*- Yates' P-value

There was a statistically significant difference in the mean PCT level among the severity groups. The mean PCT level increased with increasing severity of COVID-19: 0.0569 ± 0.0324 , 0.1736 ± 0.0594 , and 2.134 ± 3.254 ng/ml for mild, moderate, and severe COVID-19, respectively ($P=0.0000$) (Table 6). There was a statistically significant, moderate positive correlation between PCT level and disease severity ($r=0.433$, $P=0.001$) (Table 7). Linear regression coefficients (Table 8) provided the necessary information to predict COVID-19 severity from the PCT level, as well as determine whether the PCT level contributes statistically significantly to the model ($P=0.00$). So, the linear regression results revealed that PCT level is a significant factor in COVID-19 severity. Increasing the PCT level by 0.930 could lead to an increase in the severity.

Table 6.**Comparison of the mean PCT level among severity groups.**

Severity group	n	Mean \pm SD	One-way ANOVA and Tukey's HSD Post-hoc Test
Mild (1)	121	0.0569 ± 0.0324	F=35.6654 $P=0.0000$ $P_{1-2}=0.8798$ $P_{1-3}=0.0000$ $P_{2-3}=0.0000$
Moderate (2)	59	0.1736 ± 0.0594	
Severe (3)	51	2.134 ± 3.254	
Total	231	0.545 ± 1.739	

Table 7.**Correlation between the mean PCT level and disease severity.**

		PCT	Disease severity
PCT	Pearson Correlation	1	0.433
	P-value		0.001
	n	231	231
Disease severity	Pearson Correlation	0.433	1
	P-value	0.001	
	n	231	231

Table 8.**Linear regression coefficients.**

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.032	0.241		- 4.290	0.00
	PCT	0.930	0.128	0.433	7.264	0.00
a. Dependent Variable: COVID-19 severity						

Discussion

In the current study, the severe patients' group had higher PCT levels than the mild and moderate groups, suggesting that PCT could be an early marker of disease severity. Hu et al.⁽¹⁰⁾ analyzed 95 SARS-CoV-2-infected patients, including 62 moderate, 21 severe, and 12 critical COVID-19 patients (6 patients died, all critical) and showed that the mean serum PCT levels were over four times higher in severe patients than in moderate patients and were over eight times higher in critical patients than in moderate patients. The authors also found that in death cases, serum levels of PCT increased as the disease worsened.

Our study showed a statistically significant association between COVID-19 severity and patients' outcome ($P=0.005$,

with a death rate of 60% for severe COVID-19. At the same time, our results revealed no association between age and COVID-19 severity ($P=0.504$), possibly due to the smaller number of aged patients. In the current study, linear regression revealed that PCT level is a significant factor in COVID-19 severity. Our data are similar to the result of a meta-analysis by Lippi and Plebani.⁽¹¹⁾ In particular, the pooled OR of four studies⁽¹²⁻¹⁵⁾ showed that increased PCT values were associated with a nearly 5-fold higher risk of severe SARS-CoV-2 infection (OR=4.76; 95% CI: 2.74–8.29). Kotula et al.⁽¹⁶⁾ showed that the substantial increase in PCT levels reflects bacterial coinfection in pediatric patients with viral lower respiratory tract infections. Serial PCT measurement may play a role in predicting the evolution of COVID-19 toward a more severe form of the disease.

In conclusion, the current study demonstrates that the serum PCT level may be a marker of disease severity in patients infected with SARS-CoV-2.

Competing Interests

The authors declare that they have no competing interests.

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Saliva as Alternative Specimen for Measuring Inflammatory Markers Interleukins (IL10, IL-4, and IL-1 β) in Association with Disease Severity among COVID-19 Patients

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Abstract

Background: Saliva is a specimen that is easily collected by non-invasive means and does not require well-trained staff; it could be helpful in measuring inflammatory markers to determine COVID-19 severity. The aim of this study was to investigate saliva as an alternative specimen for measuring inflammatory markers IL-10, IL-4, and IL-1 β among COVID-19 patients in relation to disease severity.

Methods and Results: This cross-sectional study was conducted among COVID-19 patients in a fever clinic, isolated hotels, and hospitals providing care for positive COVID-19 patients and in public health centers for negative control patients in Jeddah, Saudi Arabia. A total of 151 subjects participated in this study, including 101 patients with COVID-19 and 50 healthy controls. Patients with COVID-19 were categorized according to the severity of their symptoms into mild (n=50) and severe cases (n=51). The salivary concentrations of IL-4, IL-10, and IL-1 β were measured using sandwich MyBioSource ELISA Test Kits.

The age of the study population ranged from 19 to 70 years old, with a mean age of 43.3 \pm 13.0 years. The distribution of the study population showed that more of the patients were men (65[64.4%]) than women (36[35.6%]) ($P=0.004$). The frequency of severe infection in men was higher than in women (35[68.6%] and 16[31.4%], respectively, $P=0.008$). The group of severe cases was significantly older than the group of mild cases (47.9 \pm 11.03 years and 38.64 \pm 13.82 years, respectively, $P=0.0007$). The volume of saliva was the smallest in severe COVID-19, compared to mild cases and controls ($P=0.0000$ in all cases). The salivary levels of IL-4, IL-10, and IL-1 β were greater in the severe cases than in mild cases and controls (46.14 \pm 11.61 pg/mL, 12.86 \pm 1.99 pg/mL, and 27.45 \pm 11.47 pg/mL versus 19.31 \pm 5.72 pg/mL, 7.96 \pm 2.12 pg/mL, and 6.59 \pm 1.90 pg/mL, respectively; $P=0.0000$ in all cases). The salivary levels of IL-4 and IL-10 in mild cases were greater than in controls (19.31 \pm 5.72 pg/mL and 7.96 \pm 2.12 pg/mL versus 15.30 \pm 4.36 pg/mL and 6.02 \pm 0.89 pg/mL, respectively; $P=0.0329$ and $P=0.0000$, respectively), but salivary IL-1 β levels in mild cases did not differ from controls (6.59 \pm 1.90 pg/mL vs. 6.03 \pm 2.28 pg/mL, $P=0.9129$).

Conclusion: Saliva could be used as an alternative sample in measuring IL-10, IL-4, and IL-1 β with the suggestion of using IL-10 and IL-4 as markers for predicting disease severity. (International Journal of Biomedicine. 2023;13(2):245-249.)

Keywords: COVID-19 • saliva • IL-4 • IL-10 • IL-1 β

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Introduction

The spectrum of COVID-19 clinical manifestation can range from asymptomatic infection to severe pneumonia, followed by multisystem failure that may lead to death. Inflammatory immune response influences the pathogenesis and clinical expression of COVID-19.^(1,2) Cytokine storms can cause a severe clinical complication known as acute respiratory distress syndrome (ARDS). ARDS is induced by an excessive immune response rather than the viral load.⁽³⁾ Researchers have revealed the association between interleukins and disease severity.⁽⁴⁾ Interleukin (IL)-10 is an inflammatory marker predicting disease severity.⁽⁵⁾ IL-10 plays an important role in regulating inflammatory response by reducing the damage caused by inflammatory cytokines. It also suppresses the expression of these cytokines and has an important role in controlling the immune response against the virus.⁽⁶⁾ Studies have shown that the concentration of serum interleukins is highly increased in cases ranging from severe and critical to mild.⁽⁷⁻¹¹⁾ It has been shown that levels of IL-10 and IL-4 are elevated in COVID-19 patients, compared to healthy individuals, and the level of IL- β has been elevated in non-survivors, compared to survivors. These findings suggest that interleukins are related to disease severity and prognosis.⁽¹²⁾

The effect of the recent COVID-19 pandemic on communities across the world is acute. There is a persistent need for non-invasive, easily self-collected specimens that do not require experienced staff to diagnose and measure inflammatory markers to determine disease severity. Saliva is a specimen that is easily collected by non-invasive means and does not require well-trained staff; it could be helpful in measuring inflammatory markers to determine COVID-19 severity.^(13,14) Slavish et al.⁽¹⁵⁾ determined the reliability of saliva specimens in measuring inflammatory markers, including IL-1 β , TNF- α , IL-6, IL-2, IL-4, IL-10, IL-12, and CRP, in response to stress across multiple studies. Galhardo et al.⁽¹⁶⁾ investigated salivary inflammatory markers, including IL-6, in hospitalized sepsis patients. They found it to be a useful specimen for diagnosing such a condition. Saliva has been used as a specimen for the detection of the SARS-CoV-2 virus. According to our knowledge, there are no published data about measuring inflammatory markers in saliva among COVID-19 patients.

The aim of this study was to investigate saliva as an alternative specimen for measuring inflammatory markers IL-10, IL-4, and IL-1 β among COVID-19 patients in relation to disease severity.

Materials and Methods

This cross-sectional study was conducted among COVID-19 patients in a fever clinic (Tetamman Clinics), isolated hotels, and hospitals providing care for positive COVID-19 patients and in public health centers for negative control patients in Jeddah, Saudi Arabia. Ethical approval was obtained from the Ministry of Health (Saudi Arabia). Formal consent was obtained from participants.

A total of 151 subjects participated in this study, including 101 patients with COVID-19 and 50 healthy

controls. Patients with COVID-19 were categorized according to the severity of their symptoms into mild ($n=50$) and severe cases ($n=51$). Open-Epi provided the statistics for sample size and power calculations.

Saliva Collection and the Measurement of the Salivary Levels of IL-4, IL-10, and IL-1 β

Subjects were given an appointment, preferably between 9 and 11 a.m. They were requested to refrain from eating, drinking, or using oral hygiene products for at least one hour before sample collection. Subjects were asked to rinse their mouth with water three times. Five minutes later, they spit into a Falcon tube kept on ice. Subjects were reminded not to cough out mucus. Saliva was collected for 30 minutes. Salivary samples were centrifuged at 2,600g for 15 min at 4°C. The supernatant was collected and frozen within 30 min from the time of collection.

The salivary concentrations of IL-4, IL-10, and IL-1 β were measured using sandwich MyBioSource ELISA Test Kits (<https://www.mybiosource.com/elisa-kits>) according to the manufacturer's manual. These kits are based on sandwich enzyme-linked immunosorbent assay technology. Standard curves were drawn to obtain the concentration of IL-4, IL-10, and IL-1 β in samples.

Statistical analysis was performed using statistical software package SPSS version 25.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean (M) \pm standard deviation (SD). Multiple comparisons were performed with one-way ANOVA with Tukey's pairwise comparisons. Group comparisons with respect to categorical variables are performed using chi-square tests. ROC curves were drawn to evaluate the ability of variables to predict disease severity. A probability value of $P < 0.05$ was considered statistically significant.

Results

The distribution of the study population showed that more of the patients were men (65[64.4%]) than women (36[35.6%]) ($P=0.004$). The frequency of severe infection in men was higher than in women (35[68.6%] and 16[31.4%], respectively, $P=0.008$). The frequency of infected men and women among mild cases was 30(60%) and 20(40%), respectively, without statistically significant differences ($P=0.157$) (Table 1).

The age of the study population ranged from 19 to 70 years old, with a mean age of 43.3 ± 13.0 years. The group of severe cases was significantly older than the group of mild cases (47.9 ± 11.03 years and 38.64 ± 13.82 years, respectively, $P=0.0007$) (Table 2).

The volume of saliva significantly differed between COVID-19 patients and the control group ($P=0.0000$), and it was the smallest in severe COVID-19, compared to mild cases and controls ($P=0.0000$ in all cases) (Table 2).

The salivary levels of IL-4, IL-10, and IL-1 β were greater in the severe cases than in mild cases and controls (46.14 ± 11.61 pg/mL, 12.86 ± 1.99 pg/mL, and 27.45 ± 11.47 pg/mL versus 19.31 ± 5.72 pg/mL, 7.96 ± 2.12 pg/mL, and 6.59 ± 1.90 pg/mL, respectively; $P=0.0000$ in all cases). The

salivary levels of IL-4 and IL-10 in mild cases were greater than in controls (19.31 ± 5.72 pg/mL and 7.96 ± 2.12 pg/mL versus 15.30 ± 4.36 pg/mL and 6.02 ± 0.89 pg/mL, respectively; $P=0.0329$ and $P=0.0000$, respectively), but salivary IL-1 β levels in mild cases did not differ from controls (6.59 ± 1.90 pg/mL vs. 6.03 ± 2.28 pg/mL, $P=0.9129$) (Table 2).

Table 1.

Gender and disease severity among COVID-19 patients.

	Male	Female	P-value
Mild cases	30 (60%)	20 (40%)	0.157
Sever cases	35 (68.6%)	16 (31.4%)	0.008
Total number	65 (64.4%)	36 (35.6%)	0.004

Table 2.

The volume of saliva and inflammatory markers among COVID-19 patients in relation to disease severity.

Variable	Group	Mean \pm SD	Statistics
Age, years	(1) Control	36.16 ± 12.91	$P=0.0000$ $P_{1-2}=0.5799$, $P_{1-3}=0.0000$ $P_{2-3}=0.0007$
	(2) Mild	38.64 ± 13.28	
	(3) Severe	47.94 ± 11.03	
Salivary volume, ml	(1) Control	3.70 ± 0.75	$P=0.0000$ $P_{1-2}=0.2953$, $P_{1-3}=0.0000$ $P_{2-3}=0.0000$
	(2) Mild	3.46 ± 0.98	
	(3) Severe	2.41 ± 0.64	
IL-4, pg/mL	(1) Control	15.30 ± 4.36	$P=0.0000$ $P_{1-2}=0.0329$, $P_{1-3}=0.0000$ $P_{2-3}=0.0000$
	(2) Mild	19.31 ± 5.72	
	(3) Severe	46.14 ± 11.61	
IL-10, pg/mL	(1) Control	6.02 ± 0.89	$P=0.0000$ $P_{1-2}=0.0000$, $P_{1-3}=0.0000$ $P_{2-3}=0.0000$
	(2) Mild	7.96 ± 2.12	
	(3) Severe	12.86 ± 1.99	
IL-1 β , pg/mL	(1) Control	6.03 ± 2.28	$P=0.0000$ $P_{1-2}=0.9129$, $P_{1-3}=0.0000$ $P_{2-3}=0.0000$
	(2) Mild	6.59 ± 1.90	
	(3) Severe	27.45 ± 11.47	

Analysis of ROC curves for severe COVID-19 patients showed an excellent area under the curve for all cytokines and salivary volume (Figures 1 and 2). For IL-10, it was 97.3% with the best cutoff (≥ 10.11); for IL-1B, it was 100% with the best cutoff (≥ 11.35); for IL-4 - 98.7% with the best cutoff (≥ 18.25); and for saliva it was 85.0%, with best cutoff (≤ 3.35).

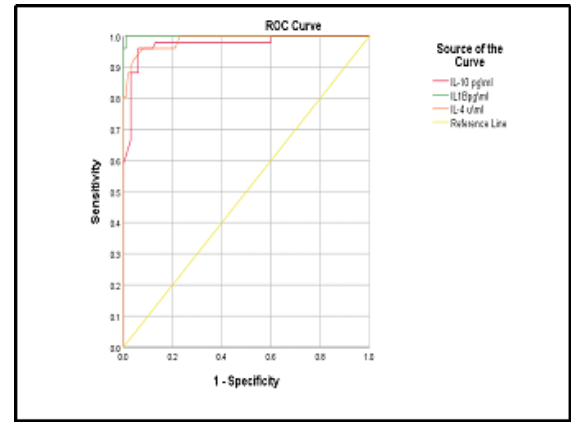


Fig. 1. ROC curve for the performance of IL-10, IL-4, and IL-1 β among severe COVID-19 patients.

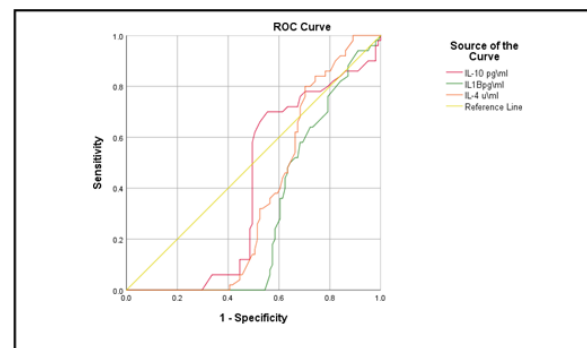


Fig. 2. ROC curve for the performance of IL-10, IL-4, and IL-1 β among mild COVID-19 patients.

Discussion

This cross-sectional study showed that the percentage of infected men was significantly higher than that of women ($65[64.4\%]$ vs. $36[35.6\%]$, $P=0.004$), consistent with the finding of Jin et al.⁽¹⁷⁾ The frequency of infected males with severe cases (68.6%) was also significantly higher ($P=0.008$) than females (31.4%), so gender could be a risk factor in exacerbating infection and possibly increasing the mortality rate, as men showed more severe clinical symptoms than women.⁽¹⁷⁾ This susceptibility may be due to high levels of ACE2 in males, which acts as a viral receptor.⁽¹⁸⁾

The mean age of the study population was 43.3 years and this finding is consistent with study by Abohamr et al.,⁽¹⁹⁾ who examined 768 COVID-19 patients with a mean age of 46.36 ± 13.7 years. The age group of severe cases is significantly older than that of mild cases. Another study reported that older patients tend to have relatively more severe clinical infections and poorer clinical outcomes associated with COVID-19 than do younger patients. Elderly patients aged 65 and older were at a much higher risk of developing severe or critical illness than other age groups.⁽²⁰⁾

The volume of saliva among COVID-19 patients was significantly less than among healthy controls and significantly less among severe COVID-19 than among mild COVID-19

cases ($P=0.000$ in all cases). This finding is consistent with previous studies that report signs of dry mouth among COVID-19 patients.⁽²¹⁻²³⁾

Immune responses have been shown to be involved in the initiation and development of COVID-19. Excessive stimulation of this response results in a cytokine storm that leads to poor prognosis in COVID-19 patients.^(7,24,25) We found that the concentration of IL-4, IL-1 β , and IL10 was significantly higher in COVID-19 patients than in healthy controls, consistent with previous reports.^(5,12,26) The concentration of IL-4, IL-1 β , and IL10 in this study was significantly higher among severe COVID-19 cases than among mild cases, which aligns with previous studies.⁽⁷⁻¹¹⁾ IL-10 is a predictor of disease severity. It is highly elevated among COVID-19 patients with severe disease compared, to mild cases and healthy controls. Also, IL-10 was elevated among mild cases, compared to healthy controls, and our finding aligns with several reports.^(5,12) Similar changes in the salivary levels were found for IL-4, which disagreed with the results of Han et al.,⁽⁵⁾ a discrepancy that may be attributed to the difference in sample size. No significant differences between mild cases and controls in the concentration of IL-1 β in our study are like the finding of the meta-analysis by Chang et al.⁽¹²⁾

In conclusion, saliva could be used as an alternative sample in measuring IL-10, IL-4, and IL-1 β with the suggestion of using IL-10 and IL-4 as markers for predicting disease severity.

Competing Interests

The authors declare that they have no competing interests.

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Distribution of Chest Computed Tomography Findings in 202 Saudi Patients with COVID-19

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Abstract

Background: Computed tomography (CT) is one radiographic imaging modality that plays an essential role in detecting, characterizing, and assessing the complications of COVID-19. The aim of this study was to determine the distribution of chest CT findings in 202 Saudi patients with COVID-19.

Methods and Results: Medical records of 202 patients diagnosed in Ohod and Al-Madinah National Hospitals (Al-Madinah Al-Monwarahwith) with positive COVID-19 infection from February 1 to March 1, 2021, were analyzed in this retrospective study. A verbal ethical agreement was obtained from the radiology department in these hospitals. Patients' demographic data and chest CT findings were evaluated.

The majority of the sample was male 128(63.4%), and the largest age group was 50–64 years (41.1%). The typical chest CT findings for COVID-19 pneumonia (ground-glass opacification) were bilateral in peripheral lung fields (91.58%), subpleural zones (1.98%), and central zones (0.59%). Among COVID-19-associated findings, septal thickening was found in 4(2.0%) cases, air bronchogram in 13(6.4%) cases, lung fibrosis in 3(1.5%) cases, the atelectatic in 5(2.5%) cases, pleural effusion in 15(7.4%) cases, and pulmonary embolism in 1(0.5%) case. There was no significant difference in the COVID-19-associated findings among different age groups and genders.

Conclusion: Pleural effusion and air bronchogram were the most common findings associated with ground-glass opacification in unenhanced chest CT in Saudi patients with COVID-19. (**International Journal of Biomedicine. 2023;13(2):250-254.**)

Keywords: COVID-19 • computed tomography • ground-glass opacification • plural effusion

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presents a substantial and extreme threat to global health.^(1,2) In Saudi Arabia, the first reported case was on March 2, 2020, according to the Ministry of Health.⁽³⁾ As of March 29, 2023, the cumulative number of confirmed COVID-19 cases in

KSA was 832,709.⁽⁴⁾ The most common clinical symptoms of COVID-19 are fever, cough, and shortness of breath. Additional to the reverse transcription polymerase chain reaction (RT-PCR) test, radiological examination of the chest has an important role in clinical diagnostic criteria for COVID-19.⁽⁵⁾ Most of the patients on chest radiography have abnormal findings, from subtle to extensive unilateral and bilateral abnormalities.^(6,7)

Computed tomography (CT) is one radiographic imaging modality that plays an essential role in detecting, characterizing, and assessing the complications of COVID-19. Chest CT is the gold standard imaging modality for COVID-19 pneumonia; in some situations, chest X-ray or ultrasound may be an effective alternative,⁽⁸⁾ but chest CT can detect abnormalities in the early stage of COVID-19, even when RT-PCR assay is negative.⁽⁹⁾ The lesions on the chest CT are distributed peripherally and considered a characteristic distribution pattern of COVID-19.^(10,11) The common findings of COVID-19 in CT images include bilateral multilobar ground-glass opacification (GGO) with a peripheral or posterior distribution; most of these opacities are in the lower lobes and less frequently within the right middle lobe.⁽¹²⁾ Furthermore, there are atypical findings considered unusual in some studies, like pleural effusion and pneumothorax, and some associated findings, like pericardial effusion, lymphadenopathy, and cavitation, considered rare and related to disease progression.⁽¹³⁾

The aim of this study was to determine the distribution of chest CT findings in 202 Saudi patients with COVID-19.

Materials and Methods

Medical records of 202 patients diagnosed in Ohod and Al-Madinah National Hospitals (Al-Madinah Al-Monwarahwith) with positive COVID-19 infection from February 1 to March 1, 2021, were analyzed in this retrospective study. A verbal ethical agreement was obtained from the radiology department in these hospitals. Patients' demographic data and chest CT findings were evaluated (location, distribution, typical and associated findings).

Statistical analysis was performed using the statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages. Group comparisons with respect to categorical variables are performed using the chi-square test with the Yates' correction. A probability value of $P < 0.05$ was considered statistically significant.

Results

The majority of the sample was male 128(63.4%), and the largest age group was 50–64 years (41.1%) (Table 1). The typical chest CT findings for COVID-19 pneumonia (GGO) were bilateral in peripheral lung fields (91.58%), subpleural zones (1.98%), and central zones (0.59%) (Figure 1). Among COVID-19-associated findings, septal thickening was found in 4(2.0%) cases, air bronchogram in 13(6.4%) cases, lung fibrosis in 3(1.5%) cases, the atelectatic in 5(2.5%) cases, pleural effusion in 15(7.4%) cases, and pulmonary embolism in 1(0.5%) case. No COVID-19-associated findings were found in 161(79.7 %) cases (Figure 2). Pleural effusion and air bronchogram were the most common findings associated with GGO. There was no significant difference in the COVID-19-associated findings among different age groups and genders (Table 2 and Table 3). We present CT images (Figure 3-5) of COVID-19 patients with permission from Ohod and Al-Madinah National Hospitals.

Table 1.

Patients' demographic data (gender and age distribution).

Variable	Group	n	%
Gender	Male	128	63.4
	Female	74	36.6
Age	20 - 34	18	8.9
	35 -49	55	27.2
	50-64	83	41.1
	65 -80	31	15.3
	>80	15	7.4
Total	202	202	100

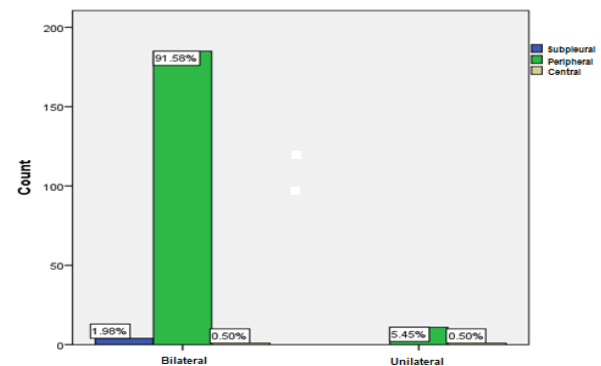


Fig. 1. Distribution and location of the typical chest CT findings.

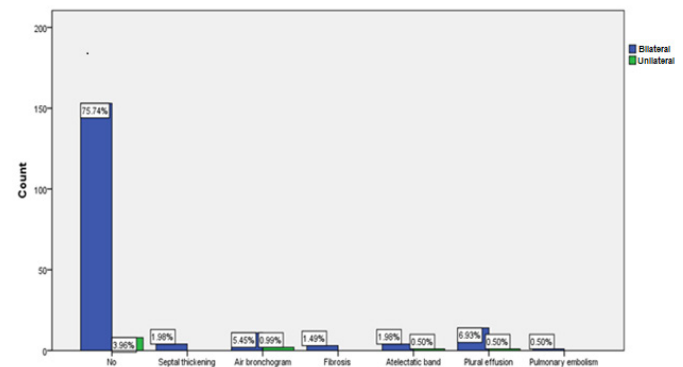


Fig. 2. Distribution of COVID-19-associated chest CT findings.

Table 2.

Relationship between COVID-19-associated findings and age.

Finding	Age					Total	Yates' P-value
	20-34	35-49	50-64	65-79	≥ 80		
No	14	41	68	24	14	161	0.814
Septal thickening	0	0	4	0	0	4	
Air bronchogram	3	5	4	1	0	13	
Fibrosis	0	0	3	0	0	3	
Atelectatic band	0	2	2	1	0	5	
Plural effusion	1	6	2	5	1	15	
Pulmonary embolism	0	1	0	0	0	1	
Total	18	55	83	31	15	202	

Table 3.

Relationship between COVID-19-associated findings and gender.

Finding	Gender		Total	Yates' P-value
	Male	Female		
No	102	59	161	0.911
Septal thickening	3	1	4	
Air bronchogram	10	3	13	
Fibrosis	2	1	3	
Atelectatic band	4	1	5	
Plural effusion	7	8	15	
Pulmonary embolism	0	1	1	
Total	128	74	202	

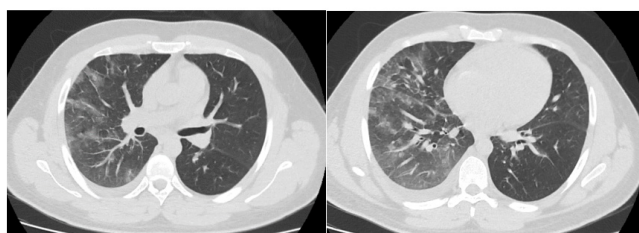


Fig. 3. A 24-year-old male patient with COVID-19. The axial non-enhanced CT scan of the chest shows diffuse bilateral patchy areas of GGO with air space opacities peripherally located with interlobar septal thickening, more pronounced at lower lung zones and bilateral pleural effusion.

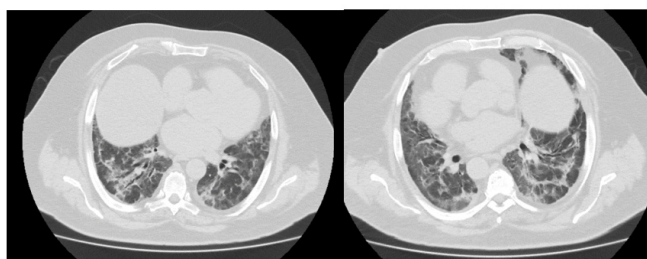


Fig. 4. A 70-year-old male patient with COVID-19. The axial non-enhanced CT scan of the chest shows bilateral diffuse GGO associated with interlobar septal thickening, reticular opacity, and traction bronchiectasis.

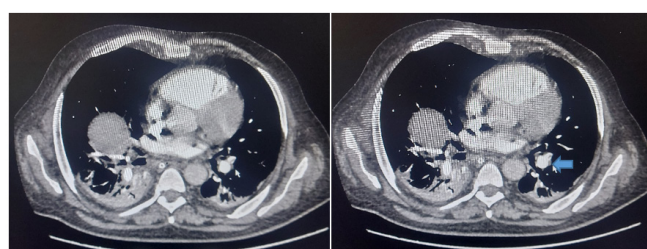


Fig. 5. A 61-year-old male patient with COVID-19. The contrast-enhanced CT scan of the chest shows a partial filling defect in the left interlobar artery and segmental branch (arrow), suggesting pulmonary embolism in the left interlobar artery and segmental branch.

Discussion

The study evaluated typical and COVID-19-associated chest CT findings in 202 patients with positive PCR tests. The most affected patients were males aged between 50 and 64; this finding matches most research on COVID-19. For example, in the studies by Zhou et al.⁽¹⁴⁾ and Aljondi R. et al.,⁽¹⁵⁾ the most infected patients were also men (62.9% and 77.9%, respectively).

Gender differences are frequently observed in many diseases. The relationship between males and COVID-19 rises from different factors, such as gender differences in the activity of the immune system and its modulation by sex hormones, coagulation patterns, and preexisting cardiovascular diseases, as well as effects deriving from smoking and drinking habits.⁽¹⁶⁾ Older patients are more affected by the disease.⁽¹⁷⁾

Our study found the typical GGO CT findings for COVID-19 pneumonia in both lungs, involving the peripheral zones, in 91.58% of cases. The result of this study is consistent with the data of Wang et al.⁽¹¹⁾ and Pakdemirli et al.,⁽¹⁸⁾ who found that multi-lobe lesions in both lungs were present in most patients. The justification for the lesion's peripheral distribution is that the virus is more likely to invade bronchioles and alveoli,⁽¹⁹⁾ which causes inflammatory reactions. Moreover, blood vessels and lymphatics rich in immune cells are more abundant in the peripheral and lower areas, so the lesions tend to distribute in the peripheral and lower area of the lungs.⁽²⁰⁾ Our study found the same atypical COVID-19-related findings as mentioned by Fang et al.⁽²¹⁾

Some studies reported the presence of subpleural sparing, bilateral pleural effusion, and septal thickening as associated findings of CT in COVID-19 patients,⁽²²⁾ and other studies consider that pleural effusion is a rare manifestation of COVID-19 infection.^(23,24) Furthermore, the presence of pleural effusion could serve as an indicator of severe inflammation and poor clinical outcomes and lead to a critical type of COVID-19.⁽²⁵⁾ Our results are consistent with the data obtained by Wei et al.,⁽²⁶⁾ who observed pleural effusion in 9.19% of the patients. Patients' circumstances with pleural effusion worsened critically and were associated with increased mortality.

Air bronchogram was defined as a pattern of air-filled (low-attenuation) bronchi on a background of opaque (high-attenuation) airless lung.⁽²⁷⁾ Air bronchogram is usually a sign of advanced disease and can be seen in both GGO and consolidation. Air bronchogram has variable incidence in different reports ranging from 28 to 80% of patients.⁽²⁸⁻³⁰⁾ We found air bronchogram in 13(6.4%) cases.

Bronchial wall thickening, subpleural line, and pericardial effusion manifested beside pleural effusion as associated findings of COVID-19 in CT images in a study by Wu et al.⁽³¹⁾ that is identical to our study as find the same associated findings in CT scan related to COVID-19. A study by Li et al.⁽³²⁾ found a significantly higher percentage of bronchial wall thickening in patients with severe/critical COVID-19.

Current literature reports pulmonary embolism in 22–30% of patients affected by COVID-19.⁽³³⁻³⁷⁾ In our

study, we reported one patient with a pulmonary embolism. Nevertheless, pulmonary embolism is considered one of the COVID-19-associated findings in severe cases.

Our study found no significant difference in the COVID-19-associated findings among different age groups and genders.

Conclusion

Pleural effusion and air bronchogram were the most common findings associated with GGO in unenhanced chest CT in Saudi patients with COVID-19.

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Competing Interests

The authors declare that they have no competing interests.

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Palatine Tonsil Measurements among Healthy Sudanese Children Using Ultrasonography

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Abstract

Background: Palatine tonsils (PT) reach their maximum normal size in early childhood; after puberty and with other body lymphatic tissue, they gradually atrophy. PT are usually easily seen in the oropharynx during the oral examination. However, clinical size assessment is difficult since the tongue's posture may significantly alter its appearance. The aim of this study was to evaluate normal PT size in healthy children by ultrasound using the anteroposterior and transverse diameters and correlate them with age, weight, and gender.

Methods and Results: This cross-sectional study was conducted at different nurseries and schools in Khartoum. A random sample of 79 Sudanese children and adolescents (39 males and 40 females) aged 1–15 years, without PT pathology, was examined. The anteroposterior diameter (APD) and transverse diameter (TD) for both tonsils were measured by ultrasound.

APD and TD were 1.46 ± 0.16 cm and 1.38 ± 0.16 cm, respectively, for the right PT and 1.51 ± 0.15 cm and 1.40 ± 0.16 cm for the left PT. The APD was significantly higher than the TD on both sides ($P < 0.01$ in both cases). In addition, L(left)PT-APD was significantly greater than R(right)PT-APD (1.51 ± 0.15 vs. 1.46 ± 0.16 , $P = 0.0044$). We found a low-to-moderate positive correlation between all PT measurements with children's age and weight ($P < 0.01$). There was no significant association between PT size and gender.

Conclusion: Ultrasound is a reliable, simple, noninvasive procedure for estimating PT size in children. Normal PT size correlates with age and weight; however, no correlation is present for gender. Normal PT sizes (APD and TD) in children and adolescents may be predicted based on age and weight using developed equations. (International Journal of Biomedicine. 2023;13(2):255-258.)

Keywords: palatine tonsils • ultrasound • transverse diameter • anteroposterior diameter

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Abbreviations

APD, anteroposterior diameter; LPT, left palatine tonsil; PT, palatine tonsils; RPT, right palatine tonsil; TD, transverse diameter.

Introduction

Palatine tonsils (PT) reach their maximum normal size in early childhood; after puberty and with other body lymphatic tissue, they gradually atrophy.⁽¹⁾ PT are usually easily seen in

the oropharynx during the oral examination. However, clinical size assessment is difficult since the tongue's posture may significantly alter its appearance.⁽²⁾ Some PT measurement techniques have been developed, the most frequently used ones being clinical grading and lateral radiography. Clinical

grading of PT size is based on the transversal extension of their midline.⁽³⁾ The depth or vertical aspects of the PT are not covered in their physical examination. The limited value of PT grading in children became evident when the actual size of the surgically removed PT tissue was compared with the clinical grading.⁽⁴⁾ Controversies exist about the value of such measurement methods and/or their significance.

The PT size cannot be confidently measured on a lateral radiograph because that technique only gives a 2D view of the PT and does not reveal the transversal extensiveness of the tissue. Moreover, no longitudinal data is gathered concerning the physiologic growth of the PT, which means the interpretation of whether the PT is hypertrophic or not cannot be accurate.⁽²⁾

Since ultrasound is an accurate, noninvasive, and inexpensive procedure and can help study PT size, shape, and appearance,⁽⁵⁾ researchers attempted to determine the mean value of PT diameters among Sudanese children. By determining the normal range of PT, early diagnosis of PT hypertrophy and disorders in children becomes possible, and laboratory blood investigation (in particular, antistreptolysin O titer) is not needed.

The aim of this study was to evaluate normal PT size in healthy children by ultrasound using the anteroposterior and transverse diameters and correlate them with age, weight, and gender.

Materials and Methods

Study sample

This cross-sectional study was conducted at different nurseries and schools in Khartoum. A random sample of 79 Sudanese children and adolescents (39 males and 40 females) aged 1–15 years, without PT pathology, was examined. The children's weight was between 8 and 58 kg. Subjects with any PT disorders or tonsillectomy were excluded.

Study design and data collection

Data were collected by scanning PT with different portable ultrasound machines, such as ALOKA SSD-500, MINDARY, and ALOKA UST-5512U, with linear transducers 5–7.5MHz. The examined person was placed in a supine position with the neck extended. The ultrasound transducer was placed transcutaneously in the transverse plane below the lower jaw angle and above the hyoid bone. In this position, the PT bed appeared as a well-defined, hypoechoic structure below the submandibular gland – superior to the constrictor muscle and lateral to the tongue. The APD (height) was calculated by measuring the distance from the tonsils' superior pole to the inferior pole; the TD (width) was calculated by measuring the distance from the tonsils' medial aspect to the lateral aspect (Figure 1).

A pre-designed data collection sheet, having the following variables, was used: age, weight, gender, APD, and TD for both PT.

Statistical analysis was performed using the statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of the distribution of continuous variables was tested by the one-sample Kolmogorov-

Smirnov test. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Minimum, maximum, and mean \pm SD were used for summarizing the data. A scatterplot was used to show the relationship between two quantitative variables measured for the same individuals. Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P < 0.05$ was considered statistically significant.

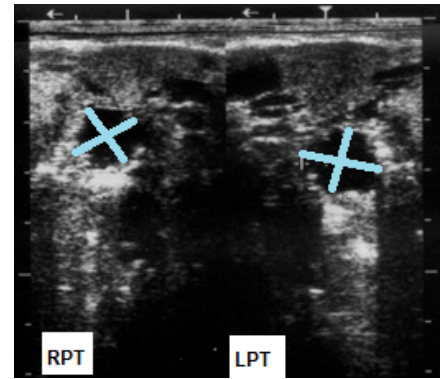


Fig. 1. A 4-year-female child (weight 11.5 kg).
RPT: 1.48 x 1.30 cm; LPT: 1.49 x 1.30 cm

Results

Table 1 describes the measurements of both PT (APD and TD). PT-APD and PT-TD were 1.46 ± 0.16 cm and 1.38 ± 0.16 cm, respectively, for the RPT and 1.51 ± 0.15 cm and 1.40 ± 0.16 cm for the LPT. The APD was significantly higher than the TD on both sides ($P < 0.01$ in both cases) (Table 2). In addition, LPT-APD was significantly greater than RPT-APD (1.51 ± 0.15 cm vs. 1.46 ± 0.16 cm, $P = 0.0044$).

Table 1.

The measurements of both PT (APD and TD) in cm

Diameter	Mean \pm SD	Minimum	Maximum
RPT - APD	1.46 ± 0.16	1.10	1.90
RPT - TD	1.38 ± 0.16	1.02	1.80
LPT - APD	1.51 ± 0.15	1.14	1.93
LPT - TD	1.40 ± 0.16	1.00	1.76

Table 2.

The comparison of diameters (in cm) of LPT and RPT

RPT-APD	P-value	RPT-TD	LPT-APD	P-value	LPT-TD
1.46 ± 0.16	0.0020	1.38 ± 0.16	1.51 ± 0.15	<0.001	1.40 ± 0.16
RPT-APD	P-value	LPT-APD	RPT-TD	P-value	LPT-TD
1.46 ± 0.16	0.0444	1.51 ± 0.15	1.38 ± 0.16	0.4333	1.40 ± 0.16

We found a low-to-moderate positive correlation between all PT measurements with children's age and weight ($P<0.01$) (Table 3). There was no significant association between PT size and gender.

Table 3.

Pearson correlation coefficients between all PT measurements and children's age and weight

	Pearson Correlation	RPT-APD	RPT-TD	LPT-APD	LPT-TD
Age	<i>r</i>	0.341	0.376	0.444	0.497
	Sig. (2-tailed)	0.002	0.001	0.000	0.000
Weight	<i>r</i>	0.371	0.384	0.426	0.442
	Sig. (2-tailed)	0.001	0.000	0.000	0.000

The scatter plots (Figures 2 and 3) show the correlation between the PT diameters and corresponding ages. In addition, a regression equation and the squared correlation coefficient were calculated. For example, the APD and TD of RPT increased by 0.0139 cm and 0.0155 cm, respectively, when age increased by a year, and the APD and TD could be predicted by using the following equations: $RPT-APD=0.0139 \times \text{age} + 1.3642$ and $RPT-TD=0.0155 \times \text{age} + 1.2586$. The APD and TD of LPT increased by 0.0175 cm and 0.0208 cm, respectively, when age increased by a year, and the APD and TD could be predicted by using the following equations: $LPT-APD=0.0175 \times \text{age} + 1.3673$ and $LPT-TD=0.0208 \times \text{age} + 1.2341$.

The scatter plots (Figures 4 and 5) show the correlation between the PT diameters and corresponding weight. The APD and TD of RPT increased by 0.0046 cm and 0.0048 cm, respectively, when weight increased by one kg, and the APD and TD could be predicted by using the following equations: $RPT-APD=0.0046 \times \text{weight} + 1.3663$ and $RPT-TD=0.0048 \times \text{weight} + 1.2686$. The APD and TD of LPT increased by 0.0051 cm and 0.0057 cm, respectively, when the weight increased by one kg, and the APD and TD could be predicted by using the following equations: $LPT-APD=0.0051 \times \text{weight} + 1.3865$ and $LPT-TD=0.0057 \times \text{weight} + 1.2673$. Thus, the linear regression models partially predict the value of PT diameters with increasing age and weight.

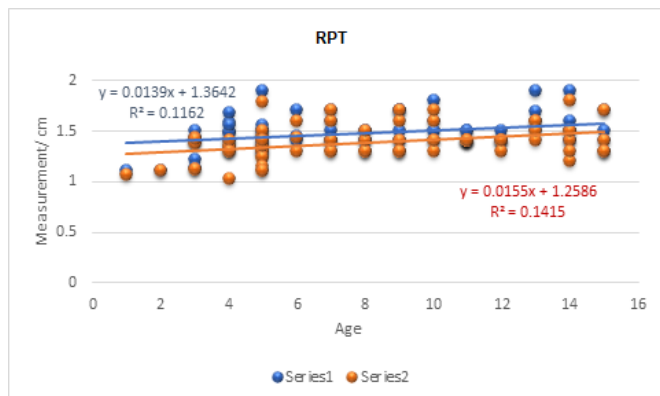


Fig. 2. Scatter plots for RPT diameters (cm) by age (years) with linear regression. Series 1: APD, Series 2: TD

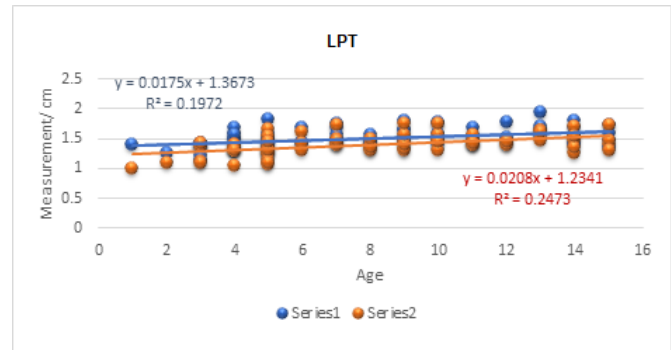


Fig. 3. Scatter plots for LPT diameters (cm) by age (years) with linear regression. Series 1: APD, Series 2: TD

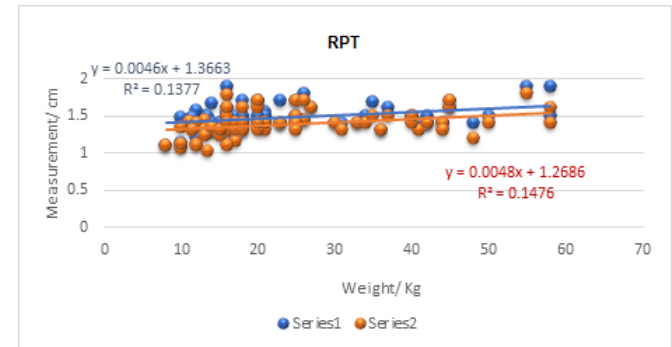


Fig. 4. Scatter plots for RPT diameters (cm) by weight (kg) with linear regression. Series 1: APD, Series 2: TD

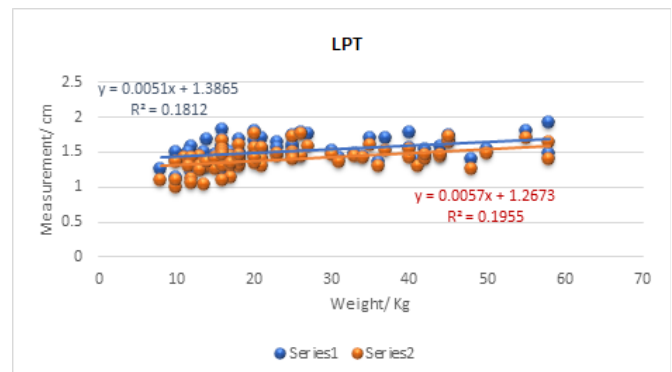


Fig. 5. Scatter plots for LPT diameters (cm) by weight (kg) with linear regression. Series 1: APD, Series 2: TD

Discussion

The present study determined the normal measurements of PT dimensions in Sudanese children. The APDs varied between 1.1 and 1.93 cm, and the TDs ranged from 1.02 to 1.76 cm. The height was more than the width, and the difference between them was significant ($P<0.0001$). The study shows considerable anatomical variation: the LPT-APD was significantly greater than RPT-APD (1.51 ± 0.15 cm vs. 1.46 ± 0.16 cm, $P=0.0044$).

Among Sudanese children, all PT measurements increased significantly with age and weight. RPT/LPT-APD and RPT/LPT-TD had a low-to-moderate positive correlation with children's age and weight. The PT size increased persistently from age 1 to 10. Some studies have considered that APD does not reliably predict PT size, and TD is the best

measure for size assessment.⁽⁶⁾ Wang et al.⁽⁴⁾ confirmed that PT size increased with weight; they found that obese children with sleep-disordered breathing had larger PT than normal-weight children with sleep-disordered breathing.

Using regression analysis, we obtained equations predicting PT size (APD and TD) in children and adolescents based on age and weight. In our study, there was no significant association between PT size and gender, which was consistent with other studies.⁽⁷⁻⁹⁾

In conclusion, ultrasound is a reliable, simple, noninvasive procedure for estimating PT size in children. Normal PT size correlates with age and weight; however, no correlation is present for gender. Normal PT sizes (APD and TD) in children and adolescents may be predicted based on age and weight using developed equations.

Competing Interests

The authors declare that they have no competing interests.

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Hepatic Artery and Portal Vein Hemodynamics in Nonalcoholic Fatty Liver Disease in Adult Saudi Patients: A Doppler Ultrasound Study

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is a growing public health problem. With the growing severity of NAFLD, there are considerable alterations in the hemodynamics of the hepatic circulation that might affect the prognosis of the condition, according to numerous reports written to assess the vasculature of the liver in patients affected with fatty liver disease. The aim of the study was to examine hemodynamic alterations in the hepatic artery (HA) and portal vein (PV) in NAFLD patients and determine how they relate to the severity of the condition, and to classify patients into various categories of NAFLD and connect the results to liver size and body mass index (BMI).

Methods and Results: One hundred and six diagnosed NAFLD patients who attended the Imaging department at King Fahad Hospital from December 2019 to January 2020 were retrospectively studied. The mean age of the patients was 45.75 ± 15.6 years, with a range of 10-79 years. The patients were examined by a TOSHIBA Xario, SSA-660A ultrasound system utilizing a multifrequency convex transducer (2–5 MHz) for all sonographic exams. B-mode assessed the liver parenchyma, and spectral Doppler estimated the HA and PV. The US appearance of hepatosteatosis, according to the severity of echogenicity, was graded (0-3). Most participants were asymptomatic (76.4%), and diabetes and diabetes with hypertension were diagnosed in 12.3% and 11.3%, respectively. It was observed that grade 1 hepatosteatosis was more prevalent than the other grades: 54 cases versus 41 cases for grade 2 and 11 cases for grade 3. The mean values of liver size and BMI in grade 3 were higher than in grade 1 ($P=0.0033$ and $P=0.0054$, respectively). A Spearman test found that the liver size ($R=0.19$, $P=0.05$) and BMI ($R=0.26$, $P=0.01$) had weak positive, but statistically significant, correlations with the severity of the hepatosteatosis grade. Doppler indices of the HA and PV in NAFLD patients did not differ significantly in hepatosteatosis grades 1-3. Only the PSV and EDV of the main PV showed a significant decrease in the hepatosteatosis grade 2 compared to grade 1 ($P=0.0065$ and $P=0.0234$, respectively). Despite the insignificant differences, the Doppler flow parameters of the HA decreased with the severity of hepatic steatosis; for example, the hepatic artery resistive index (HARI) was 0.77 ± 0.16 in grade 1, 0.72 ± 0.16 in grade 2, and 0.75 ± 0.10 in grade 3, respectively. The hepatic artery pulsatility index (HAPI) was 1.62 ± 0.49 in grade 1, 1.63 ± 0.68 in grade 2, and 1.74 ± 0.77 in grade 3. There was also a trend toward a decrease in PSV and end-diastolic velocity (EDV) of HA with the severity of hepatosteatosis.

Conclusion: The severity of hepatic steatosis is significantly correlated with liver size and BMI. The blood flow parameters of PV and HA decrease with the severity of hepatic steatosis except for the pulsatility index. (International Journal of Biomedicine. 2023;13(2):259-264.)

Keywords: NAFLD • body mass index • hepatosteatosis • hepatic circulation

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Abbreviations

BMI, body mass index; **EDV**, end-diastolic velocity; **HA**, hepatic artery; **HARI**, hepatic artery resistive index; **HAPI**, hepatic artery pulsatility index; **NAFLD**, nonalcoholic fatty liver disease; **PV**, portal vein; **PI**, pulsatility index; **PSV**, peak systolic velocity; **RI**, resistive index; **VPI**, venous pulsatility index.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a growing public health problem. A quarter to a third of the world's population is currently affected by NAFLD, which is rising due to the high incidence of diabetes and obesity.⁽¹⁻³⁾ An important liver-related complication in NAFLD is the development of steatohepatitis, cirrhosis, and hepatocellular carcinoma.^(4,5) Global prevalence of NAFLD is 25.24% (95% CI: 22.10-28.65) with highest prevalence in the Middle East and South America and lowest in Africa.⁽⁶⁾ NAFLD is the most frequent cause of aberrant liver enzyme levels in Eastern and Western nations.

The gold standard for the detection of hepatic steatosis is liver biopsy. However, liver biopsy is invasive, sometimes painful, and has rare but potentially serious complications.⁽⁷⁾ Ultrasound is the most frequently used primary imaging modality for evaluating liver disease. The basic sign of steatosis is the increased echogenicity of the liver parenchyma in comparison to the cortex of the right kidney. Calculation of the hepatorenal index based on B-Mode ultrasound images showed in some studies excellent diagnostic accuracy, even for the diagnosis of mild steatosis.⁽⁸⁻¹⁰⁾ Doppler ultrasound imaging is a non-invasive technique for evaluating the hepatic blood supply and various diseases of the liver parenchyma.⁽¹¹⁾ Due to alterations impacting vascular compliance in the liver, Doppler can provide more details about the degree of disease severity and parenchymal damage.⁽¹²⁾ With the growing severity of NAFLD, there are considerable alterations in the hemodynamics of the hepatic circulation that might affect the prognosis of the condition, according to numerous reports written to assess the vasculature of the liver in patients affected with fatty liver disease.^(13,14) There is little information on this problem in Saudi literature.

The aim of the study was to examine hemodynamic alterations in the hepatic artery (HA) and portal vein (PV) in NAFLD patients and determine how they relate to the severity of the condition, and to classify patients into various categories of NAFLD and connect the results to liver size and body mass index (BMI).

Materials and Methods

One hundred and six diagnosed NAFLD patients who attended the Imaging department at King Fahad Hospital from December 2019 to January 2020 were retrospectively studied. The mean age of the patients was 45.75±15.6 years, with a range of 10-79 years. The BMI was determined for each patient. The data was collected from the PACS system and patients' records. Exclusion criteria were cardiovascular diseases, acute or chronic hepatic disease, acute or chronic kidney disease,

alcohol consumption, use of any medications that negatively affect the liver, and a history of thoracic or abdominal surgery.

Sonographic procedure

The patients were examined by a TOSHIBA Xario, SSA-660A ultrasound system utilizing a multifrequency convex transducer (2–5 MHz) for all sonographic exams. All eight liver segments were carefully scanned, and subjects with vascular malformations or hepatic masses (cyst or hemangioma) were excluded. B-mode assessed the liver parenchyma, and spectral Doppler estimated the HA and PV. The US appearance of hepatosteatosis, according to the severity of echogenicity, was graded as follows:⁽¹⁵⁾ grade 0, normal echogenicity; grade 1 (mild): a slight diffuse increase in fine echoes in liver parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders; grade 2 (moderate): a moderate diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm; grade 3 (severe): a marked increase in fine echoes with poor or no visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver.

Three measurements of the Doppler parameters were taken at the same location, and the mean value was used in the final analysis. The PV measurements were all done in the left lateral decubitus position with breath held in inspiration; the measurements were taken at the level of the main PV before the bifurcation. The Doppler angle was always < 60°. The maximum velocity (V max), minimum velocity (V min), mean flow velocity (MFV), and portal vein diameter were measured. The portal venous pulsatility index (VPI) was calculated according to the following equation: $VPI = \frac{Vmax - Vmin}{Vmax}$.⁽¹⁶⁾ The HA indices were measured at the level of porta hepatis with the patient lying in the supine position. The main hepatic artery resistive index (HARI) was obtained with the patient in suspended respiration [$HARI = \frac{PSV - EDV}{PSV}$]. Hepatic artery pulsatility index (HAPI) was calculated according to the following equation: $HAPI = \frac{\text{peak systolic velocity} - \text{end diastolic velocity}}{\text{mean velocity}}$. A designed data collection sheet was used to gather the data.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Multiple comparisons were performed with one-way ANOVA with Tukey's pairwise comparisons. Spearman's rank correlation coefficient (R) was calculated to measure the strength and direction of the relationship between two variables. A probability value of P<0.05 was considered statistically significant.

The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee of the local hospitals. Informed consent was obtained from patients before collecting the data. All identifiable information about patients was removed, and the data were coded to ensure anonymity.

Results

Most participants were asymptomatic (76.4%), and diabetes and diabetes with hypertension were diagnosed in

12.3% and 11.3%, respectively (Table 1). The prevalence of NAFLD among different age groups was assessed (Table 2). It was most prevalent in the age group of 40-49 years, followed by the 30-39 years. The age group of 40-49 years showed a more frequent incidence of grade 1 hepatosteatosi (n=14), followed by grade 2 (n=9) and grade 3 (n=5), while the age group of 30-39 years showed almost the same frequency of grade 1 (n=13) and grade 2 (n=12); grade 3 was found only in two cases (Table 2).

Table 1.

Demographic characteristics of the study sample

Variable	n	%
Gender		
Males	55	51.9
Females	51	48.1
Age group, years		
10-19	8	7.5
20-29	7	6.6
30-39	26	24.5
40-49	29	27.5
50-59	15	14.1
60-69	17	16.0
70-79	4	3.8
Clinical history		
Asymptomatic	81	76.4
Diabetes mellitus	25	23.6
Average age: 45.75±15.6, years		
Average BMI: 27±4.02, kg/m ²		

Table 2.

Distribution of hepatosteatosi grade among different age groups.

Age group, years	Grade 1	Grade 2	Grade 3
10-19	5	2	1
20-29	7	0	0
30-39	13	12	2
40-49	14	9	5
50-59	6	6	3
60-69	7	10	0
70-79	2	2	0
Total	54	41	11
100%	51%	38.6%	10.4%

Considering the 15 cm cut-off point for liver size, the results showed that 22.6% of the participants had an enlarged

liver (16-18.99 cm [n=20] and 19-22.99 cm [n=4], respectively) (Figure 1). It was observed that grade 1 hepatosteatosi was more prevalent than the other grades: 54 cases versus 41 cases for grade 2 and 11 cases for grade 3 (Figure 2).

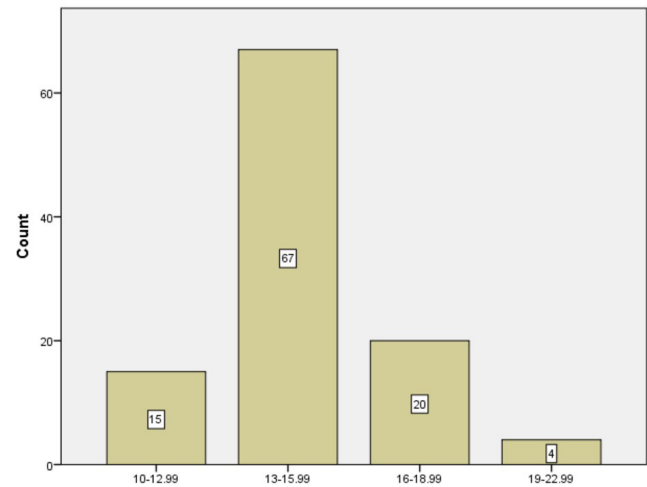


Fig. 1. Liver size (cm).

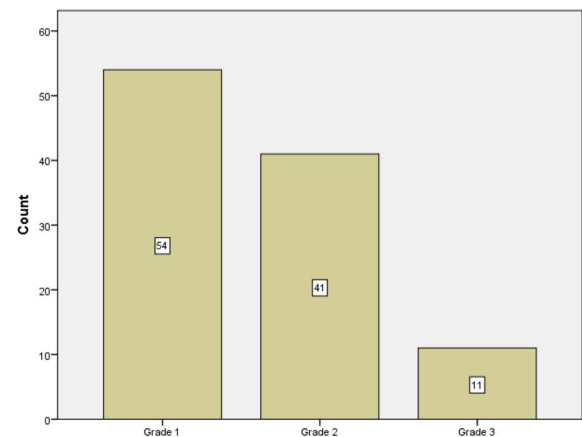


Fig. 2. Hepatosteatosi grade.

The rising hepatosteatosi grade significantly correlated with liver size and BMI. However, the PV diameter increased with the severity of fatty liver infiltration without a statistically significant difference ($P=0.9347$). The mean values of liver size and BMI in grade 3 were higher than in grade 1 ($P=0.0033$ and $P=0.0054$, respectively) (Table 3, Figure 3). A Spearman test found that the liver size ($R=0.19$, $P=0.05$) and BMI ($R=0.26$, $P=0.01$) had weak positive, but statistically significant, correlations with the severity of the hepatosteatosi grade (Table 3).

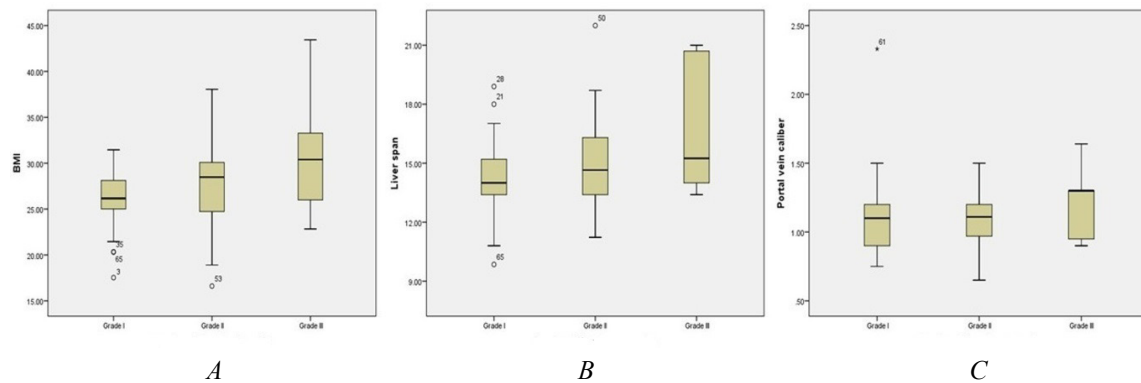
Doppler indices of the HA and PV in NAFLD patients did not differ significantly in hepatosteatosi grades 1-3. Only the PSV and EDV of the main PV showed a significant decrease in the hepatosteatosi grade 2 compared to grade 1 ($P=0.0065$ and $P=0.0234$, respectively). Despite the insignificant differences, the Doppler flow parameters of the HA decreased with the severity of hepatic steatosis; for example, the HARI was 0.77 ± 0.16 in grade 1, 0.72 ± 0.16 in grade 2, and 0.75 ± 0.10 in grade 3, respectively.

Table 3.**Comparison of hepatosteatosis grade with liver size, BMI, and PV diameter.**

Variable	Hepatosteatosis grade			Statistics	R
	Grade 1 (n=54)	Grade 2 (n=41)	Grade 3 (n=11)		
Liver size, cm	14.263±1.68	14.948±2.252	16.580±3.147	F=5.8422 P=0.0040 P ₁₋₂ =0.2591 P ₁₋₃ =0.0033 P ₂₋₃ =0.0609	0.19*
BMI, kg/m ²	26.184±2.82	27.29±4.43	30.31±6.10	F=5.2118 P=0.0070 P ₁₋₂ =0.3636 P ₁₋₃ =0.0054 P ₂₋₃ =0.0644	0.26**
PV diameter, cm	1.10±2.33	1.11±0.17	1.30±0.24	F=0.0676 P=0.9347 P ₁₋₂ =0.9948 P ₁₋₃ =0.9309 P ₂₋₃ =0.9405	0.12

R - Spearman's rank correlation coefficient; * Correlation is significant at the 0.05 level (2-tailed);

**Correlation is significant at the 0.01 level (2-tailed).

**Fig. 3.** Relationship between hepatosteatosis grade and the mean measurements of BMI (A), liver size (B), and PV diameter (C).**Table 4.****Hepatosteatosis grade and Doppler flow parameters of HA and main PV.**

Hepatosteatosis grade	HAPI	HARI	HA-PSV	HA-EDV	PV-PI	PV-PSV, cm/sec	PV-EDV, cm/sec
Grade 1 (n=54)	1.62±0.49	0.77±0.16	55.84±18.99	15.16±6.64	0.61±0.59	27.26±8.77	18.28±6.41
Grade 2 (n=41)	1.63±0.68	0.72±0.16	53.27±15.79	12.65±8.47	0.65±0.47	21.99±6.55	14.70±5.70
Grade 3 (n=11)	1.74±0.77	0.75±0.10	51.35±13.16	12.30±6.38	0.65±0.38	25.24±10.17	17.95±9.05
Statistics	F=0.1861 P=0.8304 P ₁₋₂ =0.9948 P ₁₋₃ =0.8182 P ₂₋₃ =0.8521	F=1.2100 P=0.3024 P ₁₋₂ =0.2697 P ₁₋₃ =0.9198 P ₂₋₃ =0.8367	F=0.4455 P=0.6417 P ₁₋₂ =0.7539 P ₁₋₃ =0.7132 P ₂₋₃ =0.9428	F=1.6326 P=0.2004 P ₁₋₂ =0.2331 P ₁₋₃ =0.4731 P ₂₋₃ =0.9893	F=0.0760 P=0.9269 P ₁₋₂ =0.9290 P ₁₋₃ =0.9715 P ₂₋₃ =1.0000	F=4.8876 P=0.0094 P ₁₋₂ =0.0065 P ₁₋₃ =0.7343 P ₂₋₃ =0.4703	F=3.7549 P=0.0267 P ₁₋₂ =0.0234 P ₁₋₃ =0.9869 P ₂₋₃ =0.3038

The HAPI was 1.62±49 in grade 1, 1.63±.68 in grade 2, and 1.74±0.77 in grade 3. There was also a trend toward a decrease in PSV and EDV of HA with the severity of hepatosteatosis (Table 4).

Discussion

In the study, participants' average age was 45.75±15.6 years and BMI was 27±4.02 kg/m². The study found a

significant difference in BMI among the grades of fatty liver infiltration, with the highest BMI (30.31±6.10 kg/m²) at grade 3 hepatosteatosis. Abangah et al.⁽¹⁷⁾ also detected a significant association between hepatosteatosis grades and BMI. Doppler US is an important non-invasive method in evaluating hepatic vasculature in NAFLD, as diffuse fatty infiltration in the liver alters hemodynamics in the PVB as well as HA resistance.^(18,19) In our study, we investigated the characteristic flow parameters of the PV and HA to assess the

impact of NAFLD on hepatic vascular compliance. Several reports show that the PV flow velocity,⁽²⁰⁻²²⁾ HARI,⁽²³⁻²⁵⁾ and HAPI⁽²⁶⁾ correlate negatively with NAFLD severity. Solhjoo et al.⁽²⁷⁾ showed that patients with NAFLD had a high rate of abnormal hepatic vein Doppler waveform patterns, and decreased VPI is suggestive of reduced vascular compliance in the liver. We found that only PV-PSV statistically significantly decreased with the severity of hepatosteatosis. HA Doppler velocities and HARI showed a tendency toward lower values with increasing hepatosteatosis severity. Our Doppler finding is consistent with Balasubramanian,⁽²⁵⁾ Sabry et al.,⁽²⁸⁾ and Tana et al.⁽²⁹⁾ who reported that the Doppler indices were significantly lower in NAFLD and inversely associated with the severity of fatty infiltration. Mihmanli et al.⁽¹⁸⁾ also found that HARI decreases as the severity of diffuse fatty infiltration increases. Another Iranian study⁽²⁴⁾ found that HARI was 0.75 in patients with grade 1 fatty liver, 0.68 in grade 2, and 0.64 in grade 3. There was an inverse relationship between HARI and different grades of fatty liver in NAFLD patients ($P=0.001$). In contrast, a study by Aslan et al.⁽³⁰⁾ did not find characteristic changes in HARI in NAFLD patients.

The study revealed a significant positive correlation between the changes in liver size with the severity of fatty infiltration: The liver size increased significantly as the grading of fatty infiltration increased ($P=0.005$). Similar to this finding, Khan et al.⁽³¹⁾ found a significant correlation between rising grades of fatty liver, liver size, and BMI.

The current study showed that the liver size and BMI had weak, but statistically significant, positive correlations with the severity of the hepatosteatosis grade. Aslan et al.⁽³⁰⁾ also found that liver size was increased significantly with the degree of fatty infiltration ($P<0.001$). In a study by Khanal et al.,⁽³¹⁾ 22.9% of patients with NAFLD had increased liver size, and a significant association between increasing grades of fatty liver was found with increasing liver size ($P=0.001$) and BMI ($P=0.045$).

In conclusion, the severity of hepatic steatosis is significantly correlated with liver size and BMI. The blood flow parameters of PV and HA decrease with the severity of hepatic steatosis except for the PI.

This study has significant limitations, notwithstanding our findings—first, the study's 1-year duration and small sample size of participants; the second factor is the absence of liver biopsy, the gold standard for diagnosing NAFLD. However, liver biopsy is an invasive procedure, and most patients decline to do it. Last, we did not use healthy volunteers as the control group. Further evaluating hepatic vascular Doppler indices in the NAFLD population is advocated through case-control studies with bigger sample sizes and longer follow-up intervals.

Competing Interests

The authors declare that they have no competing interests.

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Sonographic Study of Normal Common Bile Duct Diameter in Saudi Subjects

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Abstract

Background: The aim of this study was to measure the normal parameters of the common bile duct (CBD) in Saudi subjects using ultrasound modality.

Methods and Results: This descriptive cross-sectional study was carried out in Alazhar Medical Group (Alqunfoda town, Saudi Arabia) from July 2022 to October 2022. The study included 150 subjects (43 males and 107 females) aged between 10 and 73 years without known biliary/pancreatic disease or surgery. The data was collected using the ultrasound machine Samsung Medison (SonoAce R7) using the lower frequency [2-8 MHz] curvilinear probe. The age, gender, body height, weight, and body mass index (BMI) were also recorded. The mean weight, height, and BMI levels were 71.64 ± 17.32 kg, 165.06 ± 11.7 cm, and 26.19 ± 5.66 kg/m², respectively. The mean CBD diameter was 3.26 ± 0.82 mm, ranging from 1.8 mm to 5.8 mm. There was no statistically significant difference in CBD diameter between male and female subjects. Our study found that the linear regression model partially predicted the value of the CBD diameter with increasing age and weight. Further research on CBD measurement with a larger population sample and using various body positions is needed for more accurate results. (International Journal of Biomedicine. 2023;13(2):265-268.)

Keywords: common bile duct • ultrasound • body mass index

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Introduction

Many people worldwide are affected by biliary diseases.

⁽¹⁾ The common bile duct (CBD) size is key in evaluating and assessing the status of the biliary system obstruction.⁽²⁾ Ultrasonography is a reliable, safe, economical, non-invasive imaging technique that can detect a variety of biliary tree illnesses due to its excellent sensitivity and specificity.⁽³⁾ Oral cholecystography, radionuclide investigations, computerized tomography, and MRI are more accurate than ultrasound, but ultrasound is more affordable. With the development of high-resolution ultrasound, the luminal of CBD can be measured

correctly. The CBD diameter can typically range from 2 to 7 mm.^(4,5)

Literature has revealed a variety of viewpoints on the size of the CBD. The CBD may lose some elasticity with aging, and the normal duct may be somewhat extended in an elderly participant due to the loss of elastic recoil. As a rule of thumb, the duct may dilate about only 1mm each decade after age 60. Perret et al.⁽⁶⁾ demonstrated a slight, although statistically significant, increase in the caliber of CBD with increasing age. Although the CBD did increase in size with aging, 98% of all ducts remained below 6 to 7 mm, the commonly accepted upper range of normal.

As is known, variations exist in the anthropometric features of various populations, races, and regions. Despite technological advancements, the association of anthropometric measurements with the CBD diameters has remained controversial.^(7,8)

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The aim of this study was to measure the normal parameters of the CBD in Saudi subjects using ultrasound modality.

Materials and Methods

This descriptive cross-sectional study was carried out in Alazhar Medical Group (Alqunfoda town, Saudi Arabia) from July 2022 to October 2022. The study included 150 subjects (43 males and 107 females) aged between 10 and 73 years without known biliary/pancreatic disease or surgery.

The data was collected using the ultrasound machine Samsung Medison (SonoAce R7) using the lower frequency [2-8 MHz] curvilinear probe. The CBD was identified through its association with the portal vein in the long axis of the gallbladder. At this location, the CBD and hepatic artery appear as two smaller circles anterior to the portal vein, giving an appearance of a face with two ears – also called a “Mickey Mouse” sign. With the indicator directed toward the patient’s right, the right ear is the CBD, and the left ear the hepatic artery. CBD was measured at three locations – at the porta hepatis, in the most distal aspect of the pancreas head, and midway between these points, and the overall mean for all measures was calculated.

The age, gender, body height, weight, and body mass index (BMI) were also recorded.

This study was approved by the Scientific Ethics Committee of the College of Medical Radiological Sciences, Sudan University of Science and Technology (Khartoum, Sudan). Written informed consent was obtained from each patient.

Statistical analysis was performed using the statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). For data with normal distribution, inter-group comparisons were performed using Student’s t-test. Minimum, maximum, and mean \pm SD were used for summarizing the data. A scatterplot was used to show the relationship between two quantitative variables measured for the same individuals. A probability value of $P < 0.05$ was considered statistically significant.

Results

The mean age of participants was 33.37 ± 9.26 years, and the majority (65.3%) were in the age range from 26 to 41 years (Table 1). The mean weight, height, and BMI levels were 71.64 ± 17.32 kg, 165.06 ± 11.7 cm, and 26.19 ± 5.66 kg/m², respectively.

The mean CBD diameter was 3.26 ± 0.82 mm, ranging from 1.8 mm to 5.8 mm (Table 2). There was no statistically significant difference in CBD diameter between male and female subjects (Table 3).

The scatter plots (Figures 1 and 2) show the correlation between the CBD diameter and corresponding weight and age. The CBD increased by 0.0015 cm and 0.0012 cm, respectively, when age increased by a year and weight increased by one kg. Our study found that the linear regression model partially predicted the value of the CBD diameter with increasing age and weight.

Table 1.

Gender and age groups of the study participants.

Gender / Age groups	n	%
Female	43	28.7
Male	107	71.3
Age, years		
10-25	28	18.7%
26-41	98	65.3%
42-57	21	14%
58-73	3	2%

Table 2.

Anthropometric characteristics and CBD diameter in the study participants.

Variable	Mean	Maximum	Minimum	SD
Age, years	33.37	71	11	9.26
Weight, kg	71.64	120	35	17.32
Height, cm	165.06	193	130	11.70
BMI, kg/m ²	26.19	46	16	5.66
CBD diameter, mm	3.26	5.8	1.8	0.82

Table 3.

CBD diameter in males and females.

	Gender	n	Mean	Std. Error Mean	SD
CBD diameter, mm	Male	107	3.3	0.07	0.74
	Female	43	3.1	0.15	0.97
$P = 0.1746$					

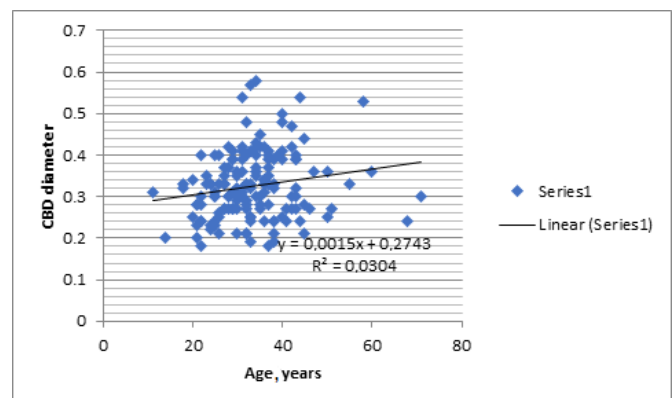


Fig. 1. Scatter plots for CBD diameters (cm) by age (years) with linear regression.

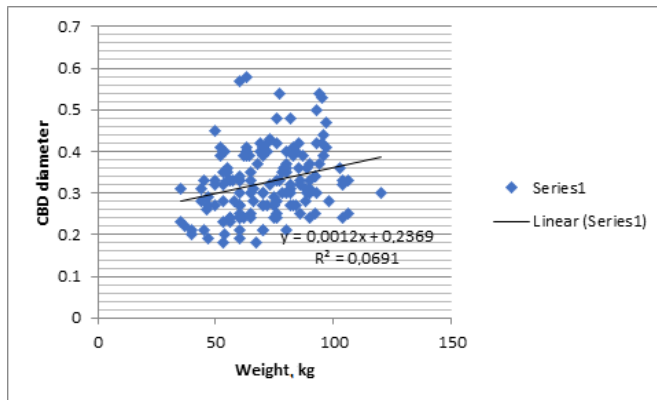


Fig. 2. Scatter plots for CBD diameters (cm) by weight (kg) with linear regression.

Discussion

The mean CBD diameter obtained in our study (3.26 ± 0.82 mm with range from 1.8 mm to 5.8 mm) differed from data in a study by Lal et al.,⁽¹⁾ who conducted sonographic measurements of CBD diameters in healthy Rajasthani residents ($n=200$) and found that the average diameter ranged from 2.0 mm to 7.9 mm, with a mean level of 4.1 mm (SD 1.01 mm).

In a study by Admassie⁽²⁾ involving 293 adult patients without known biliary disease, pancreatic disease, or surgery, the mean CBD diameter was found to be 3.9 mm with a range from 2.1 to 6 mm, and there was also a positive correlation between the CBD diameter and age and weight.

In a study by Kratzer et al.,⁽⁹⁾ the average maximum diameter of the CBD amounted to 5.3 ± 3.0 mm, and there was a positive correlation between age and CBD diameter. Our study found the linear regression model partially predicts the value of CBD diameter with increasing age and weight.

Lal et al.⁽¹⁾ applied a test for linear trend on the age-wise distribution of CBD diameter. This was found to be statistically significant ($P=0.003$). The diameter increased progressively from 3.9 mm among those aged 18-25 years to 4.7 mm among those aged more than 55 years. In contrast, CBD was not observed to have a statistically significant correlation with weight and height.

Perret et al.⁽¹⁰⁾ prospectively evaluated the CBD diameter in 1,018 patients without a history of biliary disease aged 60 to 96 over four years to determine if there was a significant change in CBD with aging (60 years old or less, mean diameter 3.6 mm \pm 0.2 mm, versus over 85 years old, mean diameter 4 mm \pm 0.2 mm, $P=0.009$). Although the common bile duct did increase in size with aging, 98% of all ducts remained below 6 to 7 mm, the commonly accepted upper range of normal.

In a study by Akochi et al.,⁽¹¹⁾ there was also a statistically significant positive correlation between CBD diameter and age of males and females ($r=0.804$ and $r=0.706$ respectively; $P=0.001$) but a mild positive correlation with weight and BMI ($r<0.35$).

In a study by Adibi and Givechian⁽¹²⁾ involving 375 patients (>16 years) with no evident hepatobiliary or

pancreatic disease in abdominopelvic ultrasonography, the mean CBD diameters (proximal and distal) were significantly ($P<0.05$) correlated with age ($r=0.55$ and $r=0.54$, respectively) and BMI ($r=0.25$ and $r=0.27$, respectively).

Worku et al.⁽¹³⁾ performed a cross-sectional study on 206 subjects without any history of hepatobiliary abnormality. The CBD measured at the proximal part just caudal to the porta hepatis. The mean age of the study participants was 39.4 (range 18-87) and the mean diameter of the CBD was 3.64 mm (range 1.8-5.9 mm). The diameter of CBD was significantly associated with age with a linear trend.

The EUS study⁽¹⁴⁾ showed that the CBD dilates significantly after the age of 70 years, but even in most elderly patients, with an intact gallbladder, the normal CBD does not exceed 7.6 mm, thus a wider CBD warrants further investigation.

Conclusion

In Saudi subjects, the mean diameter of the normal CBD was 3.26 ± 0.82 mm, ranging from 1.8 mm to 5.8 mm. There was no statistically significant difference in CBD diameter between male and female subjects. CBD diameter has a slight positive correlation with increasing age and weight. Our study found that the linear regression model partially predicted the value of the CBD diameter with increasing age and weight.

Further research on CBD measurement with a larger population sample and using various body positions is needed for more accurate results.

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Competing Interests

The authors declare that they have no competing interests.

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Tissue Expansion in Reconstructive Surgery: A 10-Year Experience in Kosovo

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Abstract

Background: Tissue expansion (TE) is one of the major developments in reconstructive surgery. The objective of this research was a retrospective analysis of our 10-year experience in correcting burn sequelae, traumas, and scars by the method of TE.

Methods and Results: A retrospective study was conducted at the Clinic of Plastic and Reconstructive Surgery at the University Clinical Center of Kosovo (UCCK, Prishtina) from January 2009 to December 2019. The sample included 67 patients (43[64.2%] females and 24[35.8%] males) treated with tissue expanders for reconstructive purposes. The most common indication for TE was burn sequelae, trauma, and scars from previous surgery. One hundred and thirty-five expanders were placed on 67 patients, and 128 operative interventions were performed. Burn sequelae (55.2%) were the main reason for TE. The age of patients was in the range of 0-50 years (mean age of 20.5 years). The predominant age groups were 11-20 years (49.2%) and 21-30 (40.3%). The most common anatomical region for TE was the head, including the face and neck (47.8%), followed by the trunk (22.4%), the lower extremities (16.4%), and the upper extremities (13.4%). Most patients underwent only one (61.2%) or two surgical interventions (31.3%); three surgical interventions were performed in 7.5% of cases. One expander was placed in the vast majority of our patients (83.6%), two expanders in 13.4% of cases, and three expanders in 3.0%. Patients with expanders on the head and neck experienced more major complications (12.5%), which ended with the removal of the expander, than those with expanders on the trunk (6.7%) and lower extremities (9.1%). Minor complications most often (18.2%) occurred on the lower extremities. These complications were evidenced by pain and transient ischemia, which did not preclude the attainment of reconstructive goals. Patients of 0 to 10 years of age had only minor complications. Occurrences of major complications were the most frequent in the age groups of 11-20 (9.1%), 21-30 (11.1%), and 31-40 (33.3%) years.

Conclusion: The results of our retrospective study on the efficacy of cutaneous expansion procedures are broadly in line with other research reported in the literature. Attention to different aspects of TE, including careful patient selection and meticulous attention to intervention detail, are the main conditions for the success of this reconstructive surgery. (International Journal of Biomedicine. 2023;13(2):269-272.)

Keywords: tissue expansion • complication • burn sequelae • scar

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Introduction

Tissue expansion (TE) is one of the major developments in reconstructive surgery. In 1956, Neumann was the first to recognize the potential of tissue expanders in this type of surgery. He placed a balloon beneath the temporal region

to reconstruct the absent ear. In 1976, Radovan developed the concept of implanted silicone balloons as expanders for breast reconstruction after mastectomy. Subsequently, TE has become a treatment method for many anomalies, as well as congenital and acquired defects in children and adults.⁽¹⁻³⁾

TE uses customized artificial implants in the tissue planes below the skin. Most tissue expanders are designed and made using inflatable balloons or osmotically active hydrogel, and the expansion rate is controlled by fluid entry or various porosities.⁽⁴⁻⁶⁾ The inflation of the implant exerts constant pressure on the skin, causing it to expand. Skin expansion

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allows the surgeon to generate additional amounts of precious tissue, thin the flap, and increase its vascularity.^(7,8)

TE allows surgeons to close the defects using the skin around them, which has the same color, texture, and adnexal structure. This is important in the case of aesthetic face corrections when damaged skin can be replaced with the skin of identical or similar qualities to avoid using the donor skin tissue. Sensitive and hairy skin can be extended and used for specific areas of the head and face to ensure adequate reconstruction that fully meets the texture of the regions. Despite the great benefit of the TE technique, complication rates are 20% to 40% when TE is performed in children.⁽⁹⁾ Patients who have a high risk of complications should always be identified.^(10,11)

The objective of this research was a retrospective analysis of our 10-year experience in correcting burn sequelae, traumas, and scars by the method of TE.

Materials and Methods

A retrospective study was conducted at the Clinic of Plastic and Reconstructive Surgery at the University Clinical Center of Kosovo (UCCCK, Prishtina) from January 2009 to December 2019. The sample included 67 patients (43[64.2%] females and 24[35.8%] males) treated with tissue expanders for reconstructive purposes. The most common indication for TE was burn sequelae, trauma, and scars from previous surgery. The medical records and operative reports of all patients were analyzed for such characteristics as gender, age, anatomical regions, types of expanders, number of expanders applied during an operating session, complications by age group, and anatomical regions.

Results were statistically processed using Microsoft Office Excel. Baseline characteristics were summarized as frequencies and percentages. Group comparisons were performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P < 0.05$ was considered statistically significant.

Results

One hundred and thirty-five expanders were placed on 67 patients, and 128 operative interventions were performed. Table 1 shows that burn sequelae (55.2%) were the main reason for TE.

Table 1.

Indications for TE.

Indications	n	%
Burn sequelae	37	55.2
Trauma	14	20.9
Scar	11	16.4
Other	5	7.5
Total	67	100.0

The age of patients was in the range of 0-50 years (mean age of 20.5 years). The predominant age groups were 11-20 years (49.2%) and 21-30 (40.3%). The age groups of 0-10 years and over 40 years were the smallest (3.0% and 3.0%, respectively) (Table 2). The most common anatomical region for TE was the head, including the face and neck (47.8%), followed by the trunk (22.4%), the lower extremities (16.4%), and the upper extremities (13.4%) (Table 3). The most common type of expander applied to our patients was crescent (43.3% of the cases), followed by round (29.8%) and rectangular (26.9%) (Table 4). Most patients underwent only one (61.2%) or two surgical interventions (31.3%); three surgical interventions were performed in 7.5% of cases. One expander was placed in the vast majority of our patients (83.6%), two expanders in 13.4% of cases, and three expanders in 3.0% (Table 5).

Table 2.

The age groups.

Age (yrs)	n	%
0-10	2	3.0
11-20	33	49.2
21-30	27	40.3
31-40	3	4.5
41-50	2	3.0
Total	67	100.0

Table 3.

Anatomic region for TE.

Anatomical region	n	%
Head and Neck	32	47.8
Trunk	15	22.4
Upper extremities	9	13.4
Lower extremities	11	16.4
Total	67	100.0

Table 4.

Type of expander and number of surgical interventions.

	n	%
Type of expander		
Crescent	29	43.3
Round	20	29.8
Rectangular	18	26.9
Surgical interventions		
1	41	61.2
2	21	31.3
3	5	7.5

Table 5.**Number of expanders used in patients.**

Number of expanders	n	%
1	56	83.6
2	9	13.4
3	2	3.0
Total	67	100.0

Patients with expanders on the head and neck experienced more major complications (12.5%), which ended with the removal of the expander, than those with expanders on the trunk (6.7%) and lower extremities (9.1%) (Table 6). Minor complications most often (18.2%) occurred on the lower extremities. These complications were evidenced by pain and transient ischemia, which did not preclude the attainment of reconstructive goals. Differences between groups were not statistically significant.

Table 6.**Complications according to anatomical regions.**

Anatomical region	n (%)	Major complications n (%)	Minor complications n (%)	Total complications n (%)
Head and neck	32 (47.8)	4 (12.5)	2 (6.2)	6 (18.8)
Trunk	15 (22.4)	1 (6.7)	1 (6.7)	2 (13.3)
Upper extremities	9 (13.4)	1 (11.1)	1 (11.1)	2 (22.2)
Lower extremities	11 (16.4)	1 (9.1)	2 (18.2)	3 (27.3)
Total		7 (10.4)	6 (9.0)	13 (19.4)

Patients of 0 to 10 years of age had only minor complications. Occurrences of major complications were the most frequent in the age groups of 11-20(9.1%), 21-30(11.1%), and 31-40(33.3%) years. No complications were found in ages over 40 (Table 7). Differences between groups were not statistically significant.

Table 7.**Complications and age groups.**

Age group (yrs)	n (%)	Major complications n (%)	Minor complications n (%)	Total complications n (%)
0 – 10	2 (3.0)	0	1 (50)	1 (50)
11 – 20	33 (49.2)	3 (9.1)	3 (9.1)	6 (18.2)
21 – 30	27 (40.3)	3 (11.1)	2 (7.4)	5 (18.5)
31 – 40	3 (4.5)	1 (33.3)	0	1 (33.3)
41 – 50	2 (3.0)	0	0	0

Discussion

TE is one of the major achievements in plastic surgery.⁽¹²⁾ Pediatric and burned populations have been the most studied patients. The present study analyzed a population in the range of 0-50 years (mean age of 20.5 years). The most common indication for TE was burn sequelae, trauma, and scars from previous surgery. These epidemiological factors are similar to those in other series.

In our study, female patients were predominant (64.2%), as in investigations by Almeida et al.⁽¹³⁾ and Nakamoto et al.,⁽¹⁴⁾ perhaps due to a stronger concern about aesthetics. Yeşilada et al.⁽¹⁵⁾ analyzed their clinical experience in the treatment of burn scars and complex defects by TE in 25 patients (14 females and 11 males) with a mean age of 9.26 years.

The anatomical region most involved in our study was the head and neck (47.8%). Cunha et al.⁽⁸⁾ reported that the scalp was the site of TE in 22.8% of cases. In a study by Mohanty et al.,⁽¹⁶⁾ the scalp, face, and neck were involved in 71.7% of cases.

The selection of the shape of the tissue expander is very important. Motamed et al.⁽¹⁷⁾ used rectangular tissue expanders and claimed that using these expanders might increase the options for flap design. The rectangular-shaped expanders were used in 69.6% of cases in a study by Mohanty et al.⁽¹⁶⁾ and 58.82% in a survey by Motamed et al.⁽¹⁷⁾ In contrast, in our study, rectangular-shaped expanders were used only in 26.9% of cases; at the same time, crescent and round expanders were used in 43.3% and 29.8% of cases, respectively.

In our study, the overall, minor, and major complication rates were 19.4%, 9.0%, and 10.4%, respectively, as in other reported series. Expander complication rates in pediatric burn patients range from 9% to 37%.⁽¹⁸⁾ Friedman et al.⁽⁹⁾ showed that major and minor complications each occurred in 9% of children who underwent TE. Between two of our children aged 0-10 years, a minor complication was found in one case, amounting to a complication rate of 50%.

In a study by Tavares Filho et al.,⁽¹⁹⁾ the rate of complications was 24.07%, of which 30.8% were considered to be absolute and 69.2% relative. In a study by Yeşilada et al.,⁽¹⁵⁾ out of 25 patients with TE, minor and major complications occurred in 1(4%) and 5(20%) cases, respectively. In our study, patients with tissue expanders on the head and neck experienced more major complications (12.5%) than those with expanders in other anatomical regions, and this rate was in line with the data of Cunha et al.⁽⁸⁾

In conclusion, the results of our retrospective study on the efficacy of cutaneous expansion procedures are broadly in line with other research reported in the literature. Attention to different aspects of TE, including careful patient selection and meticulous attention to intervention detail, are the main conditions for the success of this reconstructive surgery.

Competing Interests

The authors declare that they have no competing interests.

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Chronic Endometritis in Patients with Failure of IVF

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Abstract

Background: Chronic endometritis (CE) is highly prevalent in patients with unexplained infertility. This study aimed to determine the associations between CE and infertility.

Methods and Results: We performed a prospective study of 197 women with implantation failure and pregnancy loss after undergoing in vitro fertilization (IVF). The endometrium was examined with a hysteroscope, and a sample was taken for biopsy in areas suspected of having inflammation. Immunohistochemistry was performed for CD138 antibodies. Diagnostic criterion was more than 5 plasma cells per 10 high-power fields. Patients who were positive for CE were treated with antibiotics.

Among the 197 patients in our study, 115(58.4%) were positive for CE. Regarding the success of CE treatment and pregnancy after treatment, among the 115 CD138-positive patients, 32 became pregnant without IVF after treatment, while another 43 had successful pregnancies and childbirth with IVF. Thus, with treated CE, a successful pregnancy and childbirth was detected in 65.2% of cases, including spontaneous pregnancies and IVF.

Conclusion: Patients with infertility and those who have pregnancies lost in IVF have a high rate of CE. Failure to treat CE results in infertility and IVF failure. An efficient diagnosis and treatment of CE are important for a successful pregnancy. (International Journal of Biomedicine. 2023;13(2):273-276.)

Keywords: chronic endometritis • CD138 • infertility • IVF

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Introduction

Chronic endometritis (CE) is defined as inflammation of the endometrial mucosa characterized by the presence of leukocytes, plasma cell infiltration in the stroma, edema, and increased stromal density.^(1,2) Patients with this condition are usually asymptomatic but can present with chronic pelvic pain, dyspareunia, abnormal uterine bleeding, or persistent vaginal discharge.⁽³⁾ The presence of endometrial micropolyps in fluid hysteroscopy is significantly associated with endometrial inflammation and can be considered a reliable diagnostic sign for this pathology.^(4,5) In our experience, endometrial micropolyps are always associated with stromal edema,

homogeneous endometrial thickening, or, more frequently, non-homogeneous endometrial thickening and focal or diffuse periglandular hyperemia. These signs are also found in cases of CE.^(4,6)

The prevalence rate of CE is approximately 10%–11%, based on biopsies of patients who underwent hysterectomies because of benign gynecological conditions. Gynecologists and pathologists do not usually pay much clinical attention to CE because of the time-consuming microscopic examinations necessary to diagnose this condition, its mild clinical manifestations, and its benign nature.⁽⁷⁾

The possible relationship of CE with infertility and/or perinatal complications has recently emerged as an area of ongoing research.⁽⁸⁾ However, the definitive diagnosis for CE must be made by histopathology and immunohistochemistry.

Histopathological diagnosis is by the presence of plasma cells in the endometrium with or without accompanying acute

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inflammation and lymphocytes.⁽⁹⁾ Lymphocytes are a normal component of the endometrial stroma, and their number fluctuates with the phase of the cycle.^(10,11) Quantifying CD138+ cells by immunohistochemistry in women with a history of recurrent pregnancy loss is helpful in diagnosing CE and predicting subsequent reproductive outcomes.⁽¹²⁾

This study aimed to determine the associations between CE and infertility.

Materials and Methods

We performed a prospective study of 197 women with implantation failure and pregnancy loss after undergoing in vitro fertilization (IVF). The endometrium was examined with a hysteroscope, and a sample was taken for biopsy in areas suspected of having inflammation (Figure 1). Specimens were fixed with formaldehyde, embedded in paraffin, and subsequently sliced into 4-μm sections and stained with hematoxylin and eosin. Immunohistochemical staining was performed by incubation of endometrial samples with a 1:100 dilution of mouse monoclonal antibodies against CD138 used for immunohistochemical staining. Diagnostic criterion was more than 5 plasma cells per 10 high-power fields (HPFs) (Figure 2). Patients who were positive for CE were treated with antibiotics.

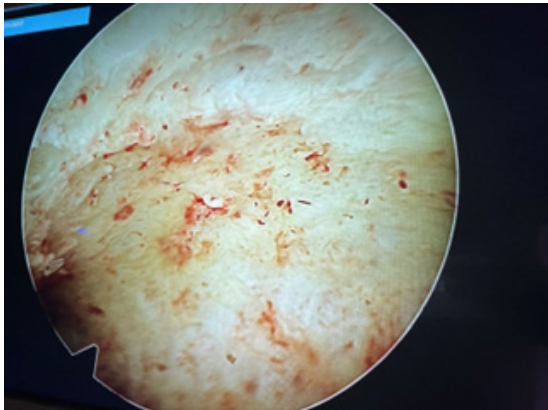


Fig. 1. Endometrial mucosa examined by hysteroscope. Endometrial mucosa appears thick, edematous, and hyperemic.

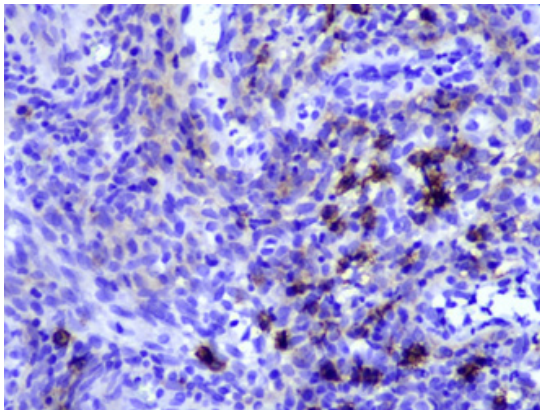


Fig. 2. Immunohistochemical CD138 expression in an endometrial biopsy: Groups of plasma cells stained with CD138 (X200).

Results

Among the 197 patients in our study, 115(58.4%) were positive for CE. When we divided the patients into age groups, the age group of ≤20 years included 3 patients (one with CE and 2 without CE), the age group of 21–30 years included 53 patients (32[60.4%] with CE and 21[39.6%] without CE), the age group of 31–40 years included 110 patients (71(64.5%) with CE and 39(35.5%) without CE), and the age group of >40 years included 31 patients (11(35.5%) with CE and 20(64.5%) without CE). These findings suggested that there was no difference in the incidence of CE between the age groups, except for the >40 group, which had fewer cases of CE than the 21–40 group.

Even after treatment at the second biopsy, among 115 patients who were CD138-positive, 20 had signs of endometritis. These patients were treated again. At the third biopsy, 3 patients still had signs of endometritis, while at the fourth biopsy, one of these patients was resistant to treatment. After treatment, this resistant patient was negative at the fourth biopsy (Table 1). Regarding the success of CE treatment and pregnancy after treatment, among the 115 CD138-positive patients, 32 became pregnant without IVF after treatment, while another 43 had successful pregnancies and childbirth with IVF. Another 40 patients had pregnancy loss even after IVF for other reasons. Thus, with treated CE, a successful pregnancy and childbirth was detected in 65.2% of cases, including spontaneous pregnancies and IVF.

Table 1. Clinical characteristics of the CD138-positive/negative patients and successful pregnancy after treatment among the 115 CD138-positive patients.

Age (years)	Patients (n)	CD138-positive/negative	Positive for endometritis in the second biopsy	CD138-positive patients (n=115)	
				Pregnant without IVF	Pregnant with IVF
<20	3	1/2		1	0
21–30	53	32/21	3	11	14
31–40	110	70/39	13	20	23
>41	31	12/20	4	0	6
All	197	115/82	20	32	43

Discussion

CE is highly prevalent in patients with unexplained infertility. Many studies have suggested a close relationship between CE and reproductive impairment. Untreated CE may contribute to poor pregnancy outcomes, and this problem deserves further investigation in a large cohort.⁽¹³⁻¹⁵⁾ Our study found that the frequency of CE in women with unexplained infertility was higher than in the general population, and more than half of the patients had CE. We did not see a clear difference between the age groups, except for the >40 group, which had fewer cases of CE than the 21–40 group.

Most previous studies have shown that up to 40% of women with infertility are diagnosed with CE.^(13,16) The degree of inflammation of the endometrium in our study was higher than that in other studies, especially in the 21–40 age group. In addition to the treatment of other factors that cause infertility, another reason for this discrepancy between studies may be the determination of hysteroscopic criteria, such as hyperemia, a strawberry aspect as an atypical image of hyperemia, stromal edema, and micropolyps.⁽¹⁷⁻²⁰⁾

Many studies have shown that, for a more accurate diagnosis, immunohistochemical staining should be performed using CD138 antibody, which increases the sensitivity for detection in plasma cells.⁽²¹⁾ Additionally, studies have suggested the number of microscopic fields and plasma cells required to define CE. Five or more plasma cells per 10–30 HPFs were found in most previous studies.⁽²²⁻²⁴⁾ Our study agreed with the indicated data. CE is easy to diagnose even in 10 HPFs. These findings suggest that the presence of a group of plasma cells can be used to diagnose CE, while CE is an important factor in infertility.

In our study, after treating CE with antibiotics and verifying the elimination of plasma cells from the endometrium, a high pregnancy rate was observed. The greatest percentage of success was associated with age. At a younger age, curing endometritis leads to greater success in pregnancy, which suggests that endometritis was the most significant cause of pregnancy loss in these women.

Conclusion

Patients with infertility and those who have pregnancies lost in IVF have a high rate of CE. Failure to treat CE results in infertility and IVF failure. An efficient diagnosis and treatment of CE are important for a successful pregnancy.

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Competing Interests

The authors declare that they have no competing interests.

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State of Regional Blood Flow in Patients with Varying Degrees of Anatomical and Functional Disorders of Periodontal Tissues

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Abstract

Background: The aim of this research was to determine the functional state of regional blood flow in young people with varying degrees of anatomical and functional disorders of periodontal tissues.

Methods and Results: One hundred and thirty-five young patients with varying degrees of anatomical and functional disorders of periodontal tissues (AFDP) and 52 controls with intact periodontium were examined. All patients with AFDP were divided into three groups: Group 1 included 33 patients in the compensation stage (low degree of periodontal risk, intact periodontium, no clinical symptoms, pale pink gums, no bleeding on probing, dentogingival attachment is not disturbed); Group 2 included 38 patients in the sub-compensation stage (average degree of periodontal risk, disorders of the mucogingival complex, without clinical symptoms); and 64 patients in the decompensation stage (high degree of periodontal risk, disorders of the mucogingival complex, the presence of clinical symptoms, individual or combined signs of periodontal pathology, gingival recession (Class I and II according to Miller's classification)). The regional blood flow of periodontal tissues was assessed by an ultrasound Doppler graph in a non-invasive way. The parameters of linear (PSV, peak systolic velocity) and volumetric (Qs, maximum systolic volume velocity) velocities of blood flow, as well as the parameters of the wall vascularization (PI, pulsation index; RI, resistance index). The PSV increased statistically significantly with a load compared to rest in the control and sub-compensation groups and decreased in the decompensation group with a load, compared to the rest condition. The PSV increased relative to the control in the subcompensation and decompensation groups in rest and under load. The Qs indicator showed a similar trend in the groups. Qs significantly increased under load, compared to the rest condition in the control, compensation, and subcompensation groups, and decreased in the decompensation group. The Qs values, in comparison with the control, increased in the subcompensation and decompensation groups in the rest condition and under load. The PI increased with the load relative to the rest condition in the control, compensation, and decompensation group. At the same time, PI decreased under the load, compared to the rest condition, in the subcompensation group. In comparison with the control, the PI values decreased in the subcompensation and decompensation groups in the rest condition and under load. The RI significantly changed only in the subcompensation group under load, compared to the rest condition. Only RI values decreased significantly in the subcompensation group under load, compared to the control.

Conclusion: The velocity characteristics (linear and volumetric velocities) of blood flow are important diagnostic indicators, allowing the most rapid and reliable assessment of the degree of anatomical and functional disorders of periodontal tissues. (International Journal of Biomedicine. 2023;13(2):277-280.)

Keywords: periodontium • regional blood flow • blood flow velocity • resistance index • pulsation index

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Introduction

Inflammatory periodontal diseases constitute an urgent, unsolved problem of modern healthcare due to the continuing high prevalence among the population of different ages.^(1,2) There is a sharp increase in young people with severe destructive and atrophic periodontal diseases, the most common cause of tooth loss in adults.^(3,4) It has been established that the vascular bed of periodontal tissues, which includes a complex of tissues having genetic and functional commonality (periodontium, bone of the alveoli of the jaw, gum with periosteum, and tooth tissue), is represented by small arteries, arterioles, pre-capillaries, and capillaries.⁽⁵⁾ Due to the abundant vascularization of tissues and a thinner vascular surface, this area seems to be a particularly good research object.⁽⁶⁾ It has been established that changes in the functioning of the microcirculatory bed of the periodontium are the most sensitive indicators (predictors), which are the first to react to pathogenic factors even before the appearance of clinical symptoms.⁽⁷⁾ With prolonged overload and constant traumatization of periodontal tissues, the processes of local metabolism in tissues are disrupted, collagen structures are destroyed, and the level of mineralization of bone tissue is significantly reduced, ultimately leading to resorption.⁽⁸⁾

To prevent severe destructive lesions, it is relevant to study local changes in the hemodynamics of periodontal tissues for a further informed and differentiated approach to the treatment and prevention of this pathology.

The aim of this research was to determine the functional state of regional blood flow in young people with varying degrees of anatomical and functional disorders of periodontal tissues.

Materials and Methods

One hundred and thirty-five young patients (49% men and 51% women) (mean age of 30.3 ± 0.5 years) with varying degrees of anatomical and functional disorders of periodontal tissues (AFDP) and 52 controls (mean age of 28.2 ± 0.6 years) with intact periodontium were examined. All patients with AFDP were divided into three groups: Group 1 included 33 patients in the compensation stage (low degree of periodontal risk, intact periodontium, no clinical symptoms, pale pink gums, no bleeding on probing, dentogingival attachment is not disturbed);⁽⁹⁾ Group 2 included 38 patients in the sub-compensation stage (average degree of periodontal risk, disorders of the mucogingival complex, without clinical symptoms); and 64 patients in the decompensation stage (high degree of periodontal risk, disorders of the mucogingival complex, the presence of clinical symptoms, individual or combined signs of periodontal pathology, gingival recession (Class I and II according to Miller's classification)).⁽¹⁰⁾

The study was conducted in the Department of Therapeutic Dentistry at the Altai State Medical University (Barnaul, Russia).

Criteria for inclusion in the groups of patients with AFDP were reproductive age (18-44 years old) and absence of concomitant somatic pathology. Criteria for inclusion in the

controls group were the absence of acute or exacerbation of chronic diseases and pathological changes in the Ent-organs.

Clinical examination of patients was carried out according to the generally accepted method of examination of patients with mucogingival pathology.⁽¹¹⁾ In the control group, a cone-beam computed tomography was performed for a complete diagnosis of the condition of the dental system, according to the results of which no changes in the bone tissue were detected.

The regional blood flow of periodontal tissues was assessed by an ultrasound Doppler graph in a non-invasive way using Minimax-Doppler-K (MM-D-K), model NB ("Minimax SP"), and a sensor with a frequency of 20 MHz. The parameters of linear (PSV, peak systolic velocity) and volumetric (Qs, maximum systolic volume velocity) velocities of blood flow, as well as the parameters of the wall vascularization (PI, pulsation index [the Gosling index]; RI, resistance index [the Pourcelot index]). The indicators were measured at the border between the attached gum and the transitional fold since all the links of the microcirculation system are represented here.⁽¹²⁾ Blood flow parameters were studied in dynamics after functional load (standard tension of the soft tissues of the upper and lower lip and cheeks).

Statistical analysis was performed using STATISTICA 10.0 software package (Stat-Soft Inc, USA). Inter-group comparisons were performed using Student's t-test. A probability value of $P < 0.05$ was considered statistically significant.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee at the Altai State Medical University (Barnaul, Russia). Written informed consent was obtained from all participants.

Results

The PSV increased statistically significantly with a load compared to rest in the control ($P < 0.001$) and sub-compensation groups ($P < 0.001$) and decreased in the decompensation group with a load, compared to the rest condition ($P < 0.001$). The PSV increased relative to the control in the subcompensation and decompensation groups in rest and under load ($P < 0.001$) in both cases) (Figure 1). The Qs indicator showed a similar trend in the groups. Qs significantly increased under load, compared to the rest condition in the control, compensation, and subcompensation groups, and decreased in the decompensation group ($P < 0.001$ in all cases). The Qs values, in comparison with the control, increased in the subcompensation and decompensation groups in the rest condition and under load ($P < 0.001$ in all cases) (Figure 2).

The PI increased with the load relative to the rest condition in the control, compensation, and decompensation group ($P < 0.001$ in all cases). At the same time, PI decreased under the load, compared to the rest condition, in the subcompensation group ($P < 0.001$). In comparison with the control, the PI values decreased in the subcompensation and decompensation groups in the rest condition and under load ($P < 0.001$ in all cases) (Figure 3).

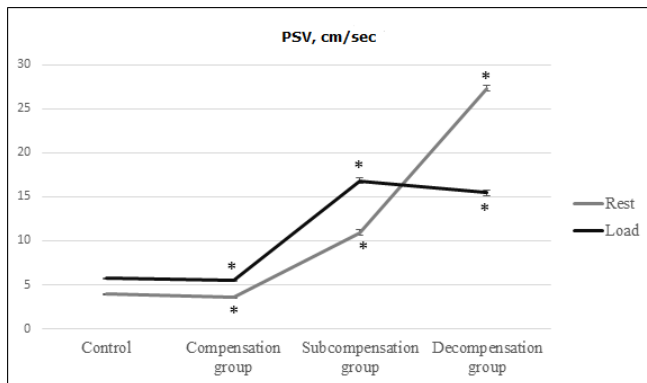


Fig. 1. PSV (cm/sec) level change in patients with different degrees of anatomical and functional disorders of periodontal tissues (* - statistically significant differences with the control group).

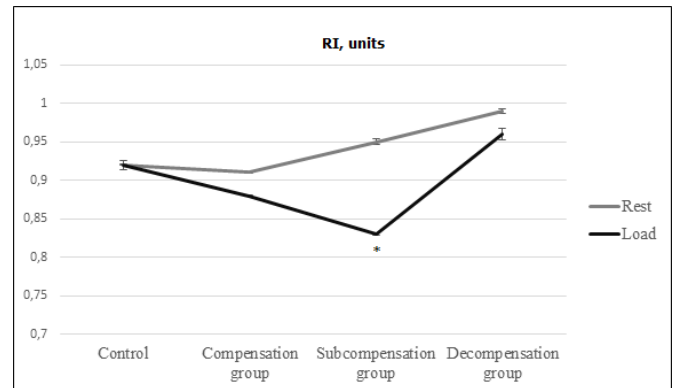


Fig. 4. RI (units) level change in patients with different degrees of anatomical and functional disorders of periodontal tissues (* - statistically significant differences with the control group).

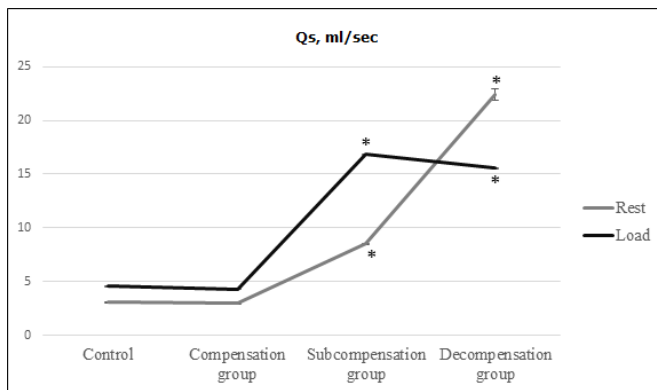


Fig. 2. Qs (ml/sec) level in patients with different degrees of anatomical and functional disorders of periodontal tissues (* - statistically significant differences with the control group).

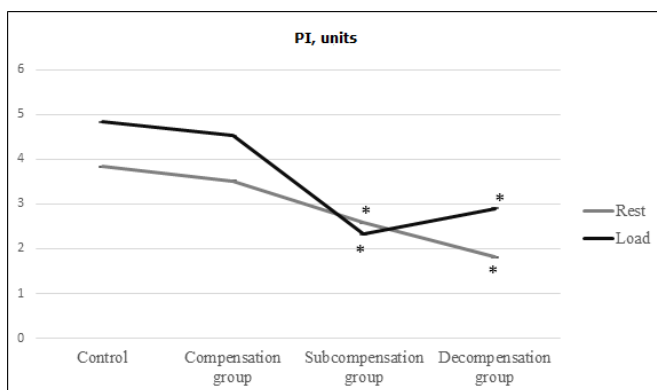


Fig. 3. PI (units) level change in patients with different degrees of anatomical and functional disorders of periodontal tissues (* - statistically significant differences with the control group).

The RI significantly changed only in the subcompensation group under load, compared to the rest condition ($P < 0.001$). Only RI values decreased significantly in the subcompensation group under load, compared to the control ($P < 0.001$) (Figure 4).

Discussion

Since the progression of pathology occurs with functional loads (lip tension and frenulum tension, eating, articulation, chewing), it is relevant to study local hemodynamics not only at rest, but also with functional load. The results showed that the reaction of periodontal tissue vessels both at rest and in a state of functional load with varying degrees of anatomical and functional disorders has its characteristics. The differences consisted of a significant change in the indicators of local hemodynamics in the study area. One of the most important indicators of the state of blood flow is its velocity, which is based on the determination of quantitative parameters of blood flow—linear (PSV) and volumetric (Qs) velocities. RI (resistance index) reflects the resistance to blood flow distal to the measurement site due to vascular occlusion. PI (pulsation index) reflects the elastic—elastic properties of the arteries.⁽⁷⁾ In the control group at rest and under functional load, the indicators' changes were normative. In the patients of the compensation group and subcompensation group (without clinical symptoms), similar changes in PSV and Qs were observed in the upward direction. However, if in the compensation group, the changes in PSV and Qs were close to the indicators of the control group, then in the subcompensation group, the values of PSV and Qs increased by more than two times. It can be said that these changes occur due to the inclusion of local mechanisms of regulation and compensation to maintain the necessary level of circulation in periodontal tissues in conditions of initial disorders.⁽¹³⁾ The decompensation group was distinguished by clinical symptoms of the disease, among which there was a positive symptom of bleeding and a symptom of the departure of the interdental papilla. In this regard, there was a decrease in the PSV velocity along the curve of the maximum velocity and the Qs velocity along the curve of the maximum volumetric velocity during the functional test. A decrease in blood flow rates in patients may be associated with arteriole spasms, stagnation in the microcirculatory bed, and pronounced rheological disorders.⁽¹⁴⁾ We also analyzed the values of the RI and PI in our study. Similar changes

were noted in compensation group patients, as in the control group. However, in the subcompensation group, the PI values decreased significantly at rest and under load. This means that in the study area, the elastic properties of the vessels are less pronounced, which confirms a decrease in compensatory mechanisms to maintain the necessary circulation level in this area. The changes in the RI value were insignificant and were noted only in the subcompensation group under load, which indicated a drop in peripheral resistance during the functional test.

Conclusion

The use of Doppler ultrasonography in assessing the state of regional blood flow of periodontal tissues is a highly informative and non-invasive method. The velocity characteristics (linear and volumetric velocities) of blood flow are important diagnostic indicators, allowing the most rapid and reliable assessment of the degree of anatomical and functional disorders of periodontal tissues.

Competing Interests

The authors declare that they have no competing interests.

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The Occlusal Characteristics of Kosovar Adolescents

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Abstract

Background: This study aimed to evaluate the occlusal characteristics of the maxillary and mandibular dental arches in Kosovar adolescents in relation to gender.

Methods and Results: In a cross-sectional study, dental casts of 400 Kosovar schoolchildren (184 males and 216 females, mean age of 15.17 years) were evaluated. Little's index of incisor irregularity (LII), overjet, and overbite were measured with a digital caliper, and then analyzed and compared by gender. In both the maxilla and mandible, LII for males and females was significantly different, with male values being higher than female values. In contrast, no significant differences were observed in overbite and overjet.

Conclusion: In Kosovar adolescents, our findings show significant gender differences in Little's irregularity index. The overjet and overbite were higher in males than in females, but there were no statistical differences between genders in Kosovar adolescents for these parameters. (*International Journal of Biomedicine*. 2023;13(2):281-285.)

Keywords: adolescents • dental occlusion • Little's irregularity index • gender

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Introduction

Irregularity Incisor Index

Crowding of the anterior teeth is one of the most common signs of malocclusion and is one of the primary reasons for patients seeking orthodontic treatment.⁽¹⁻³⁾ Although crowding alone does not determine the orthodontic treatment needed, it is an important factor to consider.⁽⁴⁾ Generally, it seems agreed that dental crowding is a multifactorial condition that cannot be produced by one specific cause alone. It is nevertheless important that the orthodontic treatment plan addresses as many of the patient's concerns as possible.⁽⁵⁾

There is considerable demand for orthodontic treatment due to aesthetic expectations related to the crowding of the maxillary incisors.⁽⁶⁾ However, their exposure decreases with age, making the mandibular incisors' crowding more visible. This can also affect the appearance of the smile.⁽⁷⁾

On the other hand, according to Buschang, crowding of the lower incisors is common, with up to 40% of the general population having moderate to severe crowding.⁽⁸⁾ An analysis of changes in occlusal characteristics in 27 individuals by Tibana and colleagues found no significant sexual dimorphism.⁽⁹⁾

The literature describes several techniques for quantifying crowding for epidemiological purposes. The irregularity index was proposed by R.M. Little⁽¹⁾ as a viable and consistent quantitative technique for measuring anterior crowding. Using this index, he calculated the rate of initial mal relationship and the outcome of initial crowding post-treatment and post-retention.

Overjet and Overbite

Orthodontists usually measure the overjet and overbite as part of the routine orthodontic diagnosis. Nonetheless, both values need to be assessed accurately, as they indicate the sagittal and vertical relationship between the incisors in the upper and lower jaws.

Some studies have found that the relationship between the upper and lower incisors from a vertical and horizontal perspective varies at different stages of facial development.⁽⁹⁾

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A study of occlusal changes at every stage of a person's growth can be beneficial to orthodontists.⁽¹⁰⁾ According to Tibana et al.,⁽⁹⁾ orthodontists should know how occlusion varies during all growth phases.

The bite structure and position of the teeth in the mouth continually change throughout a person's growth until adulthood, according to Björk.⁽¹¹⁾ Teeth, dental arches, and their relationship, i.e., occlusion, change, as do their shapes and sizes.

A number of longitudinal studies published in the past few years have revealed increases in overjets and overbites between genders during the replacement of dentition.⁽¹¹⁻¹³⁾ A study by Sinclair and Little⁽¹³⁾ revealed that the occlusal characteristics of females changed more severely than those of males. In their longitudinal study of 33 sets of plaster casts of 18 females and 15 males aged 7 to 32, Heikinheimo et al.⁽¹⁴⁾ reported that overjet increased in Finns with normal occlusions between ages 7 and 10. Thereafter, in males and females, there was a continual decrease up to age 32, peaking between 12 and 15. In contrast, they found that among females aged 7 to 32, overbite increased, while it decreased in males. However, in both genders, overbite increased between ages 7 and 12.

In his longitudinal study of overbites in 60 individuals (30 males and 30 females) from ages 8 to 20, Bergersen⁽¹⁵⁾ noted that overbites decreased as the second and the third molars erupted during the teenage years. Furthermore, he found no differences between the overbites of males and females aged 8 to 20.

Among 27 individuals (14 women and 13 men) aged 21 to 28 years, Tibana and colleagues⁽⁹⁾ discovered no changes in the overjet but an increase in the overbite at the completion of the observation time. However, no sexual dimorphism was observed.

Björk⁽¹¹⁾ noted that the variability of the overjet in Swedish boys was higher than the overbite at ages 12 and 20. The overjet usually alters with age, causing the mandible's anterior teeth to migrate forward relative to the maxilla. These typical aging changes are minor. However, differences among individuals, reflected in aging, are relatively high.

Meanwhile, other authors^(11,13,16,17) have studied and described the changes of the bite related to gender and aging in growing individuals, utilizing dental casts or a series of radiographs. In the opinion of Björk,⁽¹¹⁾ the variations in an individual's bite during growth are attributed to dental, facial, and cranial development. Consequently, chewing, breathing, speaking, and mimicking contribute to forming or modifying the bite structure.

To our knowledge, there is no evidence of occlusal characteristics in the Kosovar population. Hence, this study was designed to examine the occlusal characteristics of Kosovar adolescents based on their gender.

Materials and Methods

Seven major cities in Kosovo were studied cross-sectionally. In order to calculate the sample size, we used statistics from the Kosovo Agency of Kosovo. Based on a list of primary and secondary schools, 400 Kosovar adolescents

(184 males and 216 females) aged 13 to 19 years (mean age of 15.17 years) were randomly chosen using a multistage cluster sampling procedure. This study included the following participants: Kosovar nationality, ages 13 to 19, fully erupted permanent teeth, excluding third molars; no orthodontic history; no abrasions, no attrition, no big restorations; no fractured teeth; no abnormal tooth morphology; high-quality study casts. Study approval was obtained from the School of Dental Medicine Ethics Committee at the University of Zagreb (05-PA-30-XXIII-1/2021).

Materials and Methods

For the informed consent to be valid, it had to be signed either by the participant, if over 18 years old, or by a parent, if under 18. To make pre-orthodontic casts, alginate was used to take impressions of the mandible and maxilla and then poured into plaster models. A digital caliper (CD-6 ASX; Mitutoyo Corp., Kanagawa, Japan) with an accuracy of 0.01mm was used to measure 400 dental casts directly.

To assess the irregularity in the frontal upper and lower teeth, we used Little's irregularity index (LII).⁽¹⁾ The degree of anterior irregularity was estimated by summing the incisor and canine linear displacements. Overjet was defined as the distance measured in a parallel direction from the most pronounced position of the maxillary central incisor's incisal edge to the labial surface of the mandibular central incisor.⁽¹⁴⁾ Further, overbite was determined in the vertical plane of the maxillary central incisor's incisal edge, with the labial surface of the mandibular central incisor parallel to the maxillary occlusal surface having the highest overlap.⁽¹⁴⁾

Thirty participants' dental casts were randomly selected for intra-observer reliability, and premeasurement was performed after 24 hours. To calculate any inaccuracy of the method, the formula of Dahlberg was used, which varied from -0.15 mm to 0.19 mm.⁽¹⁸⁾

Statistical analysis was performed using statistical software package SPSS version 25.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For data with normal distribution, inter-group comparisons were performed using Student's t-test. For nonparametric data, the Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P < 0.05$ was considered statistically significant.

Results

The general characteristics of schoolchildren are shown in Table 1. Table 2 summarizes occlusal parameter mean values, standard deviations (SD), and variances (VAR). The results show that males and females differ significantly in the index of maxillary and mandibular LII, with males having higher dimensions than females ($P = 0.009$ and $P = 0.006$, respectively) (Table 3). In contrast, no significant differences were observed in overbite and overjet ($P > 0.05$ in both cases) (Table 3).

Table 1.**Characteristics of the participants (n=400).**

	Gender, n (%)	Mean age, yrs.
Male	184 (46%)	15.30 ± 1.91
Female	216 (54%)	15.05 ± 1.91
Total	400	15.17 ± 1.91

Table 2.**Descriptive statistics of occlusal parameters (n=400).**

	Mean	Minimum	Maximum	SD	VAR
Upper LII	4.52	1.1	13.50	1.77	3.158
Lower LII	2.74	0.7	9.6	1.24	1.543
Overjet	3.23	-3.5	12.0	1.92	3.712
Overbite	3.60	-6.0	7.8	1.70	2.908

Table 3.**Gender distribution of occlusal parameters (n=400).**

	Female (n=216)		Male (n=184)		P-value [^]
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks	
Maxilla LII	186.48	40279.50	216.96	39920.50	0.009
Mandible LII	185.94	40164.00	217.59	40036.00	0.006
Overjet	192.25	41526.50	210.18	38673.50	0.118
Overbite	196.44	42431.00	205.27	37769.00	0.446

[^] - Mann Whitney U-test

Discussion

In our study, males showed higher irregularity indexes of incisors, overjets, and overbites than females, indicating gender differences in Kosovar adolescents. According to Morrison et al., the irregularity index serves as an epidemiological tool because it: a) improves the accuracy of screening examinations conducted in nonclinical settings; b) is easy to perform; c) requires little technical skill; and d) produces rapid results.⁽¹⁹⁾

According to our findings, the mean value of crowding was higher in maxillary incisors than in mandibular incisors (Table 2). This finding is supported by some studies that confirm that the increase in the crowding of upper incisors is more distinct than the increase in lower incisors during this period of human development.^(16,20) Sampson⁽²¹⁾ stated that breathing through the mouth, decay, and removal of teeth are the causes of overcrowding of incisors. However, our study excluded the subjects with extractions, extensive caries, and large fillings, due to the possible effects on occlusion. Moreover, Tibana et al.⁽⁹⁾ stated that mouth breathing, as a

parafunctional oral habit, is known to have severe effects on stomatognathic structures. An assessment of breathing could verify the association. It would be interesting to consider this in future studies, although our methodology did not take it into account.

Our study also found differences in the incisal irregularity index in males and females, with males having higher incisal irregularity than females in both jaws (Table 3). In contrast, Carter and McNamara found that only mandibular LII differed between genders. Males exhibited more LII than females; however, the change in irregularity was the same in both genders.⁽¹⁶⁾ A few studies have reported that mandibular LII increases on average over the course of a lifetime, regardless of orthodontic therapy. As a result, tooth retention, interproximal reductions, or limited orthodontic treatment are needed when incisor alignment is required long-term.⁽²²⁾ In contrast, Buschang et al.⁽²³⁾ showed that adult females with Class II malocclusions had more maxillary incisor irregularities and fewer mandibular incisor irregularities than those with Class I malocclusion. Meanwhile, Ghaib et al.⁽²⁴⁾ found that male subjects with a Class II malocclusion had more upper LII, while female subjects with a Class I malocclusion showed a higher prevalence of crowded mandibular incisors.

Overjet values in our study differed between males and females, with males having average values higher than females but without statistical significance ($P>0.05$) (Table 3). This is in contrast with the findings of Lara-Carillo et al.⁽²⁵⁾ in the Mexican population, which reported that males had greater overjet than females in both Mazahua and Mestizo adolescents. In the most recent study, Olliver et al.⁽²⁶⁾ concluded in a review cohort study that overjet was about 0.5 mm higher, and overbite was about 0.5 mm lower in middle age than in adolescence. Regarding gender differences, females had higher overjet and overbite at age 45 than males. Several previous studies found no significant differences between men and women. In their study, Staley et al.⁽²⁷⁾ found that the genders had similar dimensions and did not differ significantly. In 1998, Carter and McNamara reported in their longitudinal study, which consisted of 53 subjects' dental casts, that untreated males had an overjet decrease of 0.6 mm between 13.8 and 17.2 years of age, whereas no difference was found in females. Furthermore, the overjet in the UMGS sample aged 17 to 48 did not change by sex.⁽¹⁶⁾ When Bishara et al.⁽²⁸⁾ studied individuals aged 5 to 15, they reported minimal changes in overjet. In a later study with a group aged 25 to 45, Bishara et al.⁽²⁰⁾ corroborated the same findings. Further, Akgül and Toygar⁽²⁹⁾ analyzed 14 females and 16 males over a period of 22 to 32 years. They reported that the overjet did not show significant changes in either males or females. More recently, Stern et al.⁽³⁰⁾ found no statistical differences in overjet between men and women from birth until 26 years of age, in their longitudinal study on untreated German children with normal occlusion.

The present study found no significant differences between males and females with respect to overbite ($P>0.05$) (Table 3). Several previous studies found similar results, such as Carter and McNamara,⁽¹⁶⁾ who found no difference in overbite between males and females, and Bauerle,⁽³¹⁾ who found no significant sex differences at any age. Fleming also

found no differences in the extent of overbite in males and females for Class I malocclusions, although the mean values for females tended to be slightly higher.⁽³²⁾ More recently, Stern et al.⁽³⁰⁾ found no statistical differences in overbite between males and females from birth to age 26 in their longitudinal study of untreated German children with normal occlusion. In contrast, in the population of Mexico, Lara-Carillo et al.⁽²⁵⁾ reported that males had a greater overbite than females. On the other hand, Akgül and Toygar⁽²⁹⁾ found that the overbite increased significantly in females. However, according to Bergersen,⁽¹⁵⁾ there is a consensus that overbite usually increases during mixed dentition and decreases during the teenage years. Researchers also agree that the rise in overbite may be caused by the increase in cuspid arch width, which typically occurs when the permanent maxillary incisors and canines erupt in the mouth.^(12,33,34) Furthermore, the differences in overbite depth between men and women are insignificant.^(31,32,35) Harris and Smith⁽³⁶⁾ revealed that, whereas genetic variation affects arch width and arch length to a substantial degree, more than genetic variability among families, environmental factors influence occlusal variables, including overjet, overbite, molar relationship, crowding, and rotations.

We are aware that our study has some limits. First, the sample was not equally distributed among the malocclusion classes. Second, the dental measurements were performed in 2D rather than using a 3D method.

While this study has some limitations, it also has some strengths. It was the first of its kind in our country and used a representative sample.

In conclusion, in Kosovar adolescents, our findings show significant gender differences in Little's irregularity index. These results provide basic information about occlusal characteristics in the young Kosovar population. In Kosovar orthodontics, they have significant clinical implications, particularly for improving diagnosis and treatment planning.

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Competing Interests

The authors declare that they have no competing interests.

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Does Socioeconomic Status Affect Mental Well-being Among Children with Asthma in Kosovo?

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Abstract

The aim of this study was to investigate the relationship between mental well-being – as assessed by the Strengths and Difficulties Questionnaire (SDQ) among children with asthma in Kosovo and socioeconomic status (SES).

Methods and Results: For this cross-sectional observational study, data were collected from five regions of Kosovo, public and private institutions, public hospitals and primary healthcare facilities in each area, a tertiary level hospital, and two private immunology clinics in the capital city. The survey included 161 Kosovar children (mean age of 11.1±2.7 years) with asthma, aged 7-16 years, and their caregivers. The survey questionnaires used were those of the American Academy of Pediatrics: The Children's Health Survey for Asthma (CHSA), the parent and child version (CHSA-C), and the Strengths and Difficulties Questionnaire (SDQ). SDQ showed conspicuous (borderline or abnormal) results in 25.2% of children. None of the scales of the SDQ, except prosocial behavior, showed statistically significant differences across SES categories. Prosocial behavior scores increased significantly with increasing SES.

Conclusion: Children with asthma from lower SES families in Kosovo have more social contact problems but do not show reduced mental well-being nor more conduct problems. (International Journal of Biomedicine. 2023;13(2):286-291.)

Keywords: children • asthma • strengths and difficulties • socioeconomic status • Kosovo

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Abbreviations

SDQ, the Strengths and Difficulties Questionnaire; **CHSA**, the Children's Health Survey for Asthma; **CHSA-C**, the CHSA-Child Version; **SES**, socioeconomic status; **TDS**, Total Difficulties Score.

Introduction

Asthma, the most common chronic condition in childhood, is a major public health issue for school-age children and children in general.⁽¹⁾ Asthma affects 14% of the world's children.⁽²⁾ In recent years, the prevalence of asthma symptoms increased in children and adolescents globally, with a consistent burden on health systems.⁽³⁾ Social determinants of

health, such as housing and access to care, significantly impact the health of children with asthma.⁽⁴⁾ In particular, children living in low socioeconomic communities are vulnerable to poor health outcomes.⁽⁵⁾

Poverty, unequal distribution of wealth, and unequal access to information, education, and healthcare services are important factors that impact life expectancy, morbidity, and mortality. Therefore, socioeconomic status (SES) is one

of the important determinants of morbidity and mortality.⁽²⁾ A wealth of evidence demonstrates that health problems are more prevalent in low SES patients.⁽⁶⁻⁸⁾

A link between asthma, stress, and psychiatric illnesses has been documented.⁽⁹⁾ The the Strengths and Difficulties Questionnaire (SDQ), as applied in this study, is a cost-effective instrument for detecting deviant behavior and psychosocial problems in children and adolescents, particularly in primary health care, which helps in decisions for referral to secondary healthcare facilities.^(10,11) It is especially appropriate for detecting behavioral disorders in countries lacking skilled experts in this area.⁽¹²⁾

The self-reporting SDQ is dedicated to youths aged 11 years and above, but it can also be used in children as young as 8.⁽¹³⁾ Several studies have shown a relationship between asthma and mental health.⁽¹⁴⁻¹⁷⁾

Family functioning is an essential predictor of health-related quality of life in asthmatic children.⁽¹⁸⁾ Family cohesion has been shown to be an important protective factor for children with asthma.⁽¹⁹⁾ Besides family structure, family well-being impacts a child's asthma.

The aim of this study was to investigate the relationship between mental well-being – as assessed by the SDQ among children with asthma in Kosovo and SES.

Materials and Methods

Study Design

For this cross-sectional observational study, data were collected from five regions of Kosovo, public and private institutions, public hospitals and primary healthcare facilities in each area, a tertiary level hospital, and two private immunology clinics in the capital city. Questionnaires were administered by trained personnel.

Although physician-diagnosed asthma was the first inclusion criterion, for selection, we additionally applied international criteria. For asthma definitions, we used: (1) wheezing in the past 12 months; (2) wheezing and waking up with breathlessness or breathlessness at rest in the past 12 months; (3) diagnosed asthma; (4) asthma severity based on GINA classification.

Patients

The survey included 161 Kosovar children with asthma, aged 7-16 years, and their caregivers. Participants were enrolled on a consecutive basis.

Enrolled children and parents were provided with the questionnaires. Data were collected over five months in the selected centers until the projected sample size was achieved. The research was completed in 2014. Parents and children over 16 years of age signed an informed consent. Children and adolescents between 10 and 16 completed the questionnaire by themselves. Children younger than 10 years were interviewed by trained medical personnel.

Inclusion criteria were age 7-16 years, physician-diagnosed asthma, and living in Kosovo for the past year. This study also included three subjects slightly outside these criteria, one aged 6 years and two subjects aged 17 years. Exclusion criteria were other chronic diseases in the child or

severe acute diseases (except asthma exacerbations) in the past two weeks.

Measurements

The survey questionnaires used were those of the American Academy of Pediatrics: CHSA, the parent and child version (CHSA-C), and SDQ.

CHSA – parent version

CHSA is an asthma-specific, health status, self-report instrument completed by parents of children with chronic asthma aged 5-16 years. It consists of 46 core items, computed for five asthma-specific domains for assessing a child's physical health (12 items), child activity (4 items), child and family emotional health (7 items); it also covers additional items about healthcare utilization, asthma triggers, and family demographics (23 items). Scores were calculated for each scale (scaled to 0-100), with a higher score indicating better health.

The questionnaire was translated into Albanian and back-translated by two physicians. Additional questions were included to cover environmental conditions in the area of living. There are different questionnaire versions available concerning the length of recall. We used the version for the two-week recall period.

CHSA-C – child version

The CHSA-C was adapted from the CHSA parent version with numerous child-friendly modifications.⁽¹⁸⁾ The resulting instrument is an interviewer-administered (children aged 7-16 years) or self-completed (children aged 10-16 years) instrument for children with asthma. CHSA-C consists of 21 core items covering asthma-specific domains: child physical health (7 items), child activities (3 items), and child emotional health (2 items); additional items cover healthcare utilization, asthma triggers, and child demographics (9 items).

Children's Strengths and Difficulties Questionnaire

The SDQ used was self-rated by children and covered five topics or scales comprising 25 items, divided into prosocial, hyperactivity, emotional symptoms, and conduct problems.^(19,20) Each question can be answered by one of three categories (Not True, Somewhat True, and Certainly True) that are coded as 0, 1, or 2. The sum of the codes gives a scale score, which is categorized into three categories: normal, borderline, and abnormal. A Total Difficulties Score (TDS) can be calculated by aggregating the scores for the emotional symptoms, conduct problems, hyperactivity-inattention, and peer problems subscales (range 0–40). For each scale, the score can reach 10 points, and TDS can earn 40 points.

Socioeconomic status

SES assignment was based on the mother's education, size of the flat (sqm per inhabitant), and living standard. These variables were categorized into three levels. The average of these three indicators defined the SES as follows: less than 2 was designated "low SES," 2 to 2.3 as "lower average," and above 2.3 as upper average. This assignment by tertiles was chosen since high SES is defined by the college/university education of the mother, 40 or more sqm flat space, and an excellent living standard was present in only three participants.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY:

IBM Corp) and Statistica version 10.0 (StatSoft Inc., USA). Comparisons between groups were made for illustrative purposes by categorizing the different scales; however, statistical comparisons were performed using the scores themselves. SDQ scales were analyzed by General Linear Models with SES as the main independent variable corrected for age, gender, family size, and asthma severity. All data obtained were processed without imputation for missing data.

Ethical approval for this study was obtained from the Medical Faculty Ethics Committee, at University of Prishtina, Kosovo. Written informed consent was obtained from each research participant (or the participant’s parent/guardian).

Results

Overall, 161 children (mean age of 11.1±2.7 years) with asthma were included in the study: 99(61.5%) males and 62(38.5%) females. Most children were from urban areas (101[62.7%]), with an average of seven family members living together (Table 1).

Table 1.

Overview of demographic data for the total sample of children with asthma

Variable	Category	n	%
Sex	Total	161	100.0
	Female	62	38.5
	Male	99	61.5
Age (years)	7-10	73	45.3
	11-14	64	39.8
	15+	24	14.9
	mean ± SD	11.1±2.7	
Residence	City	101	62.7
	Village	60	37.3
Marital status of the child’s parents or guardians	Married	157	97.5
	Divorced	2	1.2
	Widowed	2	1.2
Parent’s employment	Yes	93	57.8
	No	68	42.2
Family members in the household	0-4	32	19.9
	5-9	109	67.7
	10-14	16	9.9
	15+	4	2.5
	mean ± SD	6.7±3.1	
Living standard	Low	22	13.7
	Average	76	47.2
	Good	53	32.9
	Very good	10	6.2

Family structure was a joint household with married parents in 157(97.5%). Only 93(57.8%) interviewed parents were employed. According to the classification of the living standard, almost half of the respondents had an average living standard (76[47.2%]), and 53(32.9%) respondents had a good living standard (Table 1).

SDQ showed conspicuous (borderline or abnormal) results in 25.2% of children. Among participants, 21(13.2%) children with asthma had abnormal behavioral characteristics, and 19(11.9%) were classified as borderline. Emotional symptoms were categorized as abnormal in 10.1% and borderline in 6.9%; conduct problems in 11.9% as abnormal and 14.5% as borderline; hyperactivity in 5.7% as abnormal and 11.3% as borderline; peer problems in 10.1% as abnormal and 18.2% as borderline; and prosocial behavior was classified as abnormal and borderline in 3.1% each (Table 2).

Table 2.

Categorized scores of the subscales and total scale of SDQ in relation to SES.

Scale	Category	L-SES	LA-SES	UA-SES	Total
Conduct Problems	Normal	36(70.6%)	39(73.6%)	42(76.4%)	117(73.6%)
	Borderline	6(11.8%)	9(17.0%)	8(14.5%)	23(14.5%)
	Abnormal	9(17.6%)	5(9.4%)	5(9.1%)	19(11.9%)
Emotional Symptoms	Normal	40(78.4%)	43(81.1%)	49(89.1%)	132(83.0%)
	Borderline	2(3.9%)	6(11.3%)	3(5.5%)	11(6.9%)
	Abnormal	9(17.6%)	4(7.5%)	3(5.5%)	16(10.1%)
Hyperactivity	Normal	40(78.4%)	47(88.7%)	45(81.8%)	132(83.0%)
	Borderline	8(15.7%)	4(7.5%)	6(10.9%)	18(11.3%)
	Abnormal	3(5.9%)	2(3.8%)	4(7.3%)	9(5.7%)
Peer Problems	Normal	35(68.6%)	36(67.9%)	43(78.2%)	114(71.7%)
	Borderline	10(19.6%)	12(22.6%)	7(12.7%)	29(18.2%)
	Abnormal	6(11.8%)	5(9.4%)	5(9.1%)	16(10.1%)
Prosocial Behavior	Normal	47(92.2%)	48(90.6%)	54(98.2%)	149(93.7%)
	Borderline	2(3.9%)	2(3.8%)	1(1.8%)	5(3.1%)
	Abnormal	2(3.9%)	3(5.7%)	0(0.0%)	5(3.1%)
Total	Normal	33(64.7%)	42(79.2%)	44(80.0%)	119(74.8%)
	Borderline	7(13.7%)	6(11.3%)	6(10.9%)	19(11.9%)
	Abnormal	11(21.6%)	5(9.4%)	5(9.1%)	21(13.2%)

L-SES - low SES; LA-SES, lower average SES; UA-SES - upper average SES

None of the scales of the SDQ, except prosocial behavior, showed statistically significant differences across SES categories (Table 3). Prosocial behavior scores increased significantly with increasing SES.

Table 3.

Adjusted means and 95% CI by categories of SES for subscales of SDQ (range 0-10) and the total score (range 0-40).*

Subscale	L-SES		LA-SES		UA-SES	P (overall)
	mean (95% CI)	P vs. UA-SES	mean (95% CI)	P vs. UA-SES	mean (95% CI)	
Conduct Problems	2.81 (2.30-3.32)	0.256	2.47 (2.00-2.93)	0.821	2.39 (1.91-2.87)	0.484
Emotional Problems	3.67 (3.10-4.24)	0.375	3.67 (3.14-4.19)	0.326	3.30 (2.76-3.84)	0.557
Hyperactivity	3.63 (3.04-4.23)	0.642	3.30 (2.76-3.84)	0.738	3.43 (2.87-3.99)	0.721
Peer Problems	2.70 (2.12-3.28)	0.921	2.75 (2.22-3.28)	0.796	2.65 (2.10-3.20)	0.966
Prosocial Behavior	8.68 (8.20-9.15)	0.063	8.67 (8.23-9.10)	0.037	9.32 (8.87-9.77)	0.043
Total	12.81 (11.03-14.59)	0.424	12.19 (10.56-13.81)	0.723	11.77 (10.09-13.45)	0.725

* Adjusted for asthma severity, gender, age and family size; L-SES - low SES; LA-SES, lower average SES; UA-SES - upper average SES; P-values overall and for comparisons against upper average (UA) SES (Bonferroni corrected) from General Linear Model analysis.

Discussion

SES is associated with environmental and health inequalities, and environmental injustice is shown to be correlated with health outcomes and quality of life, especially in children, as a more vulnerable population group.⁽²⁰⁾ Low SES is often a barrier to healthcare utilization and medications, especially for asthma, when multiple and prolonged medications are needed.⁽²¹⁾ Children with asthma living in urban environments are at risk for experiencing problems like anxiety, withdrawal, depression, and difficulties at school. Psychological stress increases the risk of asthma episodes. Acute stresses, chronic family stress, and a combination of both increase the production of asthma-related cytokines, IL-4, IL-5, and IFN- γ , which explains the impact of negative life events on exacerbating asthma.⁽²²⁾ In our study, mental health among children with asthma assessed by the SDQ showed conspicuous results (borderline or abnormal) in 25% of children, a result similar to that from other studies conducted in Europe. For most SDQ scores, differences between France and the UK were smaller than those between France and the US.⁽²³⁾ The distributions of SDQ scores are very similar across Nordic countries.⁽²⁴⁾ SDQ findings in five countries of Southern Europe: Italy, Spain, Portugal, and Croatia, show many similarities, but according to their teachers' ratings, Italian pupils were classified as exhibiting less prosocial behavior than their Spanish and Portuguese age-mates, whereas the Portuguese children were rated as being more hyperactive and inattentive than comparable Italian and Spanish children.⁽²⁵⁾

SDQ scale scores showed no statistically significant difference except for prosocial behavior between SES groups. In several studies, findings indicate an association between SES and children's mental health. Lower SES increases the risk of unmet needs for services. Parents of children with psychological difficulties were less likely to seek a

consultation if household income was low.⁽²⁶⁾ In the German Health Interview and Examination Survey for Children and Adolescents - KIGGS, 20.6% were classified as abnormal in the SDQ, with low SES increasing the risk 2.6-fold.⁽²⁷⁾ In Greece, adolescents with low SES reported more difficulties than those with medium and high SES.⁽²⁸⁾ Also, in Slovenia, adolescents with a lower socioeconomic position were reported to show poorer mental health than those with a higher SES.⁽⁷⁾ Low SES consistently predicted mental health problems similarly in children and adolescents, with low SES increasing the risk for psychiatric problems and violent behaviors.^(29,30) While these results were obtained from the general population, chronic diseases such as asthma should make problems due to limited resources of families that are strongly related to SES more apparent. Hence it was expected that SES would show marked differences concerning mental health as assessed by the SDQ. The negative results could have a number of reasons: first, the population investigated did not show pronounced differences in SES, with the highest-level present in only three participants; second, a child in the family suffering from a chronic disease might increase family cohesion, thus compensating at least in part for lack of financial resources; third, in a country like Kosovo, differences in SES are of a less pronounced impact on access to health care. These reasons could, at least in part, explain the lack of a strong association between SES and mental health. That prosocial behavior was the only area related to SES is telling insofar as a stigmatizing effect of a disease like asthma may be associated with the social community in which the child develops.

Conclusion

Children with asthma from lower SES families in Kosovo have more social contact problems but do not show reduced mental well-being nor more conduct problems.

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Competing Interests

The authors declare that they have no competing interests.

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The Level of Lipid Peroxidation Products and Medium-Molecular-Weight Peptides in Adolescents with Obesity

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Abstract

Background: The aim of this research was to study the plasma content of lipid peroxidation products and MMWP in obese adolescents.

Materials and Results: The studies were conducted on 19 adolescent girls and 18 adolescent boys with an established diagnosis of exogenous constitutional obesity of the first degree. Twenty-four adolescent girls and 20 adolescent boys made up control groups. All adolescents were subjected to general clinical examination, including anamnestic data collection, physical examination, anthropometric data analysis, and nutritional status assessment. The content of primary, secondary, and final lipid peroxidation (LPO) products was evaluated, as well as medium-molecular-weight peptides (MMWP) absorbing at wavelengths 238, 254, and 280 nm by the spectrophotometric method. The group of obese adolescent girls, compared to the control, showed lower values of secondary LPO (thiobarbituric acid reactants) ($P=0.022$) and elevated levels of MMWP-238 ($P<0.0001$) and MMWP-280 ($P=0.03$). The group of obese adolescent boys, compared to the control, showed higher values of secondary LPO products (ketodienes and conjugated trienes) ($P=0.042$) and elevated levels of MMWP-238 ($P=0.03$).

Conclusion: The obtained data demonstrate the presence of activation of lipid peroxidation processes at the stage of secondary products in adolescent boys and endogenous intoxication in obese adolescents, regardless of gender. The need to monitor and correct these indicators in adolescent patients with obesity should be an important component of pathogenetic treatment. (International Journal of Biomedicine. 2023;13(2):292-295.)

Keywords: lipid peroxidation • medium-molecular peptides • adolescents • obesity

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Abbreviations

BMI, body mass index; **CD**, conjugated dienes; **KD-CT**, ketodienes and conjugated trienes; **LPO**, lipid peroxidation; **MMWP**, medium-molecular-weight peptides; **OS**, oxidative stress; **ROS**, reactive oxygen species; **SB**, Schiff bases; **TBARS**, thiobarbituric acid reactants.

Introduction

The study of the pathogenetic mechanisms of the formation of childhood obesity is becoming increasingly important due to the high prevalence and risk of developing numerous complications in adulthood.⁽¹⁾ The most common type of obesity is the exogenous-constitutional form, manifested by an imbalance between calories consumed

and expended.⁽³⁾ It has been established that obesity is associated with chronic inflammation of adipose tissue, activation of pro-inflammatory factors, dyslipidemia, the development of oxidative stress (OS), and other elements.⁽⁴⁻⁶⁾ The progression of OS is accompanied by the formation and accumulation of cytotoxic compounds, which act as mediators of damage and provoke characteristic metabolic shifts.⁽⁷⁻⁹⁾ At the same time, lipid peroxidation (LPO) processes are

activated in the biological membranes of cells, against which endogenous intoxication of the body develops.⁽¹⁰⁻¹²⁾ In this case, the damaging agents are unbalanced biologically active substances circulating in the blood and acquiring the properties of endogenous toxins.⁽¹³⁾ The level of medium-molecular-weight peptides (MMWP) is recognized as a universal biomarker of endogenous intoxication.⁽¹⁴⁾ The composition of the MMWP includes various combinations of regulatory peptides, including peptide hormones and their fragments and non-regulatory oligopeptides.⁽¹⁵⁾ Determination of the level of MMWP in biological fluids with a molecular weight of 300-5000 D makes it possible to characterize the severity of intoxication during the development of the pathological process.^(14,15)

The aim of this research was to study the plasma content of lipid peroxidation products and MMWP in obese adolescents.

Materials and Methods

The studies were conducted on 19 adolescent girls (mean age – 14.46±2.3 years) and 18 adolescent boys (mean age – 13.2±2.2 years old) with an established diagnosis of exogenous constitutional obesity of the first degree. Twenty-four adolescent girls (mean age – 13.76±1.26 years) and 20 adolescent boys (mean age – 13.89±1.41 years) made up control groups.

Criteria for inclusion in groups with exogenous-constitutional obesity of the 1st degree were excess body weight of more than the 95th percentile for a certain gender, height, and age; exclusion of acute or exacerbation of chronic diseases at the beginning of the examination or one month before it; permanent residence of a teenager on the territory of this municipality; signing by parents or legal representatives, as well as adolescents over 15 years of age informed consent to be included in the study. Height, body weight, and waist circumference were measured, body mass index (BMI) (kg/m²) was calculated, and the puberty stage, according to Tanner, was determined. Overweight was considered at a BMI >85th percentile for a given gender and age, and obesity - at a BMI >95th percentile.⁽¹⁶⁾ Exclusion criteria from the group: physical development delay, body weight deficiency, genetic and symptomatic forms of obesity, taking medications that potentially affect body weight, and the estimated biochemical characteristics.

All adolescents were subjected to general clinical examination, including anamnestic data collection, physical examination, anthropometric data (measurement of body weight, height, determination of BMI) analysis, nutritional status assessment, and determination of the concentration of total cholesterol and triglycerides in blood serum, glucose tolerance testing.

Biochemical measurements

Blood plasma was used as the material for the study. Plasma concentrations of primary/secondary/final products of LPO (conjugated dienes [CDs]/ketodienes and conjugated trienes [KD-CT]/Schiff bases [SB]) were estimated by I.A. Volchegorsky method.⁽¹⁷⁾ TBARs (secondary LPO products)

content was detected by fluorometry according to V.B. Gavrilov et al.⁽¹⁸⁾

The content of primary, secondary, and final LPO products was evaluated, as well as MMWP absorbing at wavelengths 238, 254, and 280 nm by the spectrophotometric method.

The MMP values (MMP 238, MMP 254, MMP 280) were evaluated by the spectrophotometric method.⁽¹⁹⁾ Measurements were carried out on a spectrophotometer SF-2000 (Russia) and BTS350 Analyzer (BioSystems, Spain).

Statistical analysis was performed using STATISTICA 10.0 software package (Stat-Soft Inc, USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. The F-test for equality of two variances was applied. For descriptive analysis, results are presented as median (Me), interquartile range (IQR; 25th to 75th percentiles). Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. A probability value of $P \leq 0.05$ was considered statistically significant.

The study was carried out in accordance with the Helsinki Declaration of the World Medical Association (1964, ed. 2013) and approved by the Committee on Biomedical Ethics at the Scientific Center for Family Health and Human Reproduction (Extract from the meeting No. 5 as of 16.05.2016).

Results and Discussion

The group of obese adolescent girls, compared to the control, showed lower values of secondary LPO (TBARs) ($P=0.022$) and elevated levels of MMWP-238 ($P<0.0001$) and MMWP-280 ($P=0.03$) (Table 1). The group of obese adolescent boys, compared to the control, showed higher values of secondary LPO products (KD and CT) ($P=0.042$) and elevated levels of MMWP-238 ($P=0.03$) (Table 1).

Table 1.

Parameters of LPO products and MMWP in adolescents with obesity (Me (25%;75% quartiles)).

Parameters	Girls		Boys		Statistics
	Control (1)	Obesity (2)	Control (3)	Obesity (4)	
CDs, $\mu\text{mol/L}$	0.86 [0.64;1.08]	0.98 [0.82;1.15]	0.96 [0.8;1.45]	0.89 [0.8;1.11]	
KD and CT, units	0.32 [0.13;0.60]	0.40 [0.30;0.56]	0.46 [0.23;0.66]	0.58 [0.24;0.48]	$P_{3-4}=0.042$
TBARs, $\mu\text{mol/L}$	1.54 [1.28;2.15]	1.23 [0.82;1.44]	1.44 [1.28;1.69]	1.18 [0.92;1.44]	$P_{1-2}=0.022$
SB, $\mu\text{mol/L}$	0.04 [0.03;0.04]	0.04 [0.03;0.05]	0.04 [0.03;0.05]	0.04 [0.03;0.05]	
MMWP 238, units	0.09 [0.05;0.15]	0.26 [0.22;0.28]	0.01 [0.08;0.02]	0.25 [0.10;0.28]	$P_{1-2}<0.0001$ $P_{3-4}=0.03$
MMWP 254, units	0.16 [0.12;0.18]	0.20 [0.14;0.23]	0.15 [0.14;0.20]	0.20 [0.17;0.23]	
MMWP 280, units	0.26 [0.24;0.28]	0.35 [0.25;0.38]	0.28 [0.22;0.34]	0.37 [0.30;0.38]	$P_{1-2}=0.03$

OS is an important component of the pathogenesis of obesity and its possible complications.^(20,21) Preclinical studies in vitro and in vivo have shown a stimulating effect of OS on the proliferation and differentiation of preadipocytes, as well as an increase in the size of adipocytes.⁽²²⁾ It is known that in a healthy body, reactive oxygen species (ROS) are involved in the activation of hypothalamic neurons involved in the regulation of eating behavior. In conditions of obesity, due to increased oxidative processes, the production of ROS increases, the hunger center is activated, the depot of white adipose tissue increases, and appetite increases.⁽²³⁾ In addition, the following factors associated with obesity that stimulate OS reactions can be distinguished: hyperglycemia, elevated lipid levels, bioelement deficiency, chronic inflammation, hyperleptinemia, increased activity of muscle tissue to maintain excess body weight in obesity, endothelial dysfunction, impaired respiratory function of mitochondria, etc.^(24,25) Metabolic disorders in obesity include developing insulin resistance and hyperglycemia. Intracellular increase in glucose levels leads to activation of glycolysis and the tricarboxylic acid cycle, which leads to hyperproduction of oxidized forms of dehydrogenase coenzymes — nicotinamide adenine nucleotide phosphate (NADP) and flavin adenine dinucleotide (FAD), disruption of the mitochondrial respiratory chain and, as a consequence, hyperproduction of ROS.⁽⁴⁾ Glucose autooxidation products serve as additional sources of hydroxyl radical and superoxide-anion radical production.⁽²⁶⁾ Obesity is accompanied by an increase in the level of free fatty acids in the blood, which stimulates the production of superoxide-anion radicals.⁽⁴⁾

LPO products formed at various stages of the chain process serve as significant markers of OS in the body.⁽²⁷⁾ We found higher values of secondary lipid peroxidation products (KD and CT) in obese adolescent boys. These products can damage the cell's structural components by induction of apoptotic and mutational processes, inhibition of DNA synthesis, proliferation, etc.⁽⁴⁾ A significant contribution to the accumulation of lipid peroxidation products can be made by the deficiency of vitamins and minerals observed in obesity. In addition, in obese patients, there is a decrease in the activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase) and a decrease in the overall antioxidant status of blood plasma, as well as a positive relationship between the level of these OS markers and BMI.^(28,29) There is a close relationship between the accumulation of LPO products and the development of endogenous intoxication in obesity, which is also noted in our study.

MMWPs belong to the products of cellular disorganization, incomplete decomposition, and non-enzymatic transformation of proteins, the so-called fragments of endogenous proteins. The main source of MMWP formation is non-enzymatic proteolysis, including blood proteins (fibrinogen, albumin, thrombin), as a result of which products of high functional activity are formed.^(15,30) Several MMWP fractions are determined depending on the wavelength. In our study, the fraction with the intensity of ultraviolet absorption at 238 nm was the most indicative. As a rule, substances of catabolic origin, xenobiotics, decay products of tissue cells, and particles of microbial origin are registered in this range. In a healthy person's biological fluids, such substances

might be found in small quantities (i.e., below the threshold of sensitivity of the method). Therefore, the appearance of high extinction values at a wavelength of 238 nm always indicates pathological processes in the body. As a rule, an increase in the values of this indicator may indicate an increase in catabolic processes and stimulation of the LPO processes.⁽¹⁴⁾ Due to the direct effect of MMP on biomembrane lipids, one can expect the development of pathological phenomena of a disconnecting nature: microcirculation disorders, disconnection of oxidative phosphorylation processes, inhibition of carbohydrate and energy metabolism enzymes, etc.⁽³¹⁾

Conclusion

The obtained data demonstrate the activation of lipid peroxidation processes at the stage of secondary products in adolescent boys and endogenous intoxication in obese adolescents, regardless of gender. Monitoring and correcting these indicators in adolescent patients with obesity should be an important component of pathogenetic treatment.

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Competing Interests

The authors declare that they have no competing interests.

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Iron Deficiency and Iron Storage Markers in Different Pregnancy Stages in Sudanese Women of Different Age

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Abstract

Background: The aim of this study was to evaluate the ID and iron storage markers in pregnant women suffering from anemia during various trimesters attending Omdurman Maternity Hospital in Sudan.

Methods and Results: This cross-sectional study included 205 pregnant women aged 15 to 45 in various trimesters who attended Omdurman Maternity Hospital (Sudan) between May 2022 and January 2023. All pregnant women were divided into two groups: the case group included 159 pregnant women with anemia, and the control group included 46 apparently healthy pregnant women. The blood test parameters were measured using a Sysmex XT-1800i Automated Hematology Analyzer (Japan). The levels of serum iron (SI) and total iron-binding capacity (TIBC) were measured using a Vitrous-350 Chemistry Analyzer (USA). The serum ferritin (SF) levels were measured by electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche).

Among anemic pregnant women, 33(20.8%) were in the first trimester of pregnancy, 68(42.8%) in the second trimester, and 58(36.5%) in the third. In the case group, more than six deliveries were found in 50.3% of cases, compared to 23.9% in the control group ($P=0.0015$). The blood levels of Hb and SF were significantly lower in all trimesters in the case group than in the control group. The SI level showed a significantly low level only in the third trimester in the case group, compared to the control group ($P<0.05$). In the case group, TIBC levels increased from trimester to trimester, reaching maximum values in the third trimester, indicating a low iron level in the blood. In the case group, in the first trimester, the levels of Hb and ferritin did not differ between the age groups of 15-25 years and >26 years. However, in the second trimester, the SF level was statistically lower in the age group of >26 years than in the age group of 15-25 years (45.83 ± 5.0 vs. 49.02 ± 3.71 ng/mL, $P=0.0038$), but in the third trimester, there was the opposite (37.50 ± 4.9 vs. 26.9 ± 4.5 ng/mL, $P=0.000$), which indicated a presence of IDA in the age subgroup of 15-25 years.

Conclusion: Pregnant women are at high risk of developing or worsening ID. Every pregnant Sudanese woman should be screened for IDA. (International Journal of Biomedicine. 2023;13(2):296-300.)

Keywords: iron deficiency anemia • serum ferritin • serum iron • total iron-binding capacity

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Abbreviations

CBC, complete blood count; **Hb**, hemoglobin; **ID**, iron deficiency; **IDA**, iron deficiency anemia; **RBC**, red blood cell; **SF**, serum ferritin; **SI**, serum iron; **TIBC**, total iron-binding capacity.

Introduction

Iron deficiency (ID) is the most common micronutrient deficiency in children and pregnant women worldwide.⁽¹⁾ ID can be caused by several factors. These causes are classified as follows: increased demand for iron that the diet cannot meet, increased iron loss (usually through blood loss), and nutritional deficiency. During pregnancy, physiologic iron demands increase substantially, and about 1g of iron must be acquired to preserve the maternal iron balance and support fetoplacental development.⁽²⁾

In pregnancy, there is a physiological expansion of plasma volume beginning in the first trimester and plateauing by the third,⁽³⁾ which exceeds the increased production of RBCs and hemoglobin. The resulting hemodilution contributes to the fall in Hb during pregnancy. Anemia in pregnancy can be caused by numerous other factors, including vitamin B12 and folate deficiency, the presence of thalassemia, inflammatory disorders, and, most commonly, ID. As the pregnancy advances, maternal RBC mass increases and placental and fetal growth accelerates, which result in the rise in physiologic iron requirements to 3.0–7.5mg/d in the third trimester.⁽⁴⁾

The World Health Organization (WHO) defines anemia of pregnancy as Hb<11g/dL, or hematocrit <33%, at any time during the pregnancy.⁽⁵⁾ The Centers for Disease Control and Prevention (CDC) define anemia of pregnancy as Hb<11g/dL, or hematocrit <33% during the first and third trimesters, and <10.5g/dL or a hematocrit <32% in the second trimester.⁽⁶⁾ The WHO defines severe anemia in all persons as a Hb of <7 g/dL and very severe anemia as a Hb of <4 g/dL.⁽⁷⁾

The reticuloendothelial system stores and recycles iron in the body by hemolyzing aged RBCs. Different from iron absorption and recycling, iron excretion lacks a physiologic regulatory system. Iron is stored in the bone marrow, liver, and spleen as ferritin. The ferritin stores in the liver are the body's primary physiologic origin of stockpile iron.

Serum ferritin (SF) concentration is a marker of reticuloendothelial iron stores, and SF concentration below the normal range is the most specific biochemical indicator of ID.⁽⁸⁾ Thresholds of SF concentration for identifying ID in pregnancy range from 10µg/L to 30µg/L.⁽⁹⁾ The systematic review findings by Daru et al.⁽¹⁰⁾ show that the most frequently used thresholds for defining ID in pregnancy (<12 and <15µg/L) are based on international guidelines informed by consensus meetings undertaken more than 15 years ago,^(1,2) not on published evidence. According to UK guidelines on the management of IF in pregnancy, an SF level of <30µg/L in pregnancy is indicative of ID.⁽¹¹⁾ Low maternal serum ferritin concentrations are associated with ID in neonates.^(12,13)

The aim of this study was to evaluate the ID and iron storage markers in pregnant women suffering from anemia during various trimesters attending Omdurman Maternity Hospital in Sudan.

Materials and Methods

This cross-sectional study included 205 pregnant women aged 15 to 45 in various trimesters who attended Omdurman

Maternity Hospital (Sudan) between May 2022 and January 2023. All pregnant women were divided into two groups: the case group included 159 pregnant women with anemia, and the control group included 46 apparently healthy pregnant women.

Blood sample collection and IDA diagnosis

About 6mL venous blood was drawn from each pregnant woman: 2mL were placed in an EDTA container to measure CBC using a Sysmex XT-1800i Automated Hematology Analyzer (Japan), and 4 mL were placed in gel tube to measure SI, TIBC using a Vitrous-350 Chemistry Analyzer (USA). SF levels were measured by electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche).

The normal SI level for women is 60 mcg/dL to 140 mcg/dL, and normal TIBC is 250 mcg/dL to 450 mcg/dL. A TIBC value >450 mcg/dL usually means a low blood iron level.⁽¹⁴⁾

Statistical analysis was performed using statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Inter-group comparisons were performed using Student's t-test. Categorical variables were analyzed using the chisquare test with the Yates' correction. A probability value of $P<0.05$ was considered statistically significant.

Results

Among anemic pregnant women, 33(20.8%) were in the first trimester of pregnancy, 68(42.8%) in the second trimester, and 58(36.5%) in the third. In the case group, more than six deliveries were found in 50.3% of cases, compared to 23.9% in the control group ($P=0.0015$). Four to six deliveries were found in 39.6% and 69.6% of subjects in the case and control groups, respectively ($P=0.0003$). In the case group, the interval of <2 years between pregnancies was found in 29.6% of cases versus 4.3% of cases in the control group ($P=0.0004$) (Table 1).

Table 1

The general characteristics of the study groups.

Number of deliveries	Case group n=159	Control group n=46
1-3 times	16 (10.1%)	3 (6.5%)
4-6 times	63 (39.6%)	32 (69.6%)
> 6 times	80 (50.3%)	11 (23.9%)
Interval between pregnancies		
> 2 years	112(70.4%)	44 (95.7%)
< 2 years	47(29.6%)	2 (4.3%)

The blood levels of Hb and SF were significantly lower in all trimesters in the case group than in the control group. The SI level showed a significantly low level only in the third trimester in the case group, compared to the control group ($P<0.05$). In the

case group, TIBC levels increased from trimester to trimester, reaching maximum values in the third trimester, indicating a low iron level in the blood (Table 2).

Table 2.

The levels of Hb, SF, TIBC, and SI in different pregnancy stages.

Pregnancy stage	Parameter	Case group	Control group	P-value
First trimester	Hb, g/dL	10.6±0.8	12.4±0.3	0.000
	SF, ng/mL	45.5±6.1	55.2±23.4	0.04
	TIBC, mcg/dL	443.7±108.7	331.5±29.9	0.000
	SI, µg/ dL	46.5±33.4	50.0±9.1	0.652
Second trimester	Hb, g/dl	10.4±±1.0	12.6±0.4	0.000
	SF, ng/mL	55.2±33.6	64.9±34.1	0.008
	TIBC, mcg/dL	488.6±105.1	423.2±86.6	0.026
	SI, µg/ dL	72.5±45.1	59.8±22.7	0.179
Third trimester	Hb, g/dl	10.3±0.9	12.7±0.7	0.000
	SF, ng/mL	58.3±34.5	80.2±53.2	0.001
	TIBC, mcg/dL	522.7±141.1	485.0±70.9	0.09
	SI, µg/ dL	42.9±27.8	74.6 ± 3.01	0.000

We did not find significant differences in the size of the age subgroups (15-25 years and >26 years) between the case and control groups in each trimester (Table 3). In the case group, in the first trimester, the levels of Hb and ferritin did not differ between the age groups of 15-25 years and >26 years. However, in the second trimester, the SF level was statistically lower in the age group of >26 years than in the age group of 15-25 years (45.83±5.0 vs. 49.02±3.71 ng/mL, $P=0.0038$), but in the third trimester, there was the opposite (37.50±4.9 vs. 26.9±4.5 ng/mL, $P<0.0001$), which indicated a presence of IDA in the age subgroup of 15-25 years (Table 4).

Table 3.

The size of the age subgroups in different pregnancy stages.

Pregnancy stage	Age subgroup	Case group n (%)	Control group n (%)	P-value
First trimester	15-25 yrs.	18 (54.5)	3 (60)	0.956*
	>26 yrs.	15 (45.4)	2 (40)	
Second trimester	15-25 yrs.	35 (51.5)	3 (23.1)	0.060
	>26 yrs.	33 (48.5)	10 (76.9)	
Third trimester	15-25 yrs.	26 (44.8)	7 (25.0)	0.076
	>26 yrs.	32 (55.2)	21 (75.0)	

*Yates' P-value

Table 4.

The levels of Hb and SF in anemic women of different age subgroups in different pregnancy stages.

Pregnancy stage	Parameter	Age group (years)	mean ± SD	P-value
First trimester	Hb, g/dL	15-25 (n=18)	10.5±0.7	0.2417
		>26 (n=15)	10.9±1.2	
	SF, ng/mL	15-25 (n=18)	50.1±3.88	0.9302
		>26 (n=15)	50.0±3.33	
Second trimester	Hb, g/dL	15-25 (n=35)	10.4±1.0	0.7224
		>26 (n=33)	10.3±1.3	
	SF, ng/mL	15-25 (n=35)	49.02 ±3.71	0.0038
		>26 (n=33)	45.83±5.0	
Third trimester	Hb, g/dL	15-25 (n=26)	10.2±0.9	0.6561
		>26 (n=32)	10.3±0.8	
	SF, ng/mL	15-25 (n=26)	26.9±4.5	<0.0001
		>26 (n=32)	37.50±4.9	

Discussion

IDA is still a pervasive public health issue in most developing countries. Pregnant women require iron to compensate for basic losses that cannot be compensated by food alone. The important variable of IDA is the number of pregnancies (gravidity). The present study showed that the risk of developing anemia during pregnancy is significantly associated with more than six deliveries in the patient's history. This conclusion is consistent with data from studies conducted in Saudi Arabia and India, which revealed that many pregnancies and deliveries are linked to a higher likelihood of developing IDA.⁽¹⁵⁾ This could be attributable to iron and other nutrient loss during multiple pregnancies and resource sharing with the fetus. Nevertheless, other studies in Ethiopia and Nepal revealed no link between pregnancy and anemia.⁽¹⁶⁾

Shorter time intervals between deliveries are another cause of anemia. Our results for the association of anemia of pregnancy with the interval of <2 years between pregnancies are consistent with previous reports.⁽¹⁷⁻¹⁹⁾ This is likely because mothers may not yet replenish essential nutrients, especially iron and folic acid, which were depleted by the previous pregnancy. A study by Mremi et al.⁽²⁰⁾ showed that post-partum women with less than two-year intervals between the last two pregnancies were about 18 times more likely to have anemia than women with more than two-year intervals (COR=18; 95% CI 8.617–38.617).

The prevalence of anemia in pregnancy is quite high. In a study by Kumar et al.,⁽²¹⁾ among 1000 mothers admitted for delivery, more than 50% were anemic at some point of time during their pregnancy, and 39% were anemic throughout.

Physiological changes occur in the second trimester, increasing plasma volume alongside a smaller increase in red cell mass, resulting in hemodilution – recognized as “physiological anemia.” In our study, perhaps due to the small size of the control group, we did not observe such dynamics, and in the case group, the Hb level in all semesters was <11g/dL. The incidence of anemia was highest in the second trimester (42.8%) than in the third (36.5%) and first trimesters (20.8%) due to the mother’s blood volume expanding and the fetus growing and developing.

Hemoglobin concentration alone lacks sufficient sensitivity and specificity to diagnose ID in pregnancy. Iron-specific biomarkers, such as SF and TIBC, can be utilized to distinguish IDA from other causes of anemia. In our study, the values of SI and TIBC were found to show significant variations between the various trimesters of pregnancy. TIBC levels significantly increased during pregnancy among our subjects, indicating a low iron level in the blood in the third trimester.

The best parameter of maternal iron status currently available is SF concentration. An SF<30 ng/mL is diagnostic for absolute ID, independently of any other parameter.⁽²²⁾ Clinically, the finding of low SF concentration is highly diagnostic for ID. Because ferritin is an acute phase reactant, a normal SF concentration may mask an iron-deficient state if inflammation is present. Measuring C-reactive protein is important if you have to interpret the SF level in conditions of possible inflammation.⁽²³⁾

Our study found absolute ID in 16.4% of cases among pregnant women with anemia, indicating IDA in the age group of 15-25 years in the third trimester of pregnancy. Many factors may contribute to an increased risk of anemia in people aged 15 to 25, such as rapid growth, blood loss during their monthly periods, and a low-iron diet. Young reproductive-age women differ in many ways from older reproductive-age women, including nutritional requirements, duration of menses, and contraceptive use.⁽²⁴⁾

Iron utilization is increased during pregnancy, as iron is required for fetal growth and development,⁽²⁵⁾ as well as for increased maternal erythropoiesis.^(4,11) Until now, the recommended dose of elemental iron for the treatment of ID has been 100–200mg daily.^(26,27) In most studies, supplementing anemic women with iron during pregnancy reduces the rate of iron deficiency anemia and nonanemic ID at term. In some studies, it reduces the risk of adverse outcomes, suggesting that supplementation in this population is beneficial.^(28,29) At the same time, the World Health Organization currently recommends universal daily supplementation with 30 to 60mg elemental iron during pregnancy in regions where the prevalence of anemia is 20% or higher; this recommendation also notes the need to take into account a stipulation in malaria-endemic areas where supplementation should be given in conjunction with “adequate measures to prevent, diagnose and treat malaria,”⁽³⁰⁾ which is especially important for Sudan. According to WHO’s latest World Malaria report, Sudan carried the heaviest burden of malaria in the Eastern Mediterranean Region in 2020, accounting for more than half of all cases (56%) and deaths (61%).⁽³¹⁾

In conclusion, pregnant women are at high risk of developing or worsening ID. Every pregnant woman should be screened for IDA. Moreover, the American College of Gynecology and Obstetrics⁽³²⁾ recommends low-dose iron supplementation in the first trimester for all women, regardless of their iron status.

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Competing Interests

The authors declare that they have no competing interests.

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Organization of the Fibrous Component of Connective Tissue Paraneural Structures in Different Periods of Ontogenesis

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Abstract

Background: The steady increase in road traffic accidents, seasonal injuries leading to damage to the peripheral nerves of the extremities, as well as the military conflicts that are becoming more frequent now actualize research aimed at improving the diagnosis, treatment, and prevention of post-traumatic changes in the nerve trunks of the extremities. The frequency of peripheral nerve injuries varies from 1.5% to 13% of all injuries in peacetime; and during hostilities, it reaches 20%, and disability is 60%. At the same time, specific destructive changes in the nerves of the extremities after their traumatic injury require extraordinary organizational, therapeutic, and functional approaches to their restoration. The purpose of this study was to evaluate the dynamics of changes in the fibrous component of the paraneurium connective tissue structures in different periods of ontogenesis.

Methods and Results: The study consisted of two stages. At the first stage, to study changes in the paraneurium connective tissue of the sciatic nerve in vivo, ultrasound was performed using an RS85 ultrasound scanner (Samsung Medison, South Korea, 2021) and two linear transducers, LA4-18B and LA2-9A. The thickness of the sciatic nerve and the surrounding paraneurium were measured, and their structural organization was evaluated. The inclusion criterion was the absence of pathology from the peripheral nervous system. All subjects were divided into four age groups (15 people in each group): Group 1 (0-11 years), Group 2 (12-25 years), Group 3 (26-40 years), and Group 4 (41-60 years).

The second stage was performed on cadaveric material of the paraneurium connective tissue of the sciatic nerve of persons of both sexes, of different ages, whose cause of death was not related to diseases or injuries of the nervous system. To determine the qualitative and quantitative ratio of collagen fibers of different degrees of maturity in the connective tissue structures of the paraneurium tissue of the sciatic nerve, polarization microscopy (MicMed-6, Lomo, Russia) was applied, and the basic principle of double refraction, which in combination with Sirius red staining, made it possible to differentiate types I and III collagen. The amount of each collagen type was determined by analyzing the color gamut after Sirius red staining in polarizing light. Fibers containing type I collagen had a red glow, while those containing type III collagen had a green glow. The ratio of collagen types was calculated using the Fiji program (USA, 2022).

US examination revealed the presence of a non-pronounced bilateral asymmetry in the structural organization of the paraneurium and a trend toward an increase in the thickness of the sciatic nerve with age, from 0-11 years to the age group of 41-60 years. Polarization microscopy of micro-preparations of the human sciatic nerve with paraneurium structures made it possible to analyze the density of the fibers of the paraneurium connective tissue and identify types I and III of collagen. At the age of 11, the amount of type I collagen fibers was maximum ($69.74 \pm 0.41\%$), and type III collagen fibers amounted to $30.26 \pm 0.44\%$. Further, with increasing age, the amount of collagen III significantly diminished. The ratio of type I to type III collagen increased with patient age, with the highest ratio in the age group 41-60 years.

Conclusion: The revealed dynamics of changes are quite comparable with the age-related features of connective tissue since it is this tissue that makes up the morphological substrate of the paraneurium of the sciatic nerve. (International Journal of Biomedicine. 2023;13(2):301-304.)

Keywords: paraneurium coating • connective tissue • collagen fiber • paraneurium

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Introduction

It is necessary to consider the indisputable fact that the functional activity of the conductor component of the peripheral nerve is inextricably linked with its stromal component, which includes endo-, peri-, epineurium, and paraneural connective tissue structures, or "paraneurium."⁽¹⁻³⁾ The functional role of the paraneurium lies in its indissoluble morphological connection with the trunk of the peripheral nerve. One of the conditions determining the severity of damage to peripheral nerves and the subsequent rate of their morpho-functional recovery is the degree of traumatization of the surrounding soft tissues, including paraneurium connective tissue structures.⁽⁴⁻⁶⁾ Currently, the legitimacy of using the term peripheral nerve paraneurium is being actively discussed in scientific circles. In 1995, Millesi et al. studied the mechanical properties of the peripheral nerve and convincingly demonstrated the role of paraneurium in the dynamics of changes in the length and diameter of the nerve trunk under conditions of limb flexion and extension.⁽⁷⁾ In 2007, Millesi with colleagues explained the participation of paraneural tissue in the passive motility of the trunks of the peripheral nerves in conditions of active movement, for example, in a uniaxial joint.⁽⁸⁾ In 2013, several researchers explained the need to use the term paraneurium in connection with its deepest role in introducing an anesthetic into the connective tissue membranes surrounding the nerve trunk.⁽⁹⁻¹¹⁾ At the same time, there were no data on the structural organization of paraneurium in different periods of ontogenesis in the available literary sources of domestic and foreign authors.

Materials and Methods

The study consisted of two stages. At the first stage, to study changes in the paraneurium connective tissue of the sciatic nerve in vivo, ultrasound was performed using an RS85 ultrasound scanner (Samsung Medison, South Korea, 2021) and two linear transducers, LA4-18B (a frequency range of 4.0 MHz - 18.0 MHz) and LA2-9A (a frequency range of 2.0 MHz - 9.0 MHz); the use of two transducers is due to the different mass of patients (a total of 60 patients were examined). The thickness of the sciatic nerve and the surrounding paraneurium were measured, and their structural organization was evaluated. The ultrasound images were obtained by experienced operators. The cross-sectional areas of the sciatic nerve were measured at the lower edge of the gluteus maximus in the posterior midline of the thigh (the gluteal sulcus). The cross-sectional area was measured by tracing the nerve just inside its hyperechoic rim, and three measurements were obtained with the probe repositioned.

The inclusion criterion was the absence of pathology from the peripheral nervous system. Informed consent was obtained from patients before collecting the data. All subjects were divided into four age groups (15 people in each group): Group 1 (0-11 years), Group 2 (12-25 years), Group 3 (26-40 years), and Group 4 (41-60 years).

The second stage was performed on cadaveric material of the paraneurium connective tissue of the sciatic nerve of

persons of both sexes, of different ages, whose cause of death was not related to diseases or injuries of the nervous system (REC approval No. 7 of 10/14/19). Dissection of the sciatic nerve and the surrounding paraneurium connective tissue was carried out in layers throughout – from the point 5cm above the gluteal fold and up to the upper corner of the popliteal fossa. The resulting biomaterial, a total of 94 complexes, consisting of a segment of the sciatic nerve with surrounding connective tissue structures and skeletal muscles, was placed in a 10% solution of neutral formalin, enclosed in paraffin according to the standard procedure and histological sections were made, which were stained with hematoxylin and eosin for overview light microscopy. To determine the qualitative and quantitative ratio of collagen fibers of different degrees of maturity in the connective tissue structures of the paraneurium of the sciatic nerve, polarization microscopy (MicMed-6, Lomo, Russia) was applied, and the basic principle of double refraction, which in combination with Sirius red coloration, made it possible to differentiate types I and III collagen. The amount of each collagen type was determined by analyzing the color gamut after Sirius red coloring in polarizing light. Fibers containing type I collagen had a red glow, while those containing type III collagen had a green glow. The ratio of collagen types was calculated using the Fiji program (USA, 2022).

Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. The mean (M) and standard error of the mean (SEM) were calculated. Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare means of 2 and 3 or more groups of variables not normally distributed. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

Considering the relevance of ultrasound (US) as one of the additional methods of diagnosing peripheral nerve lesions, this method was used in this work. US examination revealed the presence of a non-pronounced bilateral asymmetry in the structural organization of the paraneurium and a trend toward an increase in the thickness of the sciatic nerve with age, from 0-11 years to the age group of 41-60 years (Table 1).

When working with cadaveric material, it was revealed that the nerve in the upper third of the thigh was surrounded by its own fascial sheath (OFS), the thickness of which varied from 12 to 30 microns and was directly proportional to age. In cross-sections, the OFS was visualized at different distances from the nerve trunk and separated by a paraneurium fascial-cellular space (PFCP), the thickness of which varies from 1 to 1.5 mm and has no pronounced dependence on age. At the same time, it should be noted that the shape of such a paraneural space from the lateral and medial sides had the form of a crescent (up to 40-45 years of age) or the shape of a triangle, at the age of 50-60 years (in some cases, a polygonal shape was observed). The paraneurium fiber was divided by connective cords extending from the common fascial sheath

toward the epineurium and merging with its connective tissue fibers. Thin connective tissue, vaginal fascial sheets, branch outwards, toward the skeletal muscles from the OFS, at different angles, which then merge with the fascial cases of the surrounding muscles. The space between the fascial leaves is filled with white adipose tissue forming lobules of adipocytes. The shape, number, and size of such lobules have age-specific features. The identified morphological features are shown in Figure 1.

Polarization microscopy of micro-preparations of the human SN with paraneurium structures made it possible to analyze the density of the fibers of the paraneurium connective tissue and identify types I and III of collagen. Collagen fibers in the paraneurium had different diameters, densities, and compactness of location, and the vector of their orientation had not only topographical, but also age dependence. Collagen fibers formed a fibrous-parallel (a common fascial case with outgoing slings) and fibrous-mesh architectonics (areas of connective tissue located in the paraneurium space). Mature connective tissue fibers containing type I collagen had a red glow when passing polarization light, and immature (type III collagen) had a green glow. At the age of 11, the amount of type I collagen fibers was maximum ($69.74 \pm 0.41\%$), and type III collagen fibers amounted to $30.26 \pm 0.44\%$. Further, with increasing age, the amount of collagen III significantly diminished. The ratio of type I to type III collagen increased with patient age, with the highest ratio in the age group 41-60 years (Figure 2).

Discussion

Thus, our data are not only consistent with the conclusions of foreign colleagues but also complement the knowledge about the role of the paraneurium, of the sciatic nerve, in the practice of regional anesthesia. The study revealed qualitative and quantitative transformations in the structural organization of the paraneurium connective tissue. US revealed the presence of slight bilateral asymmetry and, with age, a thickening of the sciatic nerve and a thickening of the common fascial sheath, as well as an increase in its cross-sectional area.

The analysis of changes in the paraneurium structures of the sciatic nerve revealed a gradual complication in its structural organization, which depended on age. At the same time, the observed organization of paraneurium structures was inextricably linked with the complication of the structure of not only the conduction apparatus of the sciatic nerve but also the entire stromal apparatus. The so-called “morphological improvement” was manifested both in quantitative transformation – the appearance of new structures in the paraneurium; and in qualitative transformation – a change in the ratio of types I and III collagen toward a significant decrease in the latter to its complete absence by the age of 60. The revealed dynamics of changes are quite comparable with the age-related features of connective tissue since it is this tissue that makes up the morphological substrate of the paraneurium of the sciatic nerve. In our opinion, the data

Table 1.

Changes in the thickness of the sciatic nerve and the surrounding paraneurium in different age periods according to US data

Age group	Right limb			Left limb			P_{1-4}	P_{2-5}	P_{3-6}
	Thickness of the sciatic nerve (mm)		Paraneurium thickness (mm) (3)	Thickness of the sciatic nerve (mm)		Paraneurium thickness (mm) (6)			
	D (p-s) (1)	D (m-d) (2)		D (p-s) (4)	D (m-d) (5)				
0-11, years	2.38±0.1	5.7±0.21	0.46±0.61	2.22±0.21	5.32±0.22	0.54±0.14	0.01	0.005	0.005
12-25, years	2.61±0.3	7.01±0.12	0.62±0.11	2.54±0.13	7.84±0.11	0.62±0.61	0.01	0.001	0.10
26-40, years	2.42±0.22	6.94±0.11	0.68±0.13	2.41±0.11	7.25±0.11	0.65±0.63	0.10	0.01	0.10
41-75, years	2.53±0.20	7.12±0.22	0.74±0.12	2.32±0.33	7.22±0.21	0.74±0.11	0.005	0.01	0.12
P -value	0.1145	0.1215	0.1174	0.1182	0.1205	0.1194			

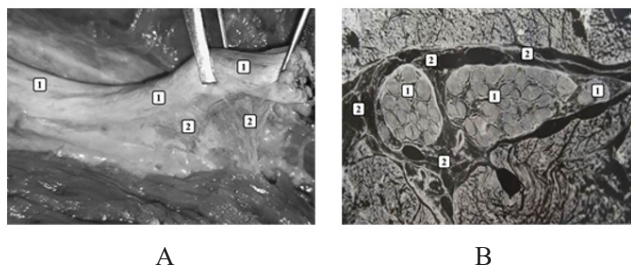


Fig. 1. Macro (A) and microphotography (B) of the sciatic nerve (1) and the surrounding paraneurial connective tissue (2) (“paraneurium”).

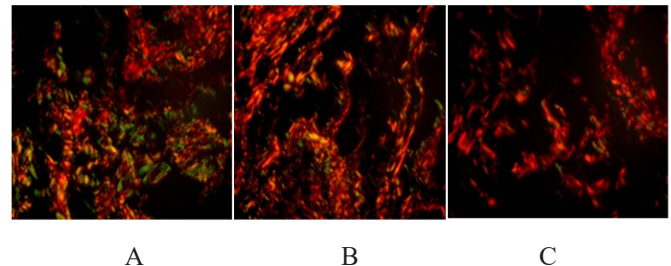


Fig. 2. Microphotography of paraneurium at the age of 0-11 years (A), 12-25 years (B), and 41-60 years (C). Polarization microscopy data. Sirius red staining. Magnification x400.

obtained can be used in practical healthcare, particularly in such areas of medicine as neurosurgery, traumatology, oncology, and neurology.

Competing Interests

The authors declare that they have no competing interests.

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Morphogenesis of Changes in the Structural Components of the Skin in Cancer Patients with COVID-19 in Older Age Groups

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Abstract

Background: Immunosuppression caused by cancer or cytotoxic drugs, aging, and comorbidities makes cancer patients not only more susceptible to COVID-19, but also more likely to progress to a severe form and increase the incidence of serious complications. The epitheliotropy of this virus is of interest to the study of skin changes and the degree of their manifestation in patients with malignant neoplasms. In this regard, the aim of our research was to study the morphogenesis of changes in the structural components of the skin in cancer patients with COVID-19.

Methods and Results: We examined the features of morpho-functional changes in skin components in 80 cancer patients who died in Kursk and the Kursk region for the period January 2021–February 2022. Group 1 included cancer patients with no history of COVID-19, whose cause of death was peritonitis due to colorectal cancer; Group 2 included cancer patients whose cause of death was viral pneumonia caused by COVID-19. Each group was further divided by sex and age. The research material was skin fragments. The results of histological and morphometric studies of the skin show that lymphocytic infiltration was typical for all age subgroups of cancer patients with COVID-19, which had higher numbers of lymphocytes per 100 cells than cancer patients without COVID-19. Infiltrative-inflammatory changes are observed in the skin, the severity of which depends on the patient's age. For the age subgroup of 76-85 years without COVID-19, a more pronounced increase in the ratio of the reticular layer to the papillary layer was characteristic, due to a decrease in the thickness of the papillary layer. Pronounced thickening of the papillary layer was found in all age subgroups of cancer patients with COVID-19.

Conclusion: It is possible to predict more frequent skin manifestations in cancer patients who have had a new COVID-19 infection, the mechanism of which is mainly due to changes in specific leukocytes, T-lymphocytes, and macrophages and their infiltration of skin tissues. (International Journal of Biomedicine. 2023;13(2):305-308.)

Keywords: COVID-19 • cancer • skin • lymphocytes

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Introduction

The novel coronavirus infection (COVID-19) pandemic has forced the global scientific and medical community to urgently develop approaches to diagnosing, treating, and preventing pneumonia associated with a COVID-19 infection. Changes that occur in the cardiovascular, digestive, and nervous systems were also of greater interest. Over time, more and more attention has been paid to the study of dermatological manifestations, particularly due to concern for certain groups

of patients, including those treated with immunosuppressants or immunomodulators. In addition, more and more data worldwide began to accumulate about skin manifestations of COVID-19, to one degree or another, occurring in almost every fifth patient and appearing more often for the first time four weeks from the onset of the main symptoms of COVID-19. Also, some authors point out that skin lesions can sometimes precede the main symptoms of COVID-19.⁽¹⁻⁴⁾

It is particularly important to study the frequency of skin manifestations of COVID-19 in cancer patients and patients with immunosuppression since these categories of patients are at high risk for both the incidence of COVID-19 and the severity of its course.⁽⁵⁾ The higher susceptibility of cancer patients to SARS-CoV-2 infection is either due to impaired immune responses characteristic of cancer and comorbidities, or to

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specific therapies that alter immune homeostasis.⁽⁶⁾ However, the structure of published descriptions of skin manifestations and analyses of risk factors, clinical course, and mortality in cancer patients infected with COVID-19 is currently heterogeneous regarding tumor formations. Data comparing cancer and non-cancer patients are scarce. The factors that make it relevant to study the morphogenesis of changes in the structural components of the skin in cancer patients under conditions of COVID-19 are (1) the high susceptibility of cancer patients to COVID-19 infection and their inclusion in the high-risk group, both in terms of incidence and severity of the infection; (2) the heterogeneity of published descriptions of the skin manifestations of COVID-19, and the paucity of data comparing cancer and non-cancer patients; and (3) the role of new data in improving the quality and timeliness of the diagnosis of COVID-19 and the skin damage caused by it.^(7,8)

The study aimed to evaluate the morphogenesis of changes in the skin’s structural components in cancer patients with COVID-19.

Materials and Methods

The practical part of our research included the study of cadaver material of 80 cancer patients who died in Kursk and the Kursk region, according to the Regional Pathological Bureau of the Kursk Region Health Committee for the period January 2021–February 2022. In this part, we examined the features of morpho-functional changes in skin components in cancer patients who had Covid-19. Excluded from the study were patients whose underlying disease was related to skin pathology and patients with severe somatic pathology. Patients were included in the study according to the selection criteria. They were divided into groups: Group 1 – cancer patients with no history of COVID-19, whose cause of death was peritonitis due to colorectal cancer, and Group 2 – cancer patients whose cause of death was viral pneumonia caused by COVID-19. Each group was further divided by sex and age (Table 1).

Table 1. Distribution of patients according to age and sex.

Sex and age groups			n
Group 1	Women	65-75 years	10
		76-85 years	10
	Men	65-75 years	10
		76-85 years	10
Group 2	Women	65-75 years	10
		76-85 years	11
	Men	65-75 years	10
		76-85 years	9

The research material was skin fragments (2x2cm) taken along the midline of the abdomen, retreating 2 cm above the navel. The histological material was sent to the

pathological laboratory. For light microscopy, the skin was fixed with 10% buffered neutral formalin and dehydrated in a frozen state in alcohols of increasing concentrations. For research, dehydrated samples were embedded in paraffin, and histological sections were made with a thickness of 4-5 μm. The sections were stained with hematoxylin and eosin (H&E) and examined in an Eclipse 80i direct light microscope, the objective magnification of which was x40, x200, and the eyepiece magnification x10. The thickness of the reticular and papillary layers, their ratio, the density of the connective tissue, and the number of interfiber spaces were determined using Altami Studio 3.0 and ImageJ 1.46h programs.

Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA). The mean (M) and standard error of the mean (SEM) were calculated. The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

After a morphological study of skin biopsies obtained from persons of Group 1 in all subgroups, the following changes were revealed: the epidermis consisted of five pronounced layers of epitheliocytes, and a rather small number of cells in a state of mitosis in the basal layer and a pronounced stratum corneum. The dermis was formed by a papillary layer, consisting of fairly thick collagen fibers with a small amount of amorphous substance. In the reticular layer, attention was also drawn to a large number of thickened collagen fibers with a small amount of elastic structures.

When studying skin biopsies obtained from males with COVID-19 in the age group of 65-75 years, we noted pathomorphological symptoms: the epidermis was represented by stratified squamous keratinized epithelium, covered with a slightly thickened stratum corneum. In the basal sections, a moderately pronounced lymphocytic infiltration was determined. Subepithelial (papillary dermis) showed signs of slight perivascular edema. The cellular composition was predominantly represented by fibroblastic different cells, lymphocytes, plasmocytes, and single mast cells. Attention was drawn to the increase in blood capillaries, compared with the control group. In the reticular layer there were thickened collagen fibers with pronounced interfiber spaces (Figure 1A).

When examining skin biopsies obtained from female COVID-19 survivors in the same age group, we found that the epidermis was enlarged with a spiny and granular layer. In the basal layer of the epidermis, there were a large number of melanocytes and lymphocytes. The papillary layer of the dermis was expressed, represented by a loose fibrous connective tissue consisting of thin collagen fibers. The cellular composition was represented by fibroblastic different cells, lymphocytes, macrophages, melanocytes, and mast cells. There were fewer blood capillaries, in comparison with biopsies obtained from males, and in the reticular layer, collagen fibers with pronounced interfiber spaces (Figure 1B).

When studying skin biopsy specimens obtained from males with COVID-19 in the age group of 76-85 years, we

found the surface relief to be smooth, due to the pronounced interstitial edema of the dermis. The epidermis was exhausted. A pronounced round cell infiltration was determined in the dermis's papillary layer. The layer itself had a small thickness. Numerous blood capillaries with dilated lumens were found. Around individual vessels, the marginal standing of lymphocytes and the exit of formed elements were determined. The reticular layer of the dermis was represented by relatively thin collagen fibers with large interfiber gaps and a large amount of amorphous substance (Figure 1C).

When studying skin biopsy specimens obtained from female patients who had COVID-19, in the same age group, we found the surface relief to be smooth, due to moderately pronounced interstitial edema of the dermis. The epidermis had focal thickened stratum corneum. Mildly expressed lymphoplasmacytic infiltration was also determined subepithelial (Figure 1D).

Thus, the results of histological and morphometric studies of the skin show that lymphocytic infiltration was typical for all age subgroups of cancer patients with COVID-19, who had higher numbers of lymphocytes per 100 cells (Table 2) than cancer patients without COVID-19. For the age subgroup of 76-85 years without COVID-19, a more pronounced increase in the ratio of the reticular layer to the papillary layer was characteristic, due to a change a decrease in the thickness of the papillary layer. Pronounced thickening of the papillary layer was found in all age subgroups of cancer patients with COVID-19, compared with the same age subgroups in cancer patients without COVID-19. A trend toward a decrease in the ratio of the cellular component to the intercellular substance was more typical for patients of all age subgroups in the group of cancer patients with COVID-19 (Table 2).

Conclusion

Based on the results of the study, it is possible to predict more frequent skin manifestations in cancer patients who have

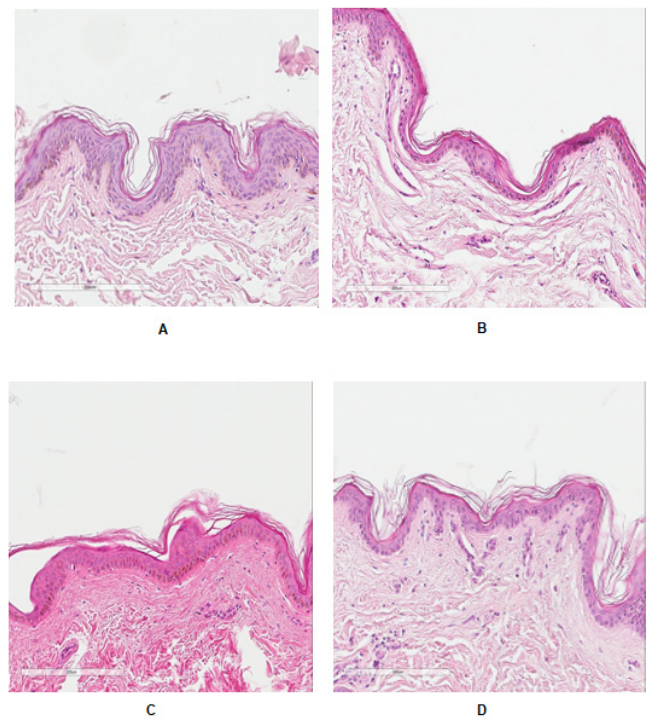


Fig. 1. Skin fragments of cancer patients with COVID-19

(A) A 71-year-old man; (B) A 77-year-old woman; (C) A 70-year-old man; (D) A 78-year-old woman. H&E staining; magnification, x200.

had a new COVID-19 infection, the mechanism of which is mainly due to changes in specific leukocytes, T-lymphocytes, and macrophages and their infiltration of skin tissues. The histological and morphometric data obtained during the study replenish the accumulated knowledge base, both about the skin features of infection with COVID-19, and about the features of its manifestations in cancer patients.

Table 2.

The quantitative data of the morphometric study

Variable	Group 1				Group 2			
	65-75 years		76-85 years		65-75 years		76-85 years	
	Men	Women	Men	Women	Men	Women	Men	Women
The number of lymphocytes per 100 cells	1.9±0.02	2.05±0.02	2.2±0.01	2.1±0.02	8.2±1.05*	7.42±0.9*	7.22±0.88*	7.02±0.82*
Thickness of the reticular layer, μm	4.8±0.3	5.1±1.02	4.91±0.8	5.4±0.5	9.12±1.52*	9.06±1.83*	9.44±1.38*	9.91±1.88*
Thickness of the papillary layer, μm	1.8±0.06	1.9±0.06	1.5±0.3	1.3±0.1	4.08±0.68*	3.49±0.76*	5.49±0.65*	4.1±0.69*
The ratio of the reticular layer to the papillary layer	2.6±0.03	2.7±0.01	3.2±0.02	4.2±0.01	2.23±0.03	2.6±0.01	1.72±0.04*	2.42±0.04
The ratio of the cellular component to the intercellular substance, %	94.7±2.2	89.6±1.2	90.51±3.74	96.3±2.9	84.69±3.27	84.18±3.20	86.57±3.74	87.31±3.87

* - $P < 0.05$ in comparison with the corresponding subgroup of Group 1

Competing Interests

The authors declare that they have no competing interests.

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Trigger Mechanisms of Cypermethrin-Induced Changes of Metabolism: An Experimental Study

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Abstract

Background: The purpose of this work was to study the triggering mechanisms of metabolic changes in experimental animals after a single injection of cypermethrin at a dose of 55 mg/kg of body weight.

Methods and Results: Sixty rats were randomly divided into four groups (15 rats in each group). Rats of the control groups (G1 and G3) were injected with saline into the stomach. Animals of the experimental groups (G2 and G4) were injected once with the synthetic pyrethroid cypermethrin at a dose of 55 mg/kg of body weight, which is 1/5 LD50.

Before being withdrawn from the experiment, blood was taken in vivo under anesthesia, and the liver was removed. The glucose, lactate, uric acid, and total bilirubin concentrations were determined in blood serum by unified research methods. The content of glutathione (GSH), malondialdehyde (MDA), and activity of glucose-6-phosphate dehydrogenase (G6PDH) was determined in erythrocyte hemolysates. In liver homogenates, the content of total protein, glycogen, uric acid, and inorganic phosphorus (Pi) was determined by unified methods, as well as MDA, GSH, activity of G6PDH, microsomal oxygenase, glutathione-S-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR).

Administration of cypermethrin to laboratory rats at a dose of 1/5 LD50 causes adaptive changes in metabolism. After one day, there was an increase in the content of glucose in the blood serum against the background of a deficiency of carbohydrates in the liver tissue. At the same time, there was an increase in anaerobic oxidation and an increase in purine catabolism, which was associated with the activation of lipid peroxidation of cell membranes and the depletion of the pool of antioxidants. GSH deficiency was exacerbated by an increase in the activity of antioxidant enzymes and xenobiotic biotransformation systems. Seven days after the administration of cypermethrin, rats retained a high rate of breakdown of purines to uric acid. This process was enhanced by a decrease in the red blood cells, a deficiency of carbohydrates, and inhibition of the activity of G6PDH, GPx, and GR. This ultimately led to the development of oxidative stress.

Conclusion: The triggers for the development of oxidative stress under cypermethrin exposure are lactic acidosis and increased catabolism of purine mononucleotides, accompanied by an increase in the production of free radicals and inhibition of the function of the antioxidant system. A decrease in the blood red blood cells, carbohydrate deficiency, and suppression of the activity of the pentose cycle 7 days after the administration of cypermethrin aggravate this condition. (International Journal of Biomedicine. 2023;13(2):309-312.)

Keywords: purine metabolism • carbohydrate metabolism • oxidative stress • cypermethrin • liver • rats

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Abbreviations

ATP, adenosine triphosphate; G6PDH, glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione-S-transferase; LP, lipid peroxidation; MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate; Pi, inorganic phosphate; RBC, red blood cells; ROS, reactive oxygen species.

Introduction

Synthetic pyrethroid cypermethrin is widely used in medicine, veterinary medicine, agriculture, and everyday life.⁽¹⁻³⁾ A large number of preparations with insecticidal and acaricidal properties have been developed on this basis.⁽⁴⁾ In recent years, a number of scientific studies have been published, noting the low selectivity and toxicity of cypermethrin for humans and warm-blooded animals.^(5,6) Many authors emphasize that under the action of synthetic pyrethroids on the mammal's body, oxidative stress has been developed.⁽⁷⁻⁹⁾ Still, they do not cover the issue of triggering mechanisms for the development of metabolic disorders.

The purpose of this work was to study the triggering mechanisms of metabolic changes in experimental animals after a single injection of cypermethrin at a dose of 55 mg/kg of body weight.

Materials and Methods

The experiment was performed on Wistar male rats weighing 230-250 g. All stages of the experiment were carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Sixty rats were randomly divided into four groups (15 rats in each group). Rats of the control groups (G1 and G3) were injected with saline into the stomach. Animals of the experimental groups (G2 and G4) were injected once with the synthetic pyrethroid cypermethrin at a dose of 55 mg/kg of body weight, which is 1/5 LD50. Animals were withdrawn from the experiment in two stages: rats of G1 and G2 - in a day and rats of G3 and G4 - seven days after the start of the experiment. During the experiment, we used a preparative form of cypermethrin with the trade name "Sharpei" (CJSC "August", Russia).

Before being withdrawn from the experiment, blood was taken in vivo under anesthesia, and the liver was removed. Then the blood was centrifuged, hemolysates were prepared from the erythrocyte mass, and homogenates were prepared from the liver at 0-2°C. Biochemical parameters were determined in the obtained blood serum, erythrocyte hemolysates, and liver homogenates. The number of RBCs was determined in whole blood. The glucose, lactate, uric acid, and total bilirubin concentrations were determined in blood serum by unified research methods. The content of glutathione (GSH),⁽¹⁰⁾ malondialdehyde (MDA),⁽¹¹⁾ and activity of glucose-6-phosphate dehydrogenase (G6PDH)⁽¹²⁾ was determined in erythrocyte hemolysates. In liver homogenates, the content of total protein, glycogen, uric acid, and inorganic phosphorus (Pi) was determined by unified methods, as well as MDA,⁽¹¹⁾ GSH,⁽¹⁰⁾ activity of G6PDH,⁽¹²⁾ microsomal oxygenase, glutathione-S-transferase (GST),⁽¹³⁾ glutathione peroxidase (GPx), and glutathione reductase (GR).⁽¹⁴⁾

Statistical analysis was performed using the STATISTICA 10 software package (Stat-Soft Inc., USA). For descriptive analysis, results are presented as median (Me),

first quartile (Q1) and third quartile (Q3). Differences of continuous variables were tested by the Mann-Whitney *U*-test. A probability value of $P < 0.05$ was considered statistically significant.

Results

The administration of cypermethrin to rats at a dose of 1/5 LD50 caused, after one day, an increase in levels of glucose, lactate, and uric acid in the blood serum (Table 1). In erythrocytes, there was a decrease in the concentration of GSH and accumulation of MDA. The activity of erythrocyte G6PDH remained at the level of control values (Table 1). The amount of RBC in the blood one day after the administration of cypermethrin did not change statistically significantly.

Table 1.

Changes in blood parameters in rats after a single injection of cypermethrin at a dose of 1/5 LD50.

Parameters	One day after		Seven days after	
	Group 1	Group 2	Group 3	Group 4
Blood				
RBC, $\times 10^{12}/L$	8.94 (8.53; 9.33)	8.66 (8.23; 8.90) $P=0.120$	8.90 (8.43; 9.62)	7.82 (7.20; 8.10) $P<0.001$
Serum				
Glucose, mg/dL	121 (108; 134)	147 (129; 160) $P=0.010$	119 (103; 137)	94.3 (76.1; 115) $P=0.003$
Lactate, mmol/L	6.27 (5.88; 7.60)	7.91 (7.11; 8.60) $P=0.002$	6.20 (5.25; 7.11)	7.64 (7.23; 8.82) $P<0.001$
Uric acid, $\mu\text{mol}/L$	71.8 (65.2; 85.3)	93.2 (83.6; 99.2) $P=0.001$	78.2 (65.6; 91.2)	94.5 (88.5; 116) $P=0.003$
Total bilirubin, $\mu\text{mol}/L$	2.73 (2.43; 3.34)	2.82 (2.45; 3.58) $P=0.672$	2.56 (2.21; 2.83)	3.72 (3.21; 3.92) $P=0.001$
Erythrocytes				
GSH, nmol/mg protein	9.27 (8.38; 10.3)	6.35 (6.01; 7.18) $P<0.001$	9.30 (8.01; 11.0)	5.99 (4.95; 7.05) $P<0.001$
MDA, nmol/mg protein	3.09 (1.87; 3.96)	4.49 (3.62; 5.96) $P=0.008$	2.66 (1.69; 4.00)	5.26 (4.21; 5.99) $P<0.001$
G6PDH, U/mg protein	8.40 (7.92; 11.9)	8.11 (7.28; 10.7) $P=0.326$	10.2 (8.61; 11.9)	7.83 (5.43; 8.61) $P=0.003$

In the liver of the G2 rats, we found a decrease in the content of glycogen and GSH against the background of an increase in the concentration of uric acid, Pi, and MDA (Table 2), which indicated an increase in the catabolism of purine mononucleotides and an increase in lipid peroxidation (LP) of the membrane structures of hepatocytes. The activity of

enzymes of the glutathione system (GST, GPx, and GR) in rats of the G2 increased, indicating activation of the detoxifying and antioxidant functions of the liver; however, the activity of liver G6PDH did not differ from the values of this indicator in the G1 (Table 2).

Table 2.

Biochemical parameters of the liver of rats after a single injection of cypermethrin at a dose of 1/5 LD50.

Parameters	One day after		Seven days after	
	Group 1	Group 2	Group 3	Group 4
Glycogen, mg/g wet wt	51.6 (35.8; 60.8)	32.3 (23.5; 36.3) <i>P</i> =0.007	49.3 (38.1; 58.9)	31.2 (28.1; 37.8) <i>P</i> =0.002
Uric acid, μ mol/g wet wt	23.8 (18.6; 30.4)	37.1 (32.1; 42.4) <i>P</i> =0.001	18.7 (16.0; 25.5)	44.2 (41.1; 53.1) <i>P</i> <0.001
Pi, μ mol/g wet wt	11.4 (8.94; 15.0)	17.0 (15.2; 19.9) <i>P</i> =0.002	9.14 (7.59; 13.2)	20.6 (18.6; 26.1) <i>P</i> <0.001
MDA, nmol/mg protein	89.5 (64.9; 91.9)	108 (95.1; 121) <i>P</i> =0.003	74.0 (65.5; 98.1)	128 (114; 151) <i>P</i> <0.001
GSH, nmol/mg protein	38.5 (31.2; 40.3)	23.3 (20.1; 27.9) <i>P</i> <0.001	33.8 (31.0; 37.6)	21.6 (18.8; 27.4) <i>P</i> <0.001
G6PDH, U/mg protein	26.8 (24.9; 35.9)	23.3 (20.2; 32.0) <i>P</i> =0.149	31.4 (25.9; 37.0)	17.2 (13.0; 20.9) <i>P</i> =0.001
MO*, nmol/mg/min	0.570 (0.510; 0.615)	0.764 (0.710; 0.852) <i>P</i> <0.001	0.574 (0.504; 0.628)	0.685 (0.622; 0.784) <i>P</i> =0.005
GST, U/mg protein	614 (504; 680)	863 (723; 945) <i>P</i> <0.001	588 (512; 706)	778 (699; 898) <i>P</i> =0.001
GPx, U/mg protein	814 (644; 853)	1162 (948; 1372) <i>P</i> <0.001	685 (620; 846)	515 (381; 731) <i>P</i> =0.024
GR, U/mg protein	438 (368; 531)	640 (530; 828) <i>P</i> <0.001	399 (357; 444)	274 (171; 331) <i>P</i> =0.001

*MO - microsomal oxygenase

In the blood, 7 days after the cypermethrin administration, we found a decrease in the number of erythrocytes and the development of glucose deficiency (Table 1). The blood concentration of lactic and uric acids, as well as the content of total bilirubin, on the contrary, statistically significantly exceeded the control values. GSH deficiency and decreased G6PDH enzymatic activity developed in erythrocytes against the background of MDA accumulation (Table 1).

In the liver of the G4 rats, a statistically significant decrease in the concentration of glycogen and GSH was detected (Table 2), while the content of uric acid, Pi, and MDA exceeded the control values. After 7 days, the activity of liver GST in rats of the G4 was still high, and the activity of GPx and GR was lower, compared to rats of the G3. In addition, we

found an inhibition of the activity of G6PDH (a key enzyme of the pentose cycle).

Discussion

The changes in the studied blood and liver parameters noted on the first day indicate the development of a stress reaction that caused an increase in glucose level in the blood serum due to increased breakdown of liver glycogen, the deficiency of which we noted during all periods of observation. The accumulation of lactate in the blood serum indicates an increase in the anaerobic oxidation of glucose by tissues, which leads to the activation of catabolism enzymes of purine mononucleotides.^(15,16) As a result, an increased formation of uric acid in organs and tissues is developed. At the same time, xanthine oxidase produces free radicals,^(16,17) which enhances the LP processes in cell membrane structures with the accumulation of MDA in them and contributes to the development of GSH deficiency due to GPx activation. Another factor contributing to the decrease in the GSH pool in liver cells is the activation of cypermethrin biotransformation systems (Figure 1), mainly due to GST. This enzyme promotes the formation of GSH conjugates with cypermethrin metabolites and LP products,⁽¹⁸⁾ which are subsequently excreted from the body. Thus, tissues lose GSH and are forced to synthesize it *de novo* from amino acids with the energy expenditure of ATP. In addition, the activation of microsomal oxidation, which is also part of the xenobiotic biotransformation system, can indirectly contribute to the development of GSH deficiency.⁽¹⁹⁾ An increase in the activity of microsomal oxygenase leads to the depletion of NADPH reserves, which is necessary for the formation of the reduced glutathione from GSSG.

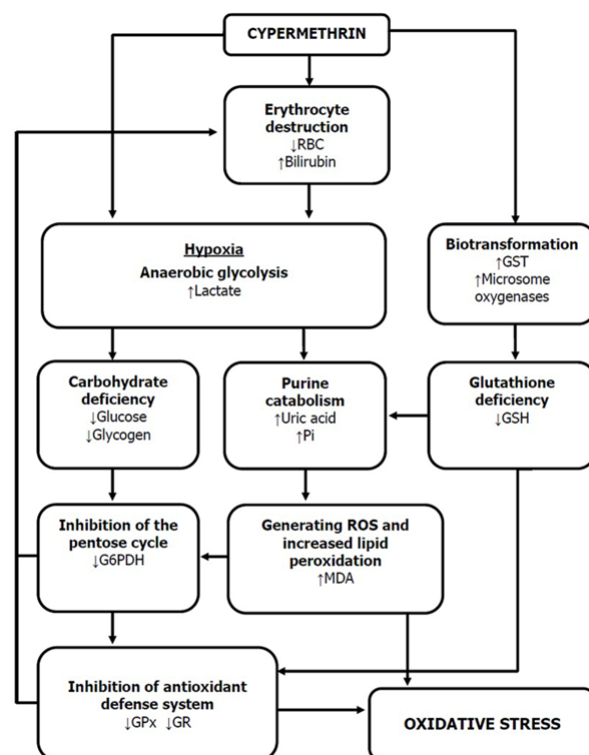


Fig. 1. Triggers of oxidative stress during the action of cypermethrin on the body.

Seven days after cypermethrin is administered to rats, glucose deficiency develops due to the depletion of glycogen stores in the liver and increased anaerobic oxidation. A decrease in RBC count enhances tissue hypoxia and further stimulates anaerobic glycolysis, leading to rapid and inefficient consumption of glucose and its conversion to lactate. The erythrocytes that are destroyed simultaneously release heme, which later turns into bilirubin, leading to its accumulation in the blood serum (Figure 1).

Carbohydrate deficiency and increased free radical oxidation in rats of the G4 lead to a decrease in the efficiency of the pentose cycle. This is evidenced by a decrease in G6PDH activity. This reduces the efficiency of the antioxidant system due to a reduction in the production of NADPH, which is necessary for the formation of the reduced glutathione from GSSG, and also leads to a deficiency of ribose-5-phosphate, which is necessary for the *de novo* synthesis of purines and their recycling. As a result, the metabolic shifts described above only increase. Suppression of G6PDH activity and a decrease in the effectiveness of the antioxidant system are the causes of damage to erythrocyte membranes and their hemolysis.

Conclusion

The triggers for the development of oxidative stress under cypermethrin exposure are lactic acidosis and increased catabolism of purine mononucleotides, accompanied by an increase in the production of free radicals and inhibition of the function of the antioxidant system. A decrease in the blood RBC, carbohydrate deficiency, and suppression of the activity of the pentose cycle 7 days after the administration of cypermethrin aggravate this condition.

Competing Interests

The authors declare that they have no competing interests.

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Prevalence of Isolated Bacteria from Urinary Tracts and Antibiotic Resistance Trend in Peja Region, Kosovo

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Abstract

Background: Urinary tract infections are the most frequent bacterial infections, causing significant morbidity at a high cost of effectiveness. The main purpose of the research was to determine the prevalence and the resistance of gram-negative bacteria in urine samples in the Peja region.

Methods and Results: This cohort longitudinal, prospective-retrospective study was conducted in the microbiological laboratories of the regional hospital in Peja and the Regional Centre of Public Health in Peja. The research includes all urine samples tested for gram-negative bacteria from 2018 to 2020. A total of 12,791 urine samples were analyzed in the study, of which 2316 (18.11%) were positive for the growth of gram-negative pathogenic strains, and 10,479 (81.89%) were negative. The most frequently isolated bacteria were *E. coli* (83.2%), followed by *Proteus* spp., *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas aeruginosa* (5.18%, 4.79%, 2.42% and 2.37% respectively). From the data of our research, we can conclude that *E. coli*, *Proteus* spp., and *Klebsiella* spp. were the three commonly isolated microorganisms in the Peja region.

A trend of increased resistance of *E. coli* to ampicillin was registered from 37.41% in 2018 to 65.58% in 2020; to tobramycin - from 3.68% in 2018 to 5.97% in 2020; to cefalexin from 29.41% in 2018 to 31.09% in 2020; to cefuroxime from 23.7% in 2018 to 28.99% in 2020; to cefotaxime from 21.32% in 2018 to 27.94% in 2020; ceftazidime from 18.84% in 2018 to 27.54% in 2020; to piperacillin from 28.73% in 2018 to 34.97% in 2020; to nitrofurantoin from 5.98% in 2018 to 8.21% in 2020; and to trimethoprim/sulfamethoxazole from 35.56% in 2018 to 42.77% in 2020. In the analyzed period, a trend of the increased resistance of *Proteus* spp. to ampicillin was registered from 31.43% in 2018 to 81.25% in 2020 and to imipenem from 4.76% in 2018 to 12% in 2020. The resistance rates of *Klebsiella* spp. strains isolated in 2020 (100% to ampicillin, 5% to amikacin, 38.46% to ofloxacin, 8.7% to imipenem, 33.33% to nitrofurantoin) were higher than those reported in 2018 (87.5%, 2.94%, 34.62%, 6.25%, and 28.21%, respectively).

Conclusion: Data from this study can be used to control antibiotic susceptibility trends, create local antibiotic policies, and help clinicians in the rational choice of antibiotic therapy, thereby preventing indiscriminate antibiotic use. (International Journal of Biomedicine. 2023;13(2):313-320.)

Keywords: urinary tract infections • gram-negative bacteria • antibiotic resistance

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Introduction

Urinary tract infections (UTIs) are the most frequent bacterial infections, causing significant morbidity at a high cost of effectiveness. They are one of the most prevalent infections in both the community (accounting for 10%-30% of infections in primary care) and hospital settings (30-40%).⁽¹⁻³⁾ The

presence of bacteria in urine more than 10⁵/ml causes UTIs.⁽⁴⁾ The pathogens that cause UTIs are different. The most common bacterial pathogens isolated from infected patients' urinary tracts are *E. coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, and *Enterococcus* species.⁽⁵⁻⁷⁾ Uropathogenic *E. coli* (UPEC) is the dominant infectious agent in both uncomplicated and complicated UTIs. *Enterococcus* spp. and *Candida* spp. are substantially

more common in complicated infections, while *Staphylococcus saprophyticus* is rare.⁽⁸⁾ Infection with UPEC increases the likelihood of recurrence within 6 months.^(9,10)

UTIs are a problem that affects all age groups and genders, but the most predisposed are women, given the anatomical construction of the urethra and the greater possibility of contamination with bacterial flora of the region. Slowing urine output at older ages makes these ages more vulnerable; greater aggressiveness of bacterial virulence in the urinary tract also increases the chance of UTIs.^(11,12) MDR infections worldwide cause 700,000 deaths, which number could increase to 10 million by 2050, depending on the discovery of new antibiotics and the type of resistance.^(13,14) Estimated costs are 3.8% of GDP.⁽¹⁵⁾ Infections caused by gram-negative bacteria resistant to antibiotics in recent years around the world and in our country have increased enormously and are endangering the lives of patients by rendering them unable to be treated. Given that for the treatment of these infections caused by pathogens resistant to more antibiotics, clinicians do not have many choices for an effective antimicrobial; then it remains important to control and monitor the spread of resistance.^(16,17) Resistance of gram-negative bacteria to antibiotics is increasing in Europe, where of particular importance is the increase of antibiotic resistance of *E. coli*, which have shown an increasing trend of antimicrobial resistance to first-line antibiotics, ciprofloxacin and trimethoprim/sulfamethoxazole. Some studies have shown an increase in the resistance of *E. coli* in UTIs for these antibiotics to 20%-45% of the isolates.^(18,19) Antibiotic use in the last 7 years in the EU decreased by 6%, but there are differences between different countries. Denmark, Latvia, the Netherlands, and Romania reduced consumption by up to 9%, while Italy and Spain increased it by 9%.⁽²⁰⁾ According to a study conducted in Kosovo in 2017, more than half of respondents (58.7%) have used antibiotics during the past year, while in the EU for 2013 - 35%. The results were higher than southern European countries such as Malta (48.0%), Cyprus (47.0%), and Romania (47.0%) while much higher than Sweden (24.0%), Poland (26.0%), and Germany (27.0%).⁽²¹⁾

The main purpose of the research was to determine the prevalence and the resistance of gram-negative bacteria in urine samples in the Peja region.

Materials and Methods

This cohort longitudinal, prospective-retrospective study was conducted in the microbiological laboratories of the regional hospital in Peja and the Regional Centre of Public Health in Peja.

The research includes all urine samples tested for gram-negative bacteria from 2018 to 2020. The epidemiological method was used, more precisely its descriptive part, to identify the results obtained from the database. The presence of gram-negative bacteria and their resistance to antibiotics was analyzed. The survey includes both male and female respondents over the age of 18. The methodology used was both quantitative and qualitative. All pathogenic isolates were studied, with a focus on gram-negative pathogenic bacteria and their medication resistance. Exclusion criteria were

isolates from patients under the age of 18; patients with more than two species of bacteria, and isolates of *Candida* spp.⁽²²⁾

Procedures

Bacteriological examination of urine samples

Microbiological laboratory examination of urine samples, urine culture (UC) is performed in the microbiological laboratories based on the standards set by EUCAST. The urine specimen for UC is usually preferred to be the morning specimen or with the patient not urinating for 4 hours prior to sampling. Significant bacteriuria is considered according to the criteria formulated by many authors, the presence of many bacteria of 100,000 and more per ml (10^5 CFU/mL of urine) with one or two pathogenic types of bacteria in culture. Then we identify the bacteria and test sensitivity to antibiotics. If both types of bacteria are non-pathogenic in the culture, then we are dealing with contamination of the sample and in this case, we only identify the bacteria and do not test sensitivity to antibiotics. A urine sample for UC is taken by spontaneous micturition. Before sampling, it is preferable: to clean the genital area to take from the middle stream of urine, not more than 2ml. in sterile plastic bottles. The time from taking to processing the sample should be 30 min to 2 hours; otherwise, the sample should be stored in the refrigerator at a temperature from +2°C to +8°C. Cultivation is done in nutrient media for urine (Blood Agar, MacConkey agar); inoculum is in the amount of 0.001 mL. The culture is incubated at 37°C for 18-24h. On the second day after incubation of the urine sample, the culture suspected of gram-negative bacteria is processed to identify these bacteria. automatic equipment (VITEK 2) is lacking, gram-negative bacteria can be identified by testing biochemical properties through a short biochemical series that consists of 1) Breakdown of carbohydrates (Kligler Iron Agar); 2) Breakdown of tryptophan (indole); 3) Decomposition of urea in deep agar; 4) Mobility test; 5) Using citrates (Simons Citrate Agar). Incubation is done aerobically at 37°C for another 18-24 hours. On the second day, in addition to processing the urine sample for identification of bacteria, antibiotic susceptibility testing is performed.⁽²³⁾

Determination of susceptibility to antibiotics

Susceptibility testing of gram-negative bacteria to antibiotics in Kosovo is done based on the standards set by EUCAST, with the method of disk diffusion in plates in the nutrient medium. From the culture grown after 24h incubation on MacConkey agar, the procedure for preparing the antibiogram is as follows:

- With sterile loops, 2-3 colonies of bacteria are taken from the medium, then placed in a test tube containing physiological saline (3 mL)
- The turbidity of the test tube is compared with the standard until turbidity is reached (0.5 McFarland)
- Sterile swabs are inserted into the suspension test tube and then removed by removing excess fluid through the walls of the test tube
- The bacteria are sown on the agar surface in three directions
- Under aseptic conditions, antibiotic discs are placed with sterile forceps at 30 mm from each other.

The media planted in this way are incubated at 37°C for 18-24 h. During this time, the antibiotic diffuses from

the disk in the agar. The bacteria will grow around the disks depending on their sensitivity to the antibiotic. The result is assessed by measuring the area of inhibition around which there is no growth of bacterial colonies. The categories of sensitivity are S-sensitive, I-intermediary, and R-resistant. Antibiotics used for antibiotic susceptibility testing of urine samples in the microbiological laboratories were ampicillin (10 µg), cefalexin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), gentamicin (30 µg), tobramycin (10 µg), amikacin (30 µg), ofloxacin (10 µg), trimethoprim-sulfamethoxazole (25 µg), nitrofurantoin (100 µg), piperacillin (30 µg), imipenem (10 µg). Microbiological analysis of urine takes 48h until final identification of bacteria and antibiotic susceptibility testing.

Data were collected from the database of Peja Hospital and Regional Center of Public Health in Peja, while for access to the data, permission was obtained from the managers, who were informed about the purpose of the research, positive aspects, and benefits of the study results.

Statistical analysis was performed using statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). The frequencies of categorical variables were compared using a chi-squared test, and a compare proportions test was applied. A probability value of $P < 0.05$ was considered statistically significant.

Results

A total of 12,791 urine samples were analyzed in the study, of which 2316(18.11%) were positive for the growth of gram-negative pathogenic strains, and 10,479(81.89%) were negative. Table 1 shows the distribution of positive and negative urine cultures in total for the period 2018-2020, as well as for each year separately.

Table 1.

Distribution of positive and negative urine cultures for the growth of gram-negative pathogenic strains by years.

Year	Urine cultures		
	Positive n (%)	Negative n (%)	Total
2018	878 (17.82)	4050 (82.18)	4928
2019	977 (18.8)	4218 (81.19)	5195
2020	461 (17.28)	2207 (82.72)	2668
Total	2316 (18.11)	10475 (81.89)	12791

From a total of 2316 gram-negative isolates, the most frequently isolated bacteria were *E. coli* (83.2%), followed by *Proteus* spp. (5.18%), *Klebsiella* spp. (4.79%), *Acinetobacter* spp. (2.42%), and *Pseudomonas aeruginosa*, the last isolated in a very small percentage of samples (2.37%). By years: the prevalence of *E. coli* was 83.49%, 83.83%, and 81.34%, respectively, in 2018, 2019, and 2020, without a statistically significant difference ($P=0.48$); the prevalence of *Klebsiella* spp. was 4.78%, 4.71%, and 4.99%,

respectively, in 2018, 2019 and 2020, without a statistically significant difference ($P=0.97$); the prevalence of *Proteus* spp. was 4.1%, 5.22%, and 7.16%, respectively, in 2018, 2019 and 2020, without a statistically significant difference ($P=0.056$); the prevalence of *Pseudomonas aeruginosa* was 2.39%, 2.46%, and 2.17%, respectively, in 2018, 2019 and 2020, without a statistically significant difference ($P=0.94$); the prevalence of *Acinetobacter* spp. was 3.42, 1.84% and 1.74%, respectively, in 2018, 2019 and 2020, with a statistically significant difference in prevalence between 2018 and 2019 ($P=0.0324$) (Table 2).

Table 2.

Prevalence of gram-negative bacteria by years

Bacteria	Total N=2316 (%)	2018 n=878 (%)	2019 n=977 (%)	2020 n=461 (%)	P-value
<i>E. coli</i>	1927 (83.2)	733 (83.49)	819 (83.33)	375 (81.34)	0.48
<i>Klebsiella</i> spp.	111 (4.79)	42 (4.78)	46 (4.71)	23 (4.99)	0.97
<i>Proteus</i> spp.	120 (5.18)	36 (4.10)	51 (5.22)	33 (7.16)	0.056
<i>P. aeruginosa</i>	55 (2.37)	21 (2.39)	24 (2.46)	10 (2.17)	0.94
<i>Acinetobacter</i> spp.	56 (2.42)	30 (3.42)	18 (1.84)	8 (1.74)	0.050 0.0324*
Others	47 (2.03)	16 (1.82)	19 (1.94)	12 (2.60)	0.610

*- 2018 vs. 2019

In the analyzed period, a trend of increased resistance of *E. coli* to ampicillin was registered from 37.41% in 2018 to 65.58% in 2020; to tobramycin - from 3.68% in 2018 to 5.97% in 2020; to cefalexin from 29.41% in 2018 to 31.09% in 2020; to cefuroxime from 23.7% in 2018 to 28.99% in 2020; to cefotaxime from 21.32% in 2018 to 27.94% in 2020; ceftazidime from 18.84% in 2018 to 27.54% in 2020; to piperacillin from 28.73% in 2018 to 34.97% in 2020; to nitrofurantoin from 5.98% in 2018 to 8.21% in 2020; and to trimethoprim/sulfamethoxazole from 35.56% in 2018 to 42.77% in 2020.

Increased resistance of *E. coli* to ampicillin in 2019 versus 2018, and in 2020 vs. 2018 and 2019 was statistically significant ($P < 0.0001$), as well as to cefalexin in 2020 vs. 2019 ($P=0.0314$), to nitrofurantoin in 2019 vs. 2018 ($P=0.017$), and to trimethoprim/sulfamethoxazole in 2020 vs. 2018 ($P=0.0247$) (Table 3).

The resistance rates of *Klebsiella* spp. strains isolated in 2020 (100% to ampicillin, 5% to amikacin, 38.46% to ofloxacin, 8.7% to imipenem, 33.33% to nitrofurantoin) were higher than those reported in 2018 (87.5%, 2.94%, 34.62%, 6.25%, and 28.21%, respectively). The difference in the resistance of *Klebsiella* spp. to ampicillin in 2020 vs. 2019 was statistically significant ($P=0.0075$) (Table 4).

Table 3.**Distribution of resistant *E. coli* strain isolates by years**

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	92	35/40 (87.5)	34/46 (73.91)	23/23 (100)	$P_{1-2}=0.1166$; $P_{1-3}=0.0796$; $P_{2-3}=0.0075$
Amikacin	2	1/34 (2.94)	0/45 (0)	1/20 (5)	$P_{1-2}=0.2501$; $P_{1-3}=0.7013$; $P_{2-3}=0.1336$
Gentamicin	16	7/41 (17.07)	6/45 (13.33)	3/23 (13.04)	$P_{1-2}=0.6306$; $P_{1-3}=0.6725$; $P_{2-3}=0.9736$
Tobramycin	7	4/25 (16)	3/23 (13.04)	0/14 (0)	$P_{1-2}=0.7739$; $P_{1-3}=0.1189$; $P_{2-3}=0.1644$
Cefalexin	47	14/26 (53.85)	22/43 (51.16)	11/21 (52.38)	$P_{1-2}=0.8296$; $P_{1-3}=0.9209$; $P_{2-3}=0.9275$
Cefuroxime	30	12/25 (48)	12/24 (50)	6/14 (42.86)	$P_{1-2}=0.8898$; $P_{1-3}=0.7605$; $P_{2-3}=0.6748$
Cefotaxime	29	12/26 (46.15)	11/24 (45.83)	6/14 (42.86)	$P_{1-2}=0.9821$; $P_{1-3}=0.8438$; $P_{2-3}=0.8609$
Ceftazidime	26	12/26 (46.15)	8/15 (53.33)	6/14 (42.86)	$P_{1-2}=0.6617$; $P_{1-3}=0.8438$; $P_{2-3}=0.5796$
Ofloxacin	17	9/26 (34.62)	3/12 (25)	5/13 (38.46)	$P_{1-2}=0.5584$; $P_{1-3}=0.8161$; $P_{2-3}=0.4800$
Imipenem	8	2/32 (6.25)	4/44 (9.09)	2/23 (8.7)	$P_{1-2}=0.6525$; $P_{1-3}=0.7324$; $P_{2-3}=0.9580$
Piperacillin	55	24/36 (66.67)	18/40 (45)	13/22 (59.09)	$P_{1-2}=0.0595$; $P_{1-3}=0.5634$; $P_{2-3}=0.2923$
Nitrofurantoin	30	11/39 (28.21)	13/37 (35.14)	6/18 (33.33)	$P_{1-2}=0.5187$; $P_{1-3}=0.6971$; $P_{2-3}=0.8956$
Trimethoprim /Sulfamethoxazole	57	21/36 (58.33)	23/39 (58.97)	13/22 (59.09)	$P_{1-2}=0.9555$; $P_{1-3}=0.9549$; $P_{2-3}=0.9928$

*R-resistance; n-number of patients***Table 4.****Distribution of resistant isolates of *Klebsiella* spp. by years**

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	92	35/40 (87.5)	34/46 (73.91)	23/23 (100)	$P_{1-2}=0.1166$; $P_{1-3}=0.0796$; $P_{2-3}=0.0075$
Amikacin	2	1/34 (2.94)	0/45 (0)	1/20 (5)	$P_{1-2}=0.2501$; $P_{1-3}=0.7013$; $P_{2-3}=0.1336$
Gentamicin	16	7/41 (17.07)	6/45 (13.33)	3/23 (13.04)	$P_{1-2}=0.6306$; $P_{1-3}=0.6725$; $P_{2-3}=0.9736$
Tobramycin	7	4/25 (16)	3/23 (13.04)	0/14 (0)	$P_{1-2}=0.7739$; $P_{1-3}=0.1189$; $P_{2-3}=0.1644$
Cefalexin	47	14/26 (53.85)	22/43 (51.16)	11/21 (52.38)	$P_{1-2}=0.8296$; $P_{1-3}=0.9209$; $P_{2-3}=0.9275$
Cefuroxime	30	12/25 (48)	12/24 (50)	6/14 (42.86)	$P_{1-2}=0.8898$; $P_{1-3}=0.7605$; $P_{2-3}=0.6748$
Cefotaxime	29	12/26 (46.15)	11/24 (45.83)	6/14 (42.86)	$P_{1-2}=0.9821$; $P_{1-3}=0.8438$; $P_{2-3}=0.8609$
Ceftazidime	26	12/26 (46.15)	8/15 (53.33)	6/14 (42.86)	$P_{1-2}=0.6617$; $P_{1-3}=0.8438$; $P_{2-3}=0.5796$
Ofloxacin	17	9/26 (34.62)	3/12 (25)	5/13 (38.46)	$P_{1-2}=0.5584$; $P_{1-3}=0.8161$; $P_{2-3}=0.4800$
Imipenem	8	2/32 (6.25)	4/44 (9.09)	2/23 (8.7)	$P_{1-2}=0.6525$; $P_{1-3}=0.7324$; $P_{2-3}=0.9580$
Piperacillin	55	24/36 (66.67)	18/40 (45)	13/22 (59.09)	$P_{1-2}=0.0595$; $P_{1-3}=0.5634$; $P_{2-3}=0.2923$
Nitrofurantoin	30	11/39 (28.21)	13/37 (35.14)	6/18 (33.33)	$P_{1-2}=0.5187$; $P_{1-3}=0.6971$; $P_{2-3}=0.8956$
Trimethoprim /Sulfamethoxazole	57	21/36 (58.33)	23/39 (58.97)	13/22 (59.09)	$P_{1-2}=0.9555$; $P_{1-3}=0.9549$; $P_{2-3}=0.9928$

R-resistance; n-number of patients

In the analyzed period, a trend of the increased resistance of *Proteus* spp. to ampicillin was registered from 31.43% in 2018 to 81.25% in 2020 and to imipenem from 4.76% in 2018 to 12% in 2020. The increased resistance of *Proteus* spp. to

ampicillin in 2020 vs. 2018 and 2020 vs. 2019 was statistically significant ($P<0.0001$, and $P=0.0037$, respectively). In addition, we found the increased resistance of *Proteus* spp. to imipenem in 2020 vs. 2019 ($P=0.0211$) (Table 5).

Table 5.

Distribution of resistant isolates of *Proteus* spp. by years

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	61	11/35 (31.43)	24/49 (48.98)	26/32 (81.25)	$P_{1-2}=0.1098$; $P_{1-3}<0.0001$; $P_{2-3}=0.0037$
Amikacin	2	0/18 (0)	1/47 (2.13)	1/27 (3.7)	$P_{1-2}=0.5358$; $P_{1-3}=0.4145$; $P_{2-3}=0.6905$
Gentamicin	18	4/35 (11.43)	9/50 (18)	5/31 (16.13)	$P_{1-2}=0.4103$; $P_{1-3}=0.5816$; $P_{2-3}=0.8298$
Tobramycin	4	1/8 (12.5)	3/10 (30)	0/6 (0)	$P_{1-2}=0.3885$; $P_{1-3}=0.3865$; $P_{2-3}=0.1495$
Cefalexin	20	0/7 (0)	13/48 (27.08)	7/26 (26.92)	$P_{1-2}=0.1185$; $P_{1-3}=0.1278$; $P_{2-3}=0.9883$
Cefuroxime	5	2/9 (22.22)	3/10 (30)	0/7 (0)	$P_{1-2}=0.7082$; $P_{1-3}=0.1967$; $P_{2-3}=0.1213$
Cefotaxime	3	1/9 (11.11)	2/10 (20)	0/6 (0)	$P_{1-2}=0.6055$; $P_{1-3}=0.4142$; $P_{2-3}=0.2568$
Ceftazidime	3	1/9 (11.11)	2/7 (28.57)	0/7 (0)	$P_{1-2}=0.3901$; $P_{1-3}=0.3778$; $P_{2-3}=0.1410$
Ofloxacin	2	0/9 (0)	1/7 (14.29)	1/7 (14.29)	$P_{1-2}=0.2568$; $P_{1-3}=0.2568$; $P_{2-3}=1.0000$
Imipenem	4	1/21 (4.76)	0/43 (0)	3/25 (12)	$P_{1-2}=0.1525$; $P_{1-3}=0.3906$; $P_{2-3}=0.0211$
Piperacillin	16	5/22 (22.73)	6/31 (19.35)	5/22 (22.73)	$P_{1-2}=0.7671$; $P_{1-3}=1.0000$; $P_{2-3}=0.7671$
Nitrofurantoin	60	21/34 (61.76)	26/44 (59.9)	13/24 (54.17)	$P_{1-2}=0.8684$; $P_{1-3}=0.5666$; $P_{2-3}=0.6499$
Trimethoprim /Sulfamethoxazole	50	16/35 (45.71)	22/47 (46.81)	12/28 (42.86)	$P_{1-2}=0.9218$; $P_{1-3}=0.8224$; $P_{2-3}=0.7413$

R-resistance; n-number of patients

Table 6.

Distribution of resistant isolates of *Pseudomonas aeruginosa* by years.

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	41	15/19 (78.95)	17/18 (94.44)	9/9 (100)	$P_{1-2}=0.1742$; $P_{1-3}=0.1443$; $P_{2-3}=0.4793$
Amikacin	6	1/12 (8.33)	4/24 (16.67)	1/9 (11.11)	$P_{1-2}=0.5012$; $P_{1-3}=0.8340$; $P_{2-3}=0.6961$
Gentamicin	12	3/21 (14.29)	7/23 (30.43)	2/10 (20)	$P_{1-2}=0.2071$; $P_{1-3}=0.6910$; $P_{2-3}=0.5426$
Tobramycin	1	0/4 (0)	1/8 (12.5)	0/2 (0)	$P_{1-2}=0.4795$; $P_{2-3}=0.6171$
Cefalexin	22	1/1 (100)	14/17 (82.35)	7/9 (77.78)	$P_{1-2}=0.6547$; $P_{1-3}=0.6171$; $P_{2-3}=0.7827$
Cefuroxime	7	3/4 (75)	4/4 (100)	0/1 (0)	$P_{1-2}=0.3173$; $P_{1-3}=0.2207$; $P_{2-3}=0.0455$
Cefotaxime	7	3/4 (75)	4/5 (80)	0/1 (0)	$P_{1-2}=0.8658$; $P_{1-3}=0.2207$; $P_{2-3}=0.1573$
Ceftazidime	9	1/4 (25)	7/9 (77.78)	1/2 (50)	$P_{1-2}=0.0828$; $P_{1-3}=0.5762$; $P_{2-3}=0.4468$
Ofloxacin	2	1/3 (33.33)	1/3 (33.33)	0/1 (0)	$P_{1-2}=1.0000$; $P_{1-3}=0.5637$; $P_{2-3}=0.5637$
Imipenem	8	2/15 (13.33)	4/24 (16.67)	2/7 (28.57)	$P_{1-2}=0.7813$; $P_{1-3}=0.3990$; $P_{2-3}=0.4903$
Piperacillin	20	6/17 (35.29)	10/16 (62.5)	4/9 (44.44)	$P_{1-2}=0.1237$; $P_{1-3}=0.6546$; $P_{2-3}=0.3922$
Nitrofurantoin	37	17/20 (85)	15/23 (65.22)	5/6 (83.33)	$P_{1-2}=0.1428$; $P_{1-3}=0.9223$; $P_{2-3}=0.4014$
Trimethoprim /Sulfamethoxazole	39	16/20 (80)	18/20 (90)	5/8 (62.5)	$P_{1-2}=0.3819$; $P_{1-3}=0.3428$; $P_{2-3}=0.0919$

R-resistance; n-number of patients

The resistance rates of *P. aeruginosa* strains isolated in 2020 (100% to ampicillin and 28.57% to imipenem) were higher than those reported in 2018 (78.95% and 13.33%,

respectively). The difference in the reduced rate of resistance of *P. aeruginosa* to cefuroxime in 2020 vs. 2019 ($P=0.0455$) was statistically significant (Table 6).

Table 7.

Distribution of resistant isolates of *Acinetobacter* spp. by years

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	38	16/30 (53.33)	16/18 (88.89)	6/8 (75)	$P_{1-2}=0.0123$; $P_{1-3}=0.2764$; $P_{2-3}=0.3743$
Amikacin	6	1/14 (7.14)	4/18 (22.22)	1/7 (14.29)	$P_{1-2}=0.2513$; $P_{1-3}=0.6076$; $P_{2-3}=0.6628$
Gentamicin	21	12/30 (40)	8/17 (47.06)	1/7 (14.29)	$P_{1-2}=0.6417$; $P_{1-3}=0.2057$; $P_{2-3}=0.1401$
Tobramycin	No resistance				
Cefalexin	15	0/2 (0)	11/17 (64.71)	4/7 (57.14)	$P_{1-2}=0.0879$; $P_{1-3}=0.1763$; $P_{2-3}=0.7332$
Cefuroxime	1	0/2 (0)	0/0	1/1 (100)	
Cefotaxime	1	0/2 (0)	0/0	1/1 (100)	
Ceftazidime	1	0/2	0/0	1/1 (100)	
Ofloxacin	2	1/2 (50)	0/0	1/1 (100)	
Imipenem	1	0/17 (0)	1/14 (7.14)	0/7 (0)	
Piperacillin	15	6/20 (30)	5/8 (62.5)	4/7 (57.14)	$P_{1-2}=0.1183$; $P_{1-3}=0.2092$; $P_{2-3}=0.8382$
Nitrofurantoin	30	14/26 (53.85)	14/17 (82.35)	2/5 (40)	$P_{1-2}=0.0581$; $P_{1-3}=0.5766$; $P_{2-3}=0.0678$
Trimethoprim /Sulfamethoxazole	32	17/26 (65.38)	11/17 (64.71)	4/8 (50)	$P_{1-2}=0.9645$; $P_{1-3}=0.4406$; $P_{2-3}=0.4926$

R-resistance; n-number of patients

Table 8.

Distribution of isolated gram-negative bacteria (%) in relation to number of antibiotics

Bacteria	Number of antibiotics to which resistance exists										
	0	1	2	3	4	5	6	7	8	9	
<i>E. coli</i> (n=1927)	39.44	23.35	16.81	9.65	4.57	2.13	0.93	1.35	1.4	0.31	0.05
<i>Klebsiella</i> spp. (n=111)	9.91	18.02	13.51	16.22	9.01	4.5	5.41	6.3	9.91	5.41	1.8
<i>Proteus</i> spp. (n=120)	15	28.33	23.33	15.83	10	4.17	1.67	0.83	0.83	0	0
<i>P. aeruginosa</i> (n=55)	3.64	9.09	10.91	20	25.45	12.73	7.27	5.45	5.45	0	0
<i>Acinetobacter</i> spp. (n=56)	14.29	19.64	5.36	12.5	26.79	17.86	1.79	0	1.79	0	0

No trend of the increased resistance of *Acinetobacter* spp. to any of the tested antibiotics was registered. The difference in the reduced resistance rate of *P. aeruginosa* to cefalexin in 2019 vs. 2018 ($P=0.034$) and 2020 vs. 2018 ($P=0.02$) was statistically significant.

The resistance rates of *Acinetobacter* spp. strains isolated in 2020 (75% to ampicillin, 14.29% to amikacin, 57.14% to cefalexin, and 57.14% to piperacillin) were higher than those reported in 2018 (53.33%, 7.14%, 0% and 30.0%, respectively). The difference in the increased rate of resistance of *Acinetobacter* spp. to ampicillin in 2019 vs. 2018 was statistically significant ($P=0.0123$) (Table 7).

Table 8 shows the distribution of isolated gram-negative bacteria in relation to the number of antibiotics to which they show resistance. Resistance to more than one antibiotic was presented by 37.21% of bacteria from the strain *E. coli*, 72.07% from the strain *Klebsiella* spp., 56.67% from the

strain *Proteus* spp., 87.27% from the strain *P. aeruginosa*, and 66.07% from the strain *Acinetobacter* spp.

Discussion

Current research examines the prevalence of UTIs, as well as the pathogens involved in infection and their sensitivity profile. There were 2316(18.11%) patients with significant bacteriuria among the 12,791 urinary specimens collected during this research. In Nigeria, the National Hospital Abuja had a lower incidence rate (13.1%).⁽²⁴⁾ In the Saudi Arabian research, the frequency was higher (32.6%).⁽¹²⁾ In Peja Region, from a total of 2316 gram-negative isolates, the most frequently isolated bacteria were *E. coli* (83.2%), followed by *Proteus* spp. (5.15%), *Klebsiella* spp. (4.79%), *Acinetobacter* spp. (2.42%), and *P. aeruginosa* (2.37%), the last isolated in a very small percentage of samples. *E. coli* was the most prevalent

aetiologic agent isolated (80.5%) in a study conducted by Raka et al.⁽²⁵⁾ in Kosovo in 2001, followed by *Proteus* spp. (6.1%), *Klebsiella* spp. (5.9%), *Citrobacter* (5.1%), and *Mycobacterium tuberculosis* (0.8%). *E. coli* was also the most common bacteria in another research. During the 2013-2014 research on Tohid Hospital in Sanandaj,⁽²⁶⁾ the most common isolated pathogen was *Escherichia coli*, which was responsible for 63.09% of positive cultures.

By years, in Peja Region, the difference in prevalence of *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Pseudomonas aeruginosa* was not significant; only for *Acinetobacter* spp. was there a statistically significant difference in prevalence between 2018 and 2019 ($P=0.033$). In the research with 28 pediatric patients, *E. coli* (53.5%) was the most commonly detected gram-negative bacteria. The number of bacteria isolates was comparable to other studies conducted in various countries. *E. coli* was the most commonly isolated organism in South America, accounting for 39.7% of UTI cases, followed by *Enterococcus* spp. (11.5%).⁽²⁷⁾ Similarly, *E. coli* was the most isolated uropathogen in China, accounting for 66.01% of UTI cases, followed by *Enterococcus* spp. (5.91%).⁽²⁸⁾

As bacterial resistance has increased in recent decades,⁽²⁹⁻³¹⁾ the isolates in this research demonstrated high resistance. In our study, 37.21% of the bacteria from the strain *E. coli*, 72.07% from the strain *Klebsiella* spp., 56.67% from the strain *Proteus* spp., 87.27% from the strain *P. aeruginosa*, and 66.07% from the strain *Acinetobacter* spp. showed resistance to more than one antibiotic.

Numerous organizations and programs are currently working to combat antibiotic resistance,⁽³¹⁾ but the first step in obtaining a proper management and good control policy for decreasing the development of antibiotic resistance among microorganisms, particularly pathogens, is the evaluation and practical assessment of antibiotic resistance patterns among specific groups of patients in a country. Results of the antibiogram test for bacterial isolates recovered from UTI revealed that amikacin and imipenem were the most effective antimicrobials against the strains. *E. coli*, as the most common pathogen of UTIs in 2020, showed the most resistance to ampicillin (65.58%) and the least resistance to imipenem (1.09%) indicated. In 2018-2020, a trend of increased resistance of *E. coli* to ampicillin was registered from 37.41% in 2018 to 65.58% in 2020.

In the research on Tohid Hospital in Sanandaj, during 2013-2014, urinary pathogens were mostly resistant to ampicillin (64.15%) and trimethoprim/sulfamethoxazole (62.67%). Resistance was lowest to imipenem (0.7%) and amikacin (1.01%). The most prevalent pathogen of UTIs, *E. coli*, demonstrated the greatest resistance to ampicillin (43.87%) and the least resistance to nitrofurantoin (3.62%).

From the data of our research, we can conclude that the *E. coli*, *Proteus* spp., and *Klebsiella* spp. were the three commonly isolated microorganisms in the Peja region. Furthermore, most isolated bacterial microbes were resistant to antibiotics used in clinical practices in the country, which can be an emerging worry for a country's health control systems. It appears that administrators should use these medications with extreme caution and precision when treating UTIs and/

or other infections. This requires health practitioners and policymakers to pay close attention to the resistance pattern in their clinical practice and policymaking processes. Data from this study can be used to control antibiotic susceptibility trends, create local antibiotic policies, and help clinicians in the rational choice of antibiotic therapy, thereby preventing indiscriminate antibiotic use.

Limitations of the study

Because only those who visited the health research laboratory through referral or on their own initiative were included in the study, this study may not reflect the general population of the Peja Region. The study included only antibiotics that have been examined in the regional health research laboratory.

Ethical considerations

Study approval was obtained from the Committee on Ethical Issues, Kosovo Doctors Chamber nr. 16/2022.

Competing Interests

The authors declare that they have no competing interests.

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Effects of the COVID-19 Pandemic on Drug Abuse Among Inmates in Kosovo Prisons

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Abstract

Background: The COVID-19 pandemic has led to an increase in drug use among inmates in prisons worldwide, including Kosovo, due to restrictions on visitation and addiction recovery programs. This study analyzed drug use among 33,144 inmates in Kosovo prisons over nine years and aimed to identify potential solutions, foster collaboration between relevant institutions, and develop better programs and treatments.

Methods and Results: The study is based on data from the medical files of inmates examined over nine years (2014-2022) in 10 Kosovo prison health units. From the total number of inmates, 8.22% were identified as drug users, and the percentage of drug use among inmates steadily increased from 5.8% in 2014 to 15.48% in 2019. In 2020, the first year of the pandemic, there was a decrease of 1.16% (from 15.48% to 14.28%), which was the first decrease in six years. The study also found a disproportional decrease in the number of inmates admitted during the same period. The Medico Psycho Therapeutic Center “Labyrinth” showed a similar trend. Suicide attempts, death under custody, and tobacco use increased linearly, with alcohol use remaining stable.

Conclusion: The study highlights the need for effective prevention, treatment, and management strategies for drug use among inmates and the general population in Kosovo. Continuous collaboration between relevant institutions and stakeholders is essential to improve the health and well-being of those affected by drug use and create a brighter future for Kosovo. (International Journal of Biomedicine. 2023;13(2):321-325.)

Keywords: inmate • drug abuse • COVID-19 • prevention • management strategies • Kosovo

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Introduction

The COVID-19 pandemic has had a profound impact on the world, and its effects have been felt in Kosovo.⁽¹⁾ Among the areas that have been particularly affected is drug use among prison inmates.⁽²⁾ Assessing the impact of the pandemic on

drug use among inmates is a complex issue that requires a comprehensive approach.⁽³⁾ The restrictions on visitation and other programs designed to help inmates with addiction have contributed to increased drug use, as inmates have turned to drugs to cope with their isolation and anxiety.⁽⁴⁾ The disruptions in the drug supply chain have also made it harder for inmates to access the usual drugs, leading to an increase in the use of more potent and dangerous drugs like fentanyl. Therefore, it is critical to take a comprehensive approach to address this issue and ensure that inmates receive the necessary support and treatment to overcome their addiction during these challenging times.

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Moreover, the issue of drug use in Kosovo is not limited to prisons. According to recent studies, there are over 30,000 confirmed drug users in Kosovo, with approximately 6,000 requiring addiction treatment.⁽⁵⁾ Additionally, drug, alcohol, and tobacco use among secondary school students in Kosovo is a cause for concern, with 43.5% of young people having smoked cigarettes, 37.8% having consumed alcohol, and 3.8% having tried marihuana. Heroin use is reported to be at 0.1% in the general population, with cannabis use at 2.1% and cocaine use at 0.3%.⁽⁶⁾ Other drugs, such as ecstasy, amphetamines, and illegally obtained prescription drugs like Tramadol and Methadone/Heptanone are also prevalent in the country, with 2.6% of respondents reporting the use of illegally obtained prescription drugs at an average age of 17.⁽⁵⁾

The national strategy against drugs in Kosovo has two components:

1) reducing the supply of drugs, a component that the Kosovo Police manage;

2) decreasing demand for drugs, a component managed by other sectors, like education, youth, health, and social sectors.

However, the COVID-19 pandemic has created additional challenges, making it crucial to reevaluate the existing strategies and develop new approaches to address the increasing rates of drug use in Kosovo.

The purpose:

- To conduct an up-to-date analysis of drug abuse among inmates in Kosovo prisons and compare it with drug abuse in other institutions, such as the Medico Psychotherapeutic Center “Labyrinth.”
- To identify potential solutions for the prevention, treatment, and management of drug abuse among inmates, particularly within the prison system, by exploring various indicators related to drug abuse in prisons and other health organizations.
- To foster greater collaboration between relevant institutions, resulting in improved and high-quality services for drug abusers based on follow-up results.
- To persuade stakeholders to develop better programs and treatments that are aligned with the current indicators and number of drug abusers in Kosovo, both inside and outside the prison system.

Materials and Methods

The methodology of this survey

Data were taken from the medical files of 33,144 inmates examined for 9 years (2014-2022). The data were obtained from the medical protocol of all 10 Kosovo prison health units with coverage of 100% of all inmates admitted into the prisons during the above-mentioned period.

Data obtained were compared to some of the data from MPTC “Labyrinth” which is the private medical center dealing with the most drug abusers in the country.

Our aim was to process and present all those data and draw conclusions and recommendations for promoting the prevention of drug abuse in prisons, as well as to give proposals and facts for initiating the adoption of the existing

national drug strategy as a sustainable national strategy and protocols for all, based on findings and recommendations.

Results

Over the course of nine years, from 2014 to 2022, a total of 33,144 inmates were admitted to Kosovo prisons. Among them, 2,726 individuals (8.22%) were identified as drug abusers. The percentage of drug abuse among inmates steadily increased from 5.8% in 2014 to 15.48% in 2019. However, in 2020, for the first time in six years, there was a decrease of 1.16% in the percentage of drug abusers among inmates, from 15.44% to 14.28%. It is worth noting that this change occurred during the pandemic period. The decrease started immediately in the year 2020 to continue slightly in the year 2022 as well. It is notable that, despite a decrease in the number of prisoners during the analyzed period (from 2014 to 2022), the percentage of drug abusers has increased (Fig.1).

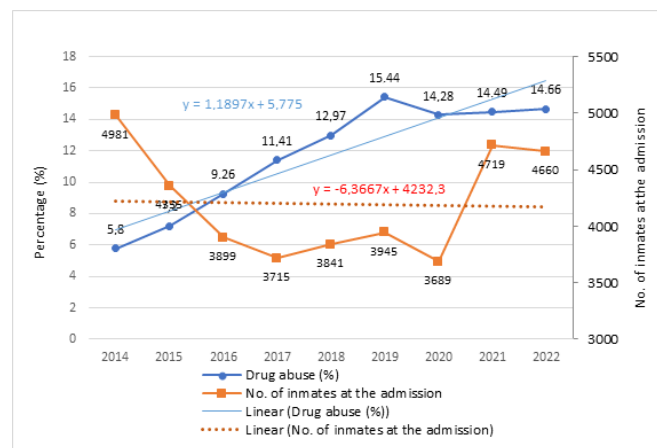


Fig. 1. The trend of drug abuse in the Kosovo Prisons (%) and number of inmates admitted in Kosovo prisons for the period 2014 -2022.

The same trend is presented at Medico Psycho Therapeutic Center “Labyrinth,” there is a decrease in new cases from the year 2019 to 2020 and 2021, touching the bottom of the trend curve, like the year 2015, and starting rapidly increasing at the year 2022.

Suicide attempts have linearly increased. Based on the linear trend equation ($y=0.39x+4.09$) the average incidence rate of suicide attempts for the period 2014-2022 was 4 cases/1000 inmates, with an increase of 0.39 per thousand for each year. There were some variations in the incidence rate, with some decrease during 2019, but an increase again in 2020, mostly caused by pandemic measures and limitations of the routine daily process, like family visits and fear of the virus (Fig.2).

Alcohol use remains almost at the same levels during the years, but tobacco use is increasing permanently at the admission by newcomers, which is a big issue in the country in general and in the prisons specifically (Fig.3).

There were variations in the HCV and HBV infection rates, with some decreases from 2014 to 2022 (Fig.4).

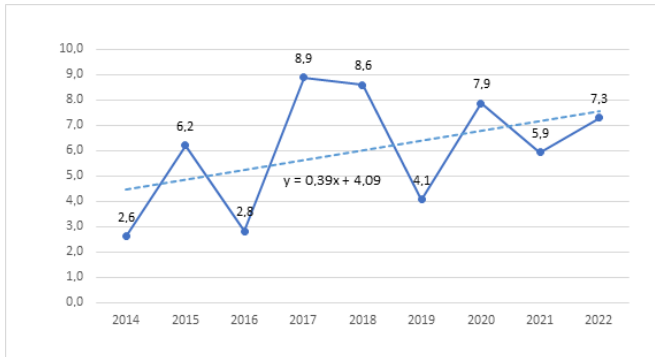


Fig. 2. The incidence rate of suicide attempts (per 1000 inmates) in Kosovo prisons for the period 2014-2022.

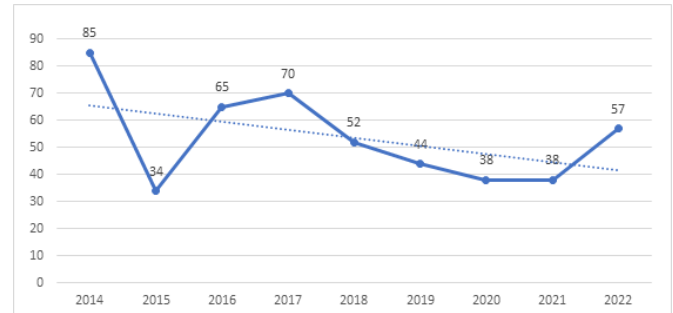


Fig. 5. The number of new cases being treated for drug addiction in MPTC "Labyrinth" (2014-2022).

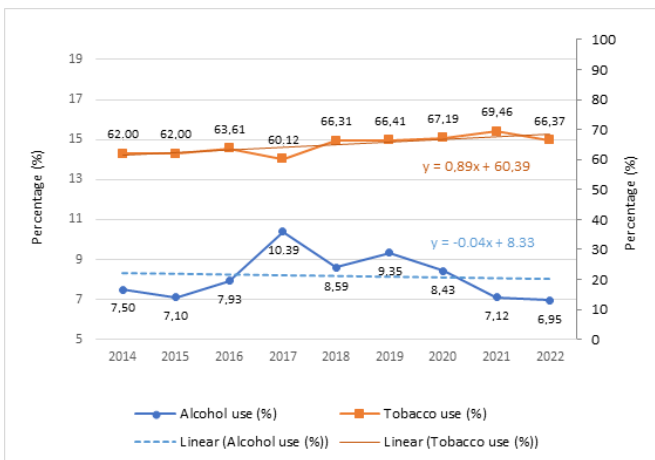


Fig. 3. The trend of alcohol and tobacco use in Kosovo prisons (2014-2022).

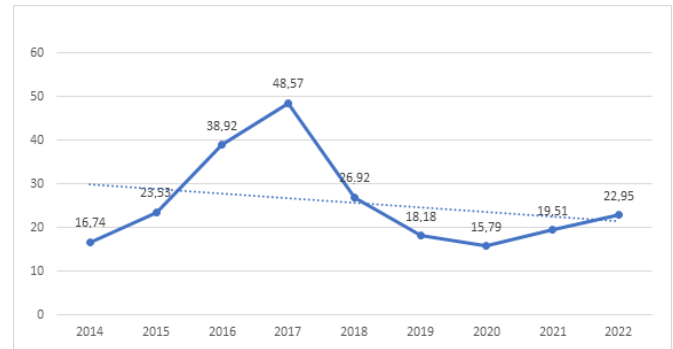


Fig. 6. The annual report and internal database of MPTC "Labyrinth" (2014-2022): percentage of marijuana users.

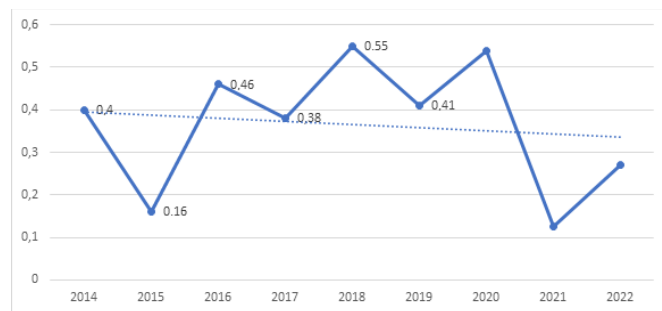


Fig. 4. The trend of HCV and HBV infection rates in Kosovo prisons (2014-2022).

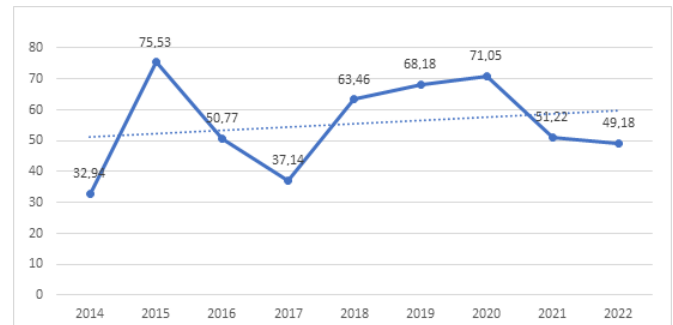


Fig. 7. The annual report and internal database of MPTC "Labyrinth" (2014-2022): percentage of heroin users.

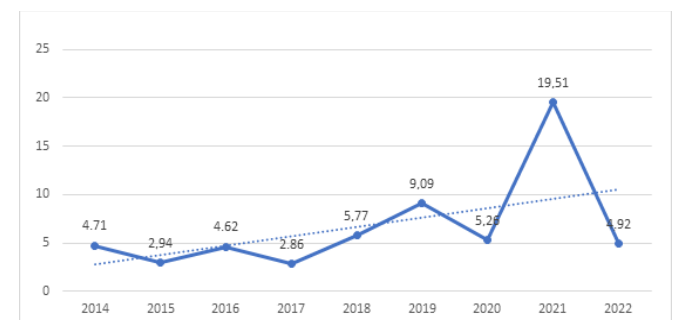


Fig. 8. The annual report and internal database of MPTC "Labyrinth" (2014-2022): percentage of cocaine users.

The annual report and internal database of MPTC "Labyrinth" have shown that during the nine-year period (2014-2022), the number of new cases being treated for drug addiction was linearly decreased from 85 cases (2014) to 38 cases (2020 – 2021), started to rapidly increase at the year 2022 (Fig.5).

During this period (2014-2022), the percentage of marijuana users has decreased but other drugs, like heroin and cocaine, have increased linearly but permanently (Fig.6-8).

Discussion

Based on the available data, the percentage of drug abusers admitted to prisons in 2020 decreased by 1.16%, compared to the previous year. However, this trend was not sustained, as the percentage of drug abusers admitted in 2021 increased again, following a linear upward trend that has been observed since 2014.

Other indicators, such as tobacco, attempting suicide and death under custody, have increased steadily with the same trend.⁽⁷⁾ The highest number of inmates who attempted suicide during the pandemic period were drug abusers.

Death under custody is not directly connected with any of the mentioned indicators but is increasing linearly, mostly caused by chronic diseases and the aging of the inmates. There were 24.40 per 100,000 inmates that committed suicide in Kosovo prisons during the period 1999-2022, in total, twenty-three (23) cases, which is much lower than in European countries,^(8,9) such as England and Wales (at 107 suicides per 100,000 prisoners, 95% CI: 96-118) and Denmark (147; 95% CI: 93-200) had rates higher than some other countries.⁽¹⁰⁾

If we compare suicides in Kosovo prisons with those in prisons in different countries, for the period 2000-2022, then we have the following data:

- In the civilian population, data show that we have 3 to 4 suicides per 100,000 inhabitants, while 18 suicides per 100,000 prisoners.
- The average number of suicides in European prisons is 62 suicides per 100,000 prisoners.
- Suicides in Kosovo prisons have been caused by different factors, different ages, and were committed in different ways.^(7,11-14)
- During the pandemic restriction time, the indicators such as tobacco and suicide attempts rapidly increased because of restriction of movement and visits, and stress caused by the strict pandemic measures.⁽¹⁵⁾
- The number of newly admitted drug users during 2020 slightly decreased because of movement restrictions, difficulties in committing the crime and difficulty finding drugs as a result of closed borders and decreased traffic in drugs.⁽¹⁶⁾ The trend of drug users entering prisons increased again after the obstacles to finding drugs were gone.

Conclusions

The study revealed a decrease in the percentage of drug abusers among inmates in Kosovo prisons during the pandemic period. However, the study also identified other concerning trends, including increases in tobacco use and suicide attempts in custody.

This report has underscored the alarming trend of increasing drug abuse among inmates in Kosovo prisons between 2014 and 2022. While a decrease was observed in 2020, it is imperative to address the underlying causes of drug

abuse and take proactive measures to prevent future spikes. The pandemic had a temporary impact on reducing drug abuse due to the restrictive measures implemented inside and outside of prisons. However, after the measures were lifted, there was a rapid increase in drug abuse.

The authors recommend a review and revision of the national strategy for drug prevention, with a particular focus on prison health services. To ensure comprehensive coverage of all necessary factors and stakeholders, including inmates, the strategy should be separated into two independent components. Additionally, current strategies and plans for preventing and treating drug abuse are inadequate.

There is a pressing need for better policies, implementation documents, and activities to provide more effective and professional drug treatment, especially in challenging situations such as pandemics and movement restrictions.

Addressing the issue of drug abuse in prisons requires a multidisciplinary approach that involves various stakeholders and a commitment to providing inmates with the necessary support and treatment to overcome drug addiction.

Competing Interests

The authors declare that they have no competing interests.

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Knowledge, Attitude, and Practice of Stroke and Its Risk Factors and Warning Signals Among the Students of the College of Applied Medicine at Majmaah University, Saudi Arabia

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Abstract

Background: Knowledge, attitude, and practice (KAP) connected to stroke risk factors, treatment, and the outcome may aid in early case diagnosis, prevention, and minimization of stroke-associated consequences through prompt hospitalization. The goal of our study was to identify the KAP of strokes and explore the relationship between KAP and stroke among Majmaah University students.

Methods and Results: Knowledge, attitude, and practice (KAP) of strokes, their risk factors, and warning signs were assessed among Majmaah University students using a convenience sampling method. The study was conducted using a self-administered questionnaire that had been pre-validated. A total of 284 students were included in the study. A majority, 230(81%), identified stroke as a disease indicating a medical emergency. The knowledge score was poor in 54.2%, satisfactory in 40.5%, and good in only 5.3% of cases. Forty-four percent of participants had positive attitudes, and 56.0% had negative attitudes concerning strokes. Most participants identified high blood pressure and high cholesterol levels (82.7% and 72.5%, respectively) as risk factors for stroke. Diabetes and irregular heartbeat were the risk factors with the lowest probability (34.5% and 33.1%, respectively).

Conclusions: This study shows that university students' knowledge, attitudes, and behaviors regarding stroke are weak, highlighting the urgent need to develop educational and awareness initiatives for better health promotion among university students. The health belief model's guidelines should be used while creating such programs. (International Journal of Biomedicine. 2023;13(2):326-332.)

Keywords: knowledge • attitude • practice • stroke • risk factor

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Introduction

A stroke, according to the World Health Organization, is defined as "rapidly developing clinical indications of focal (global) impairment of brain function, lasting more than 24 hours or leading to death, with no clear cause other than vascular origin.". Around 15 million individuals globally and 795,000 in the United States experience a stroke yearly.⁽¹⁾ About one in six people will have a stroke at some time.⁽²⁾ Strokes, as one of the leading causes of death and disability globally, have a physical, social, economic, and emotional impact on patients, their families, and healthcare systems.⁽³⁾ Numerous kinds of

strokes exist, with ischemic stroke accounting for 67%-80% of stroke cases recorded in epidemiological research. Ischemic stroke is still the most frequent lethal neurological disorder while more often being disabling than fatal.⁽⁴⁾ About 10% of ischemic stroke victims pass away within 30 days of onset, and 50% of those who survive have a permanent impairment six months later.⁽⁵⁾ Primary intracerebral hemorrhages, which account for 20% of all stroke cases, have a 50% 30-day mortality rate, and intracerebral or subarachnoid hemorrhages, are also potentially fatal conditions.⁽⁶⁻⁸⁾

Most stroke sufferers survive the initial injury but live with devastating symptoms for the remainder of their lives,

including aphasia, movement difficulties, blindness, speech problems, despair, and cognitive decline.⁽⁹⁾ The consequent decline in the patient's quality of life imposes a tremendous financial and emotional toll on the patient's family and carriers. The high economic cost is frequently consumed, as there is a great need for medical care in the initial phase after a stroke as well as a need for long-term assistance in the late phase.⁽¹⁰⁾

Stroke prevention is one of the most efficient approaches for lessening the adversarial well-being and economic outcomes of stroke.⁽¹¹⁾ Despite astounding stroke rates, there are currently only a few approved recovery techniques for reducing ischemia damage or enhancing healing in post-stroke patients' brains.⁽²⁾ There are two primary ways to enhance stroke recovery. The first is to limit acute ischemia damage by boosting reperfusion to the ischemic region.⁽¹²⁾ This can be performed with a variety of procedures, including cerebral angioplasty or thrombolysis. The second technique of recovery is to rehabilitate lost function through relearning.

Stroke prevention and treatment are significantly impacted by timely patient admission to the hospital and improved management of stroke risk factors.^(13,14) Stroke risk factors can be classified as modifiable or immutable, with the latter including age, sex, race, and a family history of stroke. Hypertension, cigarette smoking, atrial fibrillation, past stroke or transient ischemic attack (TIA), and inactivity are all regarded as risk factors. Hypertension is the most important and modifiable risk factor for stroke.⁽¹⁵⁾ The last 10 years have seen a rise in interest in improving the quality of life of stroke patients as part of the development of therapeutic approaches.⁽¹⁶⁾ Apart from a lack of basic knowledge about stroke treatment, the majority of patients are unaware of the risk factors for stroke.⁽¹⁷⁾ However, it is disappointing that many stroke patients arrive at the hospital late because of a lack of fundamental knowledge about stroke⁽¹⁸⁾ and its symptoms.⁽¹⁹⁾ Reduced time from stroke onset to hospital presentation and risk reduction depends on patients' and families and the general public's understanding of stroke.⁽¹⁹⁻²¹⁾

Knowledge, attitude, and practice (KAP) connected to stroke risk factors, treatment, and the outcome may aid in early case diagnosis, prevention, and minimization of stroke-associated consequences through prompt hospitalization. There is a shortage of research on KAP and stroke among university students, yet these studies are critical for the development of disease control and prevention methods. Understanding baseline KAP for strokes is a critical first step in developing instructional solutions. Additionally, stroke education for young pupils would be a promising method of widely disseminating stroke knowledge.⁽²²⁾ Therefore, the goal of our study was to identify the KAP of strokes and explore the relationship between KAP and stroke among Majmaah University students.

Materials and Methods

Study design

The sample size, a total of 284 participants, was calculated using a 0.50 indicator percentage, a 5% margin of error, and a 95% confidence interval. Undergraduates from the

College of Applied Medical Sciences, Majmaah University (Majmaah, KSA), were selected. Students were asked to complete a questionnaire and sign a consent form as part of the research process.

Questionnaire

The questionnaire had seven questions concerning general information about the participants, such as age, gender, study type (regular or bridging), department, educational level, employment status, and marital status. Even though the questionnaire used to assess KAP comprised three primary categories, namely knowledge, attitude, and practices, the risk factors and warning signs were also included to provide a more accurate assessment of overall illness knowledge. A list of the 12 most significant risk factors of strokes and the 15 most significant warning signs was placed in the questionnaire.

Knowledge of stroke

Eleven multiple-choice questions were used to assess understanding. Each accurate answer in the knowledge domain was worth one point, whereas each wrong or do not know response resulted in a score of zero. The knowledge portion received a total score range of 0–11 as a result of this. Therefore, Bloom's classification cut-off points for knowledge are as follows: An appropriate knowledge score of 75%–100% was regarded adequate; a suitable knowledge score of 50%–74% was considered satisfactory; and a score of less than 50% was considered poor knowledge.

Attitude toward stroke

The attitude was the belief and feeling of the respondent toward strokes. Five multiple-choice questions measured attitude. The scoring system for participants' responses was evaluated as follows: True/Yes-1 and False/No-0. The replies were totaled, and a total score was calculated for each participant, as well as an estimate of the total mean score. A positive attitude is associated with a score greater than the mean, and a negative attitude is associated with a score less than the mean.

Practice toward suspected stroke victim

Implies the immediate action of participants toward anyone showing warning symptoms of a stroke.

Authentication of the questionnaire

A committee of healthcare professionals thoroughly assessed the questionnaire. Based on the panel's findings, adjustments were made concerning how questions are arranged and structured. In addition, pilot research with 25 randomly selected university students was conducted to confirm validity. A test-retest procedure was used. After seven days, the same group of students were asked to complete the same questionnaire.

Statistical analysis was performed using statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages.

Results

Sociodemographic characteristics of the participants

The questionnaire was completed by 284 students (response rate of 100%). The average age of the participants

in this study was 28.3 ± 5.2 years. The majority (59.5%) of participants were in the 26-35 years age group. Fifty-one percent of participants were from the regular study group, and 48.9% were from bridging (Table 1). Forty-one percent of participants were from the Department of Nursing, followed by 18.3% from the Department of Medical Laboratories, 15.5% from Physical Therapy and Rehabilitation, and 13.4% from the Department of Medical Equipment. Most participants (66.9%) were studying at the baccalaureate level, while 19% were studying for a diploma, and 12% were from the master's degree program. In addition to this, 53.5% of participants were employed, and the remaining 46.5% were unemployed. The marital status data showed that 54% of participants were married, while 40.5% were single (Table 1).

Table 1.

Sociodemographic profile of the participants (n = 284)

Characteristics	No. of Participants	Percent
Age		
15-25	98	34.5
26-35	169	59.5
36-45	17	6.0
Gender		
Male	197	69.4
Female	87	30.6
Study Type		
Regular	145	51.1
Bridging	139	48.9
Department		
Physical Therapy (PHT)	44	15.5
Nursing (NRS)	117	41.2
Medical Laboratories (MDL)	52	18.3
Radiology (RMI)	23	8.1
Public Health (PBH)	10	3.5
Medical Equipment (MET)	38	13.4
Educational Level		
Diploma	54	19.0
Bachelor	190	66.9
Master	34	12.0
Doctorate	6	2.1
Employment status		
Yes	152	53.5
No	132	46.5
Marital Status		
Married	153	53.9
Divorced/Separated	15	5.3
Widowed	1	0.4
Single	115	40.5

Sources of information for achieving knowledge of stroke

The source of stroke information was obtained mostly (31.3%) from friends and relatives. Further, radio (28.2%) and television (19%) also significantly contributed to spreading the knowledge of strokes among the participants. In addition, other information sources about stroke were medical and nursing staff (11.3%), the Internet (6.3%), and books & pamphlets (3.9%).

Information regarding the history of stroke among family members, relatives, or friends

When the participants were asked about a family member, other relative, or friend who had suffered from a stroke, about 41.9% responded that they have at least one member who suffered from a stroke, and only 4.6% recalled having more than one member suffering from a stroke. Strikingly, 35.9% of participants reported that there was no one among family, friends, or relatives suffering from a stroke. More specifically, it was observed that there were more men (66.9%) than women (33.1%) relatives who had a stroke. It was noted that among the men's relatives, mostly father, uncle, and grandfather were reported as having a history of strokes, and aunt and mother were among the women's relatives.

The practice of immediate action of participants toward anyone showing warning symptoms of stroke

The majority of participants (85.9%) reported that if they found anyone showing warning symptoms of stroke, the immediate action that they would take would be to call an ambulance. Other participants reported that they would tell someone, for example, a family member, friend, or neighbor (78.9%), to drive them to the nearest healthcare center (78.5%), advise them to take rest (71.1%), and bring the person to a general practitioner (70.8%) if they find them still showing warning symptoms of stroke (Table 2).

Table 2.

The practice of immediate action of participants toward anyone showing warning symptoms of stroke

Practice Pattern	Frequency	Percent
Call an ambulance	244	85.9
Tell someone for example family member, friend, or neighbor	224	78.9
Drive them to the nearest healthcare center	223	78.5
Advise them to take rest	202	71.1
Bring the person to a general practitioner	201	70.8
Give something, for example, aspirin or a headache medication	198	69.7
Take time to allow spontaneous recovery	113	39.8
I don't know	15	5.3

Risk factors associated with stroke

Data analysis regarding the risk factors associated with stroke showed that most participants believed that high blood pressure (82.7%) is a major risk factor for stroke (Table 3). High cholesterol levels (72.5%) and cigarette smoking (70.4%) were considered the next crucial contributing risk factors. In addition

to this, the other risk factors for stroke were high blood sugar (42.2%) and a history of having a heart attack (36.6%).

Table 3.

Risk factors of stroke identified by the participants.

Risk factors	Frequency	Percent
High blood pressure	235	82.7
High cholesterol levels	206	72.5
Cigarette smoking	200	70.4
Obesity or overweight	190	66.9
High blood sugar	120	42.2
History of having a heart attack	104	36.6
Diabetes	98	34.5
Irregular heartbeat	94	33.1
Lack of physical activity	91	32
Excessive alcohol consumption	37	13.0
History of stroke among family members	24	8.5
Light to moderate coffee consumption (3-4 cups per day)	21	7.4

Sudden onset of warnings signs of stroke

The most commonly reported (56.3%) warning sign for sudden onset of stroke was a sudden loss of speech or inability to explain oneself in words and severe headache with flashing lights (48.9%). Other reported warning indications of a stroke beginning were weakness or numbness of the face, especially on one side of the body (44.0%), sudden confusion (43.3%), and inability to talk due to quick onset of back pain (43.0%) (Table 4).

Knowledge about stroke

Most (74.6%) of the students were aware of stroke from reading or hearing about it and most (81%) considered stroke a medical emergency. Nearly half of the students identified ischemic stroke as the most common type of stroke. Further, only 46.5% believed exercise is the most helpful way to reduce stroke occurrence. Also, 46.1% of participants recognized that both blood tests and CT scans could be used to diagnose stroke. Less than half (44.7%) of the respondents knew that stroke is more common in men than females. Only 41.2% of the students properly understood a stroke-affected part or organ. A blood clot in the brain was cited as a stroke sign by about 40% of individuals. About 32.7% of students knew that physiotherapy and medication could help them recover from a stroke. Less than one-third (29.2%) of students could figure out the prevalence of stroke in Saudi Arabia (Table 5A).

Attitude toward stroke

About half of the participants (54.6%) showed a positive attitude toward procedures like thrombectomy, angioplasty, and stenting done for stroke patients. An almost equal number of participants (48.2%) answered correctly for stroke survivors suffering from post-stroke depression. However, only 27.1% of students were aware of aspirin and warfarin as the most common drug prescribed for stroke. Further, 23.9% of students showed a negative attitude toward full recovery after a stroke (Table 5B).

Table 4.

Warning signs of stroke identified by the participants.

Warning signs	Frequency	Percent
Sudden loss of speech or inability to express oneself in words	160	56.3
Severe headache with flashing lights and nausea	139	48.9
Weakness or numbness of the face, especially one side of the body	125	44.0
Sudden confusion	123	43.3
Inability to talk due to the sudden onset of a backache	122	43.0
Sudden onset of vertigo, imbalance, and clumsiness of limbs	113	39.8
Sudden onset of unconsciousness with generalized convulsion and incontinence	105	37.0
Neck stiffness	95	33.4
Shoulder pain	86	30.3
Sudden trouble seeing in one or both eyes	86	30.3
Sudden loss of appetite with abdominal pain, nausea, and diarrhea	77	27.1
Urinary urgency	56	19.7
Weakness or numbness of leg, especially one side of the body	55	19.4
Weakness or numbness of the arm, especially one side of the body	54	19.0
Any mention of Face, Arm, Speech, Time (FAST)	24	8.5

Table 5.

Knowledge and Attitude toward stroke

(A). Knowledge about stroke	Frequency	Percent
Stroke is a medical emergency	230	81.0
Have you heard of a condition called 'Stroke'?	212	74.6
Stroke is due to a lack of blood supply to the brain	168	59.2
The most common type of stroke is an ischemic stroke	149	52.5
Which of these could help reduce the chance of stroke...	132	46.5
Diagnosis of stroke can be made by...	131	46.1
Stroke is more common among men	127	44.7
Which part/organ of the body is affected in Stroke?	117	41.2
Which of the following is suggestive of stroke?	115	40.5
Most useful methods for recovery are..	93	32.7
Can you estimate how many people have a stroke in Saudi Arabia?	83	29.2

(B). Attitude toward stroke

Is thrombectomy, angioplasty and stenting done for stroke?	155	54.6
Stroke survivors suffer from post-stroke depression.	137	48.2
Is there any current treatments for stroke?	121	42.6
Drugs prescribed for stroke are aspirin and warfarin	77	27.1
Nobody makes a full recovery after a stroke	68	23.9

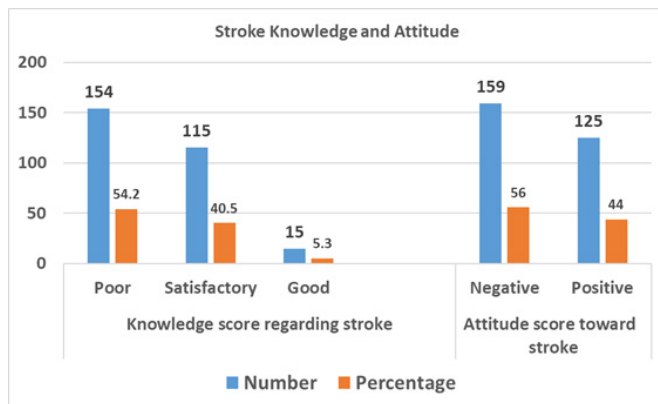


Fig. 1. Knowledge and attitude score of included subjects

The knowledge score was poor in 54.2%, satisfactory in 40.5%, and good in only 5.3% of cases. Forty-four percent of participants had positive attitudes, and 56.0% had negative attitudes concerning strokes (Figure 1).

Discussion

Despite breakthroughs in understanding the mechanism of cerebral ischemia, treatment options for ischemic stroke remain limited.⁽²¹⁾ Although modern medicine has eradicated or significantly curtailed many diseases, the incidence of stroke continues without significant change. Stroke has not been completely eliminated by preventive efforts that tackle the risk factors, and it typically still manifests as the population's first symptom.⁽²³⁾

Stroke is a significant cause of hospitalization, lengthy hospital stays, chronic disability, cognitive impairment, and death.^(24,25) Stroke causes a devastating impact on individuals, families and caregivers, primary care, acute hospitals, and purchasers of health care, along with policymakers.⁽²⁶⁾

This study is the first to study the KAP among university students at the College of Applied Medical Sciences. This study demonstrates poor attitudes, practices, and awareness of risk factors and warning signs for stroke. In this study, most students (60%) were aged 26-35 years. About two-thirds of the participants were male. An almost equal number of participants were full-time (regular) or part-time (bridging). Further, it was found that most of the students participating in this study were from nursing (41.2%) and were involved in the graduation program. Half of the participants were employed, and the remaining half were unemployed. More than half of the students (53.9%) were married, while 40.5% were single. Most of the participants could not recall having anyone in the family, or were ignorant of anyone in the family, having episodes of stroke. About 42.2% of participants had a single-family member suffering from a stroke. This study supported the idea that men are more likely than women to get a stroke. It was also evident from the current study that calling an ambulance and informing the family members, friends, or neighbors, and carrying the patient to the nearest healthcare center was the most immediate action taken by anyone suspecting a patient was having a stroke.

People with hypertension were more likely to have a sudden loss of speech or an inability to communicate verbally as a sign of an impending heart attack. Stress and smoking were the two most frequent risk factors, whereas double and fuzzy vision or complete blindness were the most frequent warning signs.⁽²⁷⁾ Similarly, in a national study of Koreans, Paresis was the most prevalent symptom, while hypertension was the most frequent risk factor.⁽²⁸⁾ Students in this study had a limited understanding of the signs and symptoms of a stroke. According to the Korean study, most participants were unaware of the warning signs of a stroke.⁽²⁹⁾

Most participants perceive stroke as a medical emergency and a brain disorder. Similar findings from research on an Australian urban population indicated that 73% of respondents accurately identified the brain as the target organ of a stroke.⁽²⁷⁾ In contrast, results obtained in a Northwest India study showed that most respondents could not identify a stroke as a brain disease.⁽³⁰⁾ About three-fifths of students knew that stroke is caused by inadequate blood flow to the brain. About half of the participants were aware of the many types of stroke, and that ischemic stroke is the most prevalent type of stroke. An equal number of students responded to exercise as the means for reducing stroke, and further, both the CT scan and blood test can be used as a means of diagnosis of stroke. Forty-four percent of participants identified stroke to be more common in men. Only one-third of the participants believed that post-stroke recovery is possible through medication and physical therapy. The science of stroke rehabilitation has made enormous strides in recent years.⁽³¹⁾ Astonishingly, one-third of the participants seem to have no clue about the prevalence of stroke in Saudi Arabia.

One-third of students acquired their basic knowledge regarding stroke from friends and relatives, and one-third gained their knowledge through the radio. Such a high degree of familiarity with stroke may be associated with more intimate interpersonal and interfamily interactions in these locations. More than half the number of participants had a positive attitude toward thrombectomy, angioplasty, and stenting done for stroke. Almost half of the participants believed that the patient who had suffered from a stroke would undergo depression. Only a quarter of the participants had a negative attitude in believing that after a stroke, the patient could have a full recovery. The knowledge score was found to be poor among most of the participants (54.2%), satisfactory in 40.5%, and good in only 5.3%. A U.K. study⁽³²⁾ had identical findings, indicating that most nursing students (91.5%) had only a basic understanding of the risk factors for stroke. More than half of the participants (56.0%) had overall negative attitudes toward stroke compared to those who showed a positive attitude (44.0%).

KAP studies have several limitations. KAP studies rely on self-reported data, which can be subject to bias and inaccuracies. In addition, the small sample size may constrain the results being generalized to a larger population. KAP studies typically focus on understanding knowledge and attitudes but do not always provide insight into the reason for certain behaviors. KAP studies are often observational and do not provide direct evidence of cause and effect. Finally,

the KAP studies may need to be more generalizable across different cultures, as knowledge, attitudes, and practices may vary significantly.

Ethical Considerations

The study was approved by the Ethics Committee of the at Majmaah University, Kingdom of Saudi Arabia. After a complete description of the study, written informed consent was obtained from all participants. All identifiable information about participants was removed, and the data were coded to ensure anonymity.

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Competing Interests

The authors declare that they have no competing interests.

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Awareness of Ethical Principles, Professional Behavior, and Ethical Treatment of Patients Among Medical and Dental Students

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Abstract

As future healthcare professionals, medical and dentistry students have an ethical responsibility to establish higher standards of ethics and professionalism in their interactions with patients. Therefore, expanding their knowledge and awareness of ethical concepts, professional behavior, and ethical patient treatment is essential. The aim of this research was to gather information about general medicine and dentistry students' awareness of ethical concepts, professional behavior, and ethical patient treatment. The questionnaire included 16 questions divided into four sections. The study found that most students agreed with basic professional health ethical principles, and that there was no significant difference between the students' directions (general medicine or dentistry) and the year of study (first and fourth). In this research, general medicine and dental students, in general, indicated a good level of awareness of ethical principles, professional behavior, and ethical patient treatment. (**International Journal of Biomedicine. 2023;13(2):333-337.**)

Keywords: bioethics • professional behavior • dentistry • general medicine • students

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Introduction

Bioethics is a field of study that focuses on ethical issues arising from advancements in technology, medicine, and biology.⁽¹⁾ The discipline of medical ethics evaluates clinical medicine and associated scientific research, based on a set of principles that healthcare professionals can refer to in times of uncertainty or disagreement.⁽²⁾ These principles include respect for autonomy, non-maleficence, beneficence, and justice. The Hippocratic Oath, the Declaration of Helsinki (1964), and The Nuremberg Code (1947) provide basic ethical guidelines for healthcare professionals.⁽³⁻⁵⁾ Bioethics aims to integrate beneficence, autonomy, and justice in issues related to euthanasia, confidentiality, consent forms, and potential conflicts of interest in healthcare.⁽⁶⁾

The Medical Faculty (Department of General Medicine and Department of Dentistry) of the University of Prishtina include study program courses in ethics during the first semester of the first year of study, comprising 15 lectures and 15 seminary practices. This enables students to learn about the aims, objectives, and phases of bioethics and deontology, as well as patient confidentiality and the responsibilities of doctors toward their patients.

Materials and Methods

This study collected data from first- and fourth-year students at the Medical Faculty of the University of Prishtina. The questionnaire included 16 questions divided into four sections. The first section contained three descriptive questions regarding basic professional ethical principles in dentistry for dentistry students (DS) and general medicine students (GMS). The second section consisted of questions related to basic professional ethical principles in dentistry for GMS and DS by years of study. The third section contained questions related

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to the professional behavior of dentists for DS and GMS. The fourth section included questions about the professional behavior of dentists for GMS and DS by years of study.

Statistical analysis was performed using statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp.). Categorical variables were analyzed using the Chisquare test or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P < 0.05$ was considered statistically significant.

Results

The study involved 131 DS and 170 GMS from the Faculty of Medicine in their first and fourth years. Of the total students enrolled in the study, 33.2% were male, while 66.8% were female (Table 1).

Table 1.

Characteristics of the students involved in the study.

	First year		Fourth year		Total	
	n	%	n	%	n	%
Total	145	100	156	100	301	100
<u>Directions</u>						
General Medicine	82	56.6	88	56.4	170	56.5
Dentistry	63	43.4	68	43.6	131	43.5
<u>Gender</u>						
Male	35	24.1	65	41.7	100	33.2
Female	110	75.9	91	58.3	201	66.8

Questions related to the basic ethical principles for dentists

Regarding the statement that «The basic professional ethical principles in dentistry are patient autonomy, do not

harm, do good, justice, and accuracy,» the rate of agreement (Strongly agree or agree) was 97.3% for the students included in this research, without significant difference according to the directions (general medicine or dentistry) ($P=1.0000$) (Table 2) or the year of study of DS ($P=0.2453$) and with significant difference according to the year of study of GMS ($P=0.0245$) (Table 3).

Regarding the statement that «Principles are abstract; their application is concrete but open to interpretation,» 70.4% of the students agreed (Strongly agree or agree) with a significant difference between GMS and DS ($P=0.0062$) (Table 2) but without significant difference according to the years of study of GMS ($P=0.6483$) and DS ($P=0.8434$) (Table 3).

Concerning the statement that «Health professionals are obliged to provide health services that are within the standards of dental health care and within the professional skills of the dentist,» 95.7% of the students agreed, without significant difference between GMS and DS ($P=0.4032$) (Table 2) or the year of study of DS ($P=0.6203$) and with significant difference according to the year of study of GMS ($P=0.0011$) (Table 3).

Questions related to the dentist's professional behavior

Regarding the statement that «Doctor/dentist has obligations to patients, coworkers, and the community wherein they live and interact,» 97.0% of the students included in the research agreed (Strongly agree or agree) without significant differences according to the directions ($P=1.0000$) (Table 4) or the year of study of DS ($P=0.6203$) and with significant difference according to the year of study of GMS ($P=0.0245$) (Table 5).

Concerning the opinion that «Doctor/dentist is obligated to inform the patient about the procedures they received and the qualified medical personnel who provided those procedures» because the patient has the right to ask

Table 2.

Questions related to basic ethical principles for dentists according to directions (general medicine or dentistry).

Basic professional health ethical principles		GMS		DS		Total		P-value
		n	%	n	%	n	%	
	Total	170	100.0	131	100.0	301	100.0	
The basic professional ethical principles in dentistry are patient autonomy, do not harm, do good, justice, and accuracy	Strongly agree/ Agree	165	97.1	128	97.7	293	97.3	*1.0000
Principles are abstract; their application is concrete but open to interpretation	Strongly agree/ Agree	109	64.1	103	78.6	212	70.4	0.0062
Health professionals are obliged to provide health services that are within the standards of dental health care and within the professional skills of the dentist	Strongly agree/ Agree	161	94.7	127	96.9	288	95.7	*0.4032

*- Fisher's exact test

questions about the treatment offered and the professional health personnel responsible for the treatment, 91.7% of the students agreed (Strongly agree or agree) without significant difference according to directions ($P=0.4281$) (Table 4) or the year of study of DS ($P=0.4952$) and with significant difference

according to the year of study of GMS ($P=0.0073$) (Table 5).

In response to the statement that «Any complications with the patient's health care or mistakes the doctor/dentist made during the health procedure should be disclosed to the patient,» 71.1% of the students agreed (Strongly agree

Table 3.

Questions related to the doctors/dentist's professional behavior according to directions and years of the study

Basic professional health ethical principles		GMS					DS				
		First year		Fourth year		<i>P</i> -value	First year		Fourth year		<i>P</i> -value
		n	%	n	%		n	%	n	%	
	Total	82	100.0	88	100.0		63	100.0	68	100.0	
The basic professional ethical principles in dentistry are patient autonomy, do not harm, do good, justice, and accuracy	Strongly agree/ Agree	77	93.9	88	100.0	*0.0245	63	100.0	65	95.6	*0.2453
Principles are abstract; their application is concrete but open to interpretation	Strongly agree/ Agree	54	65.9	55	62.5	0.6483	50	79.4	53	77.9	0.8434
Health professionals are obliged to provide health services that are within the standards of dental health care and within the professional skills of the dentist	Strongly agree/ Agree	73	89.0	88	100.0	*0.0011	62	98.4	65	95.6	*0.6203

*- Fisher's exact test

Table 4.

Questions related to the doctors/dentist's professional behavior according to directions and years of the study

Doctor/dentist's professional behavior		GMS		DS		Total		P-value
		n	%	n	%	n	%	
	Total	170	100.0	131	100.0	301	100.0	
Doctor/dentist has obligations to patients, coworkers, and the community wherein they live and interact	Strongly agree/ Agree	165	97.1	127	96.9	292	97.0	*1.0000
Doctor/dentist is obligated to inform the patient about the procedures they received and the qualified medical personnel who provided those procedures	Strongly agree/ Agree	154	90.6	122	93.1	276	91.7	0.4281
Any complications with the patient's health care or mistakes the doctor/dentist made during the health procedure should be disclosed to the patient	Strongly agree/ Agree	137	80.6	77	58.8	214	71.1	0.000
Doctor/dentist has an ethical responsibility to be truthful, and it is unethical for him to withhold information	Strongly agree/ Agree	150	88.2	112	85.5	262	87.0	0.4830
The difference between the complication of the procedure and the mistake-negligence, and the lack of professional skills of the dentist are aspects that involve legal interpretation.	Strongly agree/ Agree	154	90.6	104	79.4	258	85.7	0.0059

*- Fisher's exact test

or agree), with significant difference according to directions ($P=0.000$) (Table 4) and the year of study of GMS ($P=0.0218$) and DS ($P=0.000$) (Table 5).

The «Doctor/dentist has an ethical responsibility to be truthful, and it is unethical for him to withhold information» statement evoked agreement by 87.0% of the students (Strongly agree or agree) without significant difference between GMS and DS ($P=0.4830$) and by years of study of DS ($P=0.5722$), but a significant difference according to the years of study of GMS ($P=0.0158$).

Agreement with the statement that «The difference between the complication of the procedure, on the one hand, and the dentist's mistake/negligence or lack of professional skills, on the other, are aspects that involve legal interpretation» was reported by 85.7% of the students with a significant difference between GMS and DS ($P=0.0059$) (Table 4) and the years of study of GMS ($P=0.0011$) and a non-significant difference according to the years of study of DS ($P=0.0850$) (Table 5).

Discussion

This study highlights the importance of ethical knowledge in medical and dental education and the importance of integrating ethical education into medical and dental curricula to understand better the principles of ethics and the essential role of the doctor-patient relationship. The ethical framework of interactions between doctors, dentists, and patients is crucial in ensuring better outcomes for all parties involved.⁽⁷⁾

By integrating bioethics into the undergraduate curriculum, future generations of doctors and dentists will be better equipped to meet the high ethical standards required in their profession.⁽⁸⁾ The study also found that participants agreed with the principles of ethics, indicating adequate ethical education. Medical education is responsible for prioritizing ethics to ensure that the profession continues to uphold its high standards of professionalism.⁽⁹⁾

Table 5.

Questions related to the professional behavior of the doctors/dentist according to the directions and year of studies

Doctor/dentist's professional behavior		GMS					DS				
		First year		Fourth year		P-value	First year		Fourth year		P-value
		n	%	n	%		n	%	n	%	
	Total	82	100.0	88	100.0		63	100.0	68	100.0	
Doctor/dentist has obligations to patients, coworkers, and the community wherein they live and interact	Strongly agree/ Agree	77	93.9	88	100.0	*0.0245	62	98.4	65	95.6	*0.6203
Doctor/dentist is obligated to inform the patient about the procedures they received and the qualified medical personnel who provided those procedures	Strongly agree/ Agree	69	84.1	85	96.6	*0.0073	60	95.2	62	91.2	*0.4952
Any complications with the patient's health care or mistakes the doctor/dentist made during the health procedure should be disclosed to the patient	Strongly agree/ Agree	73	89.0	86	97.7	*0.0218	63	100.0	52	76.5	*0.000
Doctor/dentist has an ethical responsibility to be truthful, and it is unethical for him to withhold information	Strongly agree/ Agree	67	81.7	83	94.3	*0.0158	55	87.3	57	83.8	0.5722
The difference between the complication of the procedure and the mistake-negligence, and the lack of professional skills of the dentist are aspects that involve legal interpretation.	Strongly agree/ Agree	68	82.9	86	97.7	*0.0011	54	85.7	50	73.5	0.0850

*- Fisher's exact test

Conclusion

By integrating bioethics into the undergraduate curriculum, future generations of dentists and doctors will achieve high professionalism and ethical standards. Ethical education should be an ongoing process throughout a healthcare professional's career to ensure they maintain high ethical standards that benefit patients and the broader community.

Competing Interests

The authors declare that they have no competing interests.

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Association between the Proinflammatory Cytokine IL-17F and *Helicobacter Pylori* Infection in a Sample of Iraqi Patients

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Abstract

Background: Infection of the gastric mucosa with *Helicobacter pylori* (*Hp*) is characterized by the induction of a number of proinflammatory cytokines, including IL-8, IL-6, and TNF- α , involved in *Hp*-related gastric inflammation. The functions of members of the IL-17 cytokine family, other than IL-17A, in *Hp* infection remain understudied. The aim of our study was to assess the association between the proinflammatory cytokine IL-17F and *Hp* infection in a sample of Iraqi patients.

Methods and Results: This study included 50 Iraqi patients (18 males and 32 females; a mean age of 36 ± 1.74 years) infected with *Hp*. The healthy control group consisted of 16 subjects (3 males and 13 females), with a mean age of 31 ± 2.44 years. For the qualitative detection of antibodies (IgG, IgM, and IgA) against *Hp* in the serum, we used the OnSite H. pylori Ab Combo Rapid Test (CTK Biotech). ELISA was used to detect levels of human IL-17F in serum using ABTS ELISA Development Kit (Pepro Tech, USA). The serum level of IL-17F in patients with *Hp* infection was significantly higher than in the control group (238.9 ± 7.64 pg/mL vs. 114.00 ± 3.66 pg/mL, $P=0.0001$). However, the serum level of IL-17F in *Hp* patients was not significantly different between men and women (237 ± 12.12 pg/mL and 239 ± 9.94 pg/mL, respectively, $P=0.9015$). In addition, no significant difference was found between age subgroups: 240 ± 13.18 pg/mL, 231 ± 10.17 pg/mL, and 252 ± 18.35 pg/mL in age subgroups of <30 years, 30-45 years, and >45 years, respectively, ($P>0.05$).

Conclusion: Patients infected with *Hp* were characterized by higher serum levels of IL-17F than non-*Hp* subjects. IL-17F plays an important role in the inflammatory response to *Hp* infection in a sample of Iraqi patients. (International Journal of Biomedicine. 2023;13(2):338-341.)

Keywords: *Helicobacter pylori* • IL-17F • ELISA

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Introduction

Helicobacter pylori (*Hp*), a Gram-negative bacterial pathogen, colonizes the gastric epithelium of at least half of the world's population.⁽¹⁻⁴⁾ *H. pylori* isolates possess substantial genotypic diversity, which engenders differential host inflammatory responses. (5) In some individuals, *Hp*-related inflammation contributes to the development of peptic ulcers and gastric cancer.⁽⁶⁾ *H. pylori* strains that possess the cag pathogenicity island and secrete a functional cytotoxin

induce more severe gastric injury and further augment the risk for developing distal gastric cancer.^(7,8) In 1994, the IARC/WHO identified *Hp* as a Group 1 carcinogen.⁽⁹⁾ Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is also closely associated with *Helicobacter pylori* (HP) infection.⁽¹⁰⁾ Eradication of *H. pylori* infection has the potential to reduce the risk of gastric cancer development.^(5,11)

Infection of the gastric mucosa with *Hp* is characterized by the induction of a number of proinflammatory cytokines, including IL-8, IL-6, and TNF- α , involved in *Hp*-related gastric inflammation.⁽¹²⁻¹⁴⁾ The functions of members of the IL-17 cytokine family, other than IL-17A,⁽¹⁵⁻¹⁷⁾ in *Hp* infection remain understudied.

IL-17F is a member of the IL-17 cytokine family, which contains six members (IL-17A-F).⁽¹⁸⁾ IL-17F and IL-17A are

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closely related cytokines that exist as homodimers and IL-17A:IL-17F heterodimers.⁽¹⁹⁾ These cytokines play crucial roles in host defense against bacterial infections by recruiting neutrophils through the induction of CXC chemokines and inducing anti-microbial proteins in infected sites.^(20,21) IL-17F, first identified in 2001,⁽²²⁾ is mainly expressed by a distinct type of T cells, T helper 17 cells and $\gamma\delta$ T cells.^(23,24) Due to strong sequence homology with IL-17A, IL-17F can induce the production of proinflammatory cytokines (IL-6, granulocyte colony-stimulating factor [G-CSF], and granulocyte-macrophage colony-stimulating factor [GM-CSF]) and chemokines (CXCL1, CXCL2, and CXCL5) and promote granulopoiesis and neutrophil recruitment, albeit less potently than IL-17A.⁽²³⁻²⁶⁾ Increased levels of Th17 cytokines, including the production of IL-17A and IL-17F, are associated with more detrimental outcomes of *Hp* infection.⁽²⁸⁾ Data obtained by Dixon et al.⁽²⁷⁾ showed that IL-17A and IL-17F might have overlapping roles in maintaining the gastric mucosal response to *Hp* infection.

The aim of our study was to assess the association between the proinflammatory cytokine IL-17F and *Hp* infection in a sample of Iraqi patients.

Materials and Methods

This study included 50 Iraqi patients (18 males and 32 females; a mean age of 36 ± 1.74 years) infected with *Hp*, who were admitted to Baquba Teaching Hospital and some city outpatient departments during the period from January to June 2022. The healthy control group consisted of 16 subjects (3 males and 13 females), with a mean age of 31 ± 2.44 years.

Serum Samples

We collected 5 mL of venous blood samples in a plain tube and left for 30 min to allow clotting at room temperature ($20-25^\circ\text{C}$). Samples were centrifuged for 15 min at 3000rpm. After that, the serum was collected in polypropylene microfuge tubes and stored at -20°C for further analysis.

Immunological Tests

For the qualitative detection of antibodies (IgG, IgM, and IgA) against *Hp* in the serum, we used the OnSite H. pylori Ab Combo Rapid Test (CTK Biotech).

ELISA was used to detect levels of human IL-17F in serum using ABTS ELISA Development Kit (Pepro Tech, USA) following the manufacturer's instructions. These kits contain the key components necessary for quantitative measurement of human IL-17F in a sandwich ELISA format within the range of 6–2000 pg/ml.

Statistical analysis was performed using statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as the mean and standard error of the mean (SEM) for continuous variables. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Multiple comparisons were performed with one-way ANOVA and Tukey's HSD Post-hoc Test. A probability value of $P < 0.05$ was considered statistically significant.

The study was approved by the Ethics Committee of the Technical Institute Baquba. All participants provided written informed consent.

Results

The serum level of IL-17F in patients with *Hp* infection was significantly higher than in the control group (238.9 ± 7.64 pg/mL vs. 114.00 ± 3.66 pg/mL, $P = 0.0001$). However, the serum level of IL-17F in *Hp* patients was not significantly different between men and women (237 ± 12.12 pg/mL and 239 ± 9.94 pg/mL, respectively, $P = 0.9015$). In addition, no significant difference was found between age subgroups: 240 ± 13.18 pg/mL, 231 ± 10.17 pg/mL, and 252 ± 18.35 pg/mL in age subgroups of <30 years, 30–45 years, and >45 years, respectively (Table 1).

Table 1.

The serum level of IL-17F (pg/mL) in the study groups.

Group	n	Mean ± SEM	<i>P</i> -value
Main group	50	238.90±7.64	0.0001
Control group	16	114.00±3.66	
Main group	n	Mean ± SEM	<i>P</i> -value
Male	18	237±12.12	0.9015
Female	32	239±9.94	
Main group	n	Mean ± SEM	<i>P</i> -value
Age group of <30 years	13	240±13.18	0.5267
Age group of 30-45 years	17	231 ±10.17	
Age group of >45 years	20	252±18.35	

Discussion

Hp is the dominant member of the gastric microbiota and has infected more than half of the human population, of whom 5%–15% develop gastric diseases ranging from gastritis and metaplasia to gastric cancer.⁽²⁸⁾

In a study by Fraser et al.,⁽²⁹⁾ the relative risk of *Hp* infection significantly increased with age, lower socio-economic status, and lower household income, but was not significantly associated with gender. Joshi et al.⁽³⁰⁾ showed that among the 418 patients diagnosed with peptic ulcer diseases, 213 patients were positive for *Hp* by rapid urease test. Among the positive cases, over half were male patients, and the majority of the patients were in the age group of 35–44 years.

Hp-associated gastritis is characterized by an increased number of acute and chronic inflammatory cells secreting cytokines that contribute to maintaining and expanding the local inflammation.⁽³¹⁾ Studies have reported that *Hp*-specific gastric mucosal T cell responses are usually Th1 predominant,

but recently, Th17—markedly IL-17—is believed to be one of the driving immune cells in *Hp* infection.^(17,32)

Arisawa et al.⁽³³⁾ investigated the associations between the *IL-17F* 7488T/C (rs763780) polymorphism in association with the development of inflammatory changes in the gastric mucosa in *Hp*-infected Japanese subjects. The authors found that in *Hp*-infected cases, the carriage of the T allele and TT genotype increased the risk of the development of epigastric pain syndrome (OR=11.3, 95% CI: 1.23-103.2, *P*=0.032 and OR=0.4, 95% CI: 1.17-92.3, *P*=0.036, respectively).

Data obtained by Luzzza et al.⁽³¹⁾ indicate that biologically active IL-17 production is increased during *Hp* infection, suggesting that this cytokine may play an important role in the inflammatory response to *Hp* colonization.

In our study, patients infected with *Hp* were characterized by higher serum levels of IL-17F than non-*Hp* subjects. IL-17F plays an important role in the inflammatory response to *Hp* infection in a sample of Iraqi patients.

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Competing Interests

The authors declare that they have no competing interests.

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Presurgical Nasoalveolar Molding Modified Technique in Complete Bilateral Cleft Lip and Palate Infant: A Case Report

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Abstract

Through this research, we have presented a modified presurgical nasoalveolar molding (PNAM) device, protocol, and equipment of preoperative nasal alveolar formation techniques to improve lip, nose, alveolar, and palatal deformity in the Department of Orthodontics and Maxillo-Facial Department at the University of Prishtina. A two-week-old boy was brought to our Department of Orthodontics. The baby was diagnosed with non-syndrome bilateral complete cleft of the primary and secondary palate. After a complete examination and orthodontic therapeutic planning, precaution was taken for creating a PNAM appliance and sent to the laboratory. An intraoral molding plate was established on both sides of the separate segments of the maxilla and fixed with a mini screw to approximate the maxillary segments. Parts of the strings built in the front side activated (please check) and, together with the nasal stent, were added to correct the nose deformity. By using modified NAM to close the cleft segments, both the ones of the upper lip and the ones of the palate, we achieved the same results and reduced possible tissue damage by maintaining the conventional NAM apparatus. We used an alveolar formation plate with traction stainless steel to reduce the separate parts. Under this presurgical treatment, the cleft alveolus and palatal segments were reduced considerably. Also, the parts of the flattening nasals of the nose were set up.

Through interdisciplinary work, we created a new modified NAM appliance to avoid the obstacles of the traditional NAM devices and to improve the presurgical treatment by using the benefits of the postnatal period of the child to have a more effective treatment. Despite the inability of previous studies to decide on the degree of efficacy of PNAM and the fact that scientific data is limited, we consider that our research can contribute to a better understanding of why it is essential to use PNAM and of the need to enhance the apparatus. (International Journal of Biomedicine. 2023;13(2):342-345.)

Keywords: nasoalveolar molding • mini screw • cleft lip • cleft palate

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Abbreviations

NAM, nasoalveolar molding; PNAM, presurgical nasoalveolar molding; PSIO, presurgical infant orthopedics

Introduction

Nasal alveolar molding (NAM) is an intraoral, presurgical orthopedic device used in the first weeks of newborn babies that suffer from cleft lip and cleft palate. This device is used in this

period of growth and development of the newborn baby to use the plasticity of the nose cartilage and the formation of the early alveolar process.⁽¹⁾ The cleft lip, with or without a cleft palate as a birth defect, is a congenital orofacial anomaly that is considered among the most common and seen in children born alive.⁽²⁾

From statistics in different countries, the oral clefting rates vary from 1: 500 to 1: 2000 births.⁽³⁾ Adjunctive neonatal therapy for correcting the cleft lip and the palate is a type of presurgical infant orthopedics used since the 1950s.

Although this therapy has had some improvements in the different accesses on PSIO, and through the years has developed more after MacNeil, there have not been satisfactory results in solving the problems, especially in correcting the deformations of the nose cartilages.^(1,4) Some of the problems that the traditional approach could not address can now be solved through PNAM.

In 1993, Grayson used the principle of moldable nasal cartilage to develop the new technique and, together with his partners, described the first treatment protocol for PNAM. PNAM therapy is a technique that not only can help to correct alveoli and cleft lips, but it also can improve the configuration of the nose, lips, and cleft palate in infants. Therefore, the frequency of using this technique in the presurgical treatment of children has greatly increased.⁽¹⁾

The purpose of this study was to present a modified protocol and equipment for the treatment procedure for an infant with bilateral cleft lip and palate using PNAM techniques.

The importance of the PNAM treatment in the first postnatal weeks that it takes advantage of the optimal time immediately after the infant is born since it allows us to reduce the severity of the initial cleft deformity. Minimizing the deformities using the PNAM technique helps plan further therapy for the patient with cleft lips, especially in reducing surgical rehabilitation. In cases with bilateral clefts of the lip, alveoli, and palate, the main objective of the PNAM treatment is to repair and elongate the columella, place the premaxilla along the midsagittal plane, and pull the premaxilla gradually without pressure to approach the posterior cleft segments.⁽⁵⁾

Treatment procedures

The protocol of the presurgical treatment consists in reshaping the lip, the alveolar process, and the nostrils without surgical intervention as an essential part of the treatment for the patient with cleft lip and palate PNAM. A two-week-old boy was brought to our Department of Orthodontics. The baby was diagnosed with non-syndrome bilateral complete cleft of the primary and secondary palate (Figure 1)



Figure 1. Baby with non-syndrome bilateral complete cleft of primary and secondary palate.

After being informed of the treatment procedures, both parents were asked to fill in a form to consent. After an anamnesis with all the information, including if the infant has any concomitant disease, we made an extra- and intraoral examination, taking notes, additional and intraoral photographs, and an impression of the study model and the working model (Figure 2A).



Figure 2: A) The plaster model used to create the device; B) The device wire part that is used to activate the part of the apparatus connected to acrylic.

A special infant tray was used to create an impression of the upper dental arch with alginate. To avoid possible complications of the aspiration of the material or liquid, the infant was positioned straight and provided with high-power suction. The infant was held in a reclined position in his mother's lap in a dentistry chair. A dentistry mirror was used during the examination to move the tongue and to release the respiratory system while taking the impressions.

Construction of active alveolar molding plate

The alveolar mold plate is constructed from acrylic material. The intraoral molding plate was created on both sides of the separate segments of the maxilla and fixed with a mini screw to close the maxillary segments. The segments were projected in therapeutic planning of the modified device NAM and placement of string elements, those that are glued to the acrylic plate and also to the part that is put in the columella with the opportunity to activate them (Figure 3).

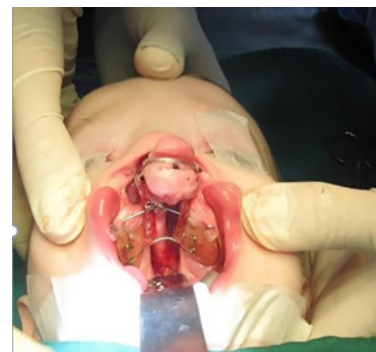


Figure 3. Fixing the device with mini-screws and connecting them with wires for activation.

Weekly visits were made based on the need to activate the strings. Later, both parts of the fabricated nasal conformers were used to keep the symmetry of the nose. Parents were

guided to place tape on the infant in the segments of the cleft lip. The acrylic plate that was mounted in maxillary segments also affects the mooring of activation of the stainless-steel string. It will also expand and help in the shaping of the nose of the infant (Figure 4). The mini screw was used after the initial close-up of the segments, and then the reduced plate of the device with accompanying active elements continued the activity. At the end of PNAM, there was a considerable reduction in the lip, alveolar and palatal cleft.



Figure 4. Baby following the placement of the modified PNAM device.

Discussion

A new approach of PNAM therapy with modified appliances is much more effective in reducing cleft deformity, and it helps in further surgical treatment. Taking into consideration three well-known techniques of NAM therapy (Grayson's technique, Figueroa's technique, and Louis's technique) and the difficult situation of bilateral cleft and palate, to improve the treatment, we used a modified NAM device, first mounted with a mini screw for the cleft palatal parts and for the creation of string elements to have an active role in closing the separate segments (Figure 2B).

The difference between the device we used and the Grayson technique is that Grayson used an orthopedic device with a wide acrylic plate that included all the parts of the palate.⁽⁶⁾ In contrast, we have reduced to the maximum the acrylic part that we used, and the difference also applies to mounting the plate. The reduced palatine plate is substituted with parts of a string with active function, where the alveoli and the cleft palate remain free, just that the cleft segments close up physiologically when light pressure is put on them. Fixation of the acrylic parts with a mini screw in the first two weeks creates stable retention of the NAM device and does not allow the device to move when the musculature is activated. Parts of the strings built earlier get active together with the nasal stent that was added to correct the nose deformity.

While the traditional Figueroa NAM asks that during the visits acrylic resin be removed from the palatal plate to shrink the segments,⁽⁷⁾ we, to reduce such a need, modified the NAM appliance by reducing parts of the acrylic plate and fixing the plate for two weeks in the cleft segments of the palatal, by also using the anchoring role of the fixed plate and activation of the string element for controlled closing up

and without the pressure of the cleft segments. A great part of the segments remains free to close up without added pressure from the plate.

In Liou's technique, the NAM plate has a reduction of the molding plate, compared to the Grayson technique, and it is made of two nasal components for forming the nose and tapes for pulling the premaxilla.⁽⁸⁾

Although the objective is the same in our case, such a technique requires strict patient monitoring. In contrast, the therapy with a modified NAM device allows control of the stability of the device in the mouth of the patient and the opportunity for adequate control of the activation of the string elements, both those that are glued to the acrylic plate and the part that is put in the columella, which enables us to achieve better effects during closing up of the segments and elongation of the columella by pulling (Figure 4).

As for keeping the nose, in our technique, we planned to use nasal conformers and place tapes. NAM also shows the ability to create the columella non-surgically by extending tissues. This is achieved by progressively extending the nasal stents and producing elastic pressures that stretch tissues next to the pro-labium. This procedure eliminates surgical repair of the columella and the resulting scar tissue from the procedure performed at the cleft palate centers.^(9,10)

Any severe form of bilateral cleft lip and palate requires presurgical care to avoid complications such as wound dehiscence or premaxilla malposition. For this aim, NAM is utilized to construct nasal cartilages, premaxilla, and alveolar ridges. But studies have identified certain limitations, among them a lack of scientific data and study, expenses, irritation of the lip and nasal tissues, threat of aspiration, mucosal ulcers, nasal and intraoral hemorrhage, fungal infection, airway blockage.^(11,12) Therefore, for this reason, the the PNAM apparatus has been modified to avoid the occurrence of these problems that may occur during the treatment of patients.

The suitable time to use NAM is from when the infant is born with CLP until he is three months old. When our patient reached this age, primary lip and nose repair was done. According to the instructions, nasal conformers must be kept for at least one year.⁽¹³⁾

Even in our case study, as a result of early intervention, we had the opportunity to use these advantages. We have also used nasal conformers to save the achieved correction with PNAM. Primary surgical closure of the lip and nose is performed from three to four months of age.⁽¹⁴⁾ NAM attempts to rectify nasal asymmetry and inadequate nasal tip projection while forcing the protruded premaxilla and enhancing the contour of the maxillary arch. NAM restores natural columellar length and avoids the need for further nose surgery.⁽¹⁵⁾ Individuals with bilateral cleft lip and nose deformity routinely undergo PNAM, primary rhinocheiloplasty, and postoperative nasal stents.⁽¹⁶⁾ Long-term, there is insufficient knowledge to support the advantage of NAM over no NAM in assessing nasal aesthetics and the shape of the alveolar arch. Long-term studies are necessary before recommending nasoskeletal shaping treatment as part of a protocol because its use is not without complications, and its true value is unproven in the long run.⁽¹⁵⁾

Conclusion

The modified NAM preoperative procedure is a better alternative for enhancing the aesthetics of the nose and the alveolar gap, as well as assisting in the approximation of the weeping segments before surgery. Despite the inability of previous studies to decide on the degree of efficacy of PNAM and the fact that scientific data is limited, we consider that our research can contribute to a better understanding of why it is essential to use PNAM and the need to enhance the apparatus.

Competing Interests

The authors declare that they have no competing interests.

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Endodontic Management of Mandibular Premolars with Root Canal Vertucci Type II and III Configuration: Two Case Reports

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Abstract

The location of all root canals (RCs) and their adequate preparation, disinfection, and obturation play an important role in successful endodontic treatment (ET). Furthermore, in addition to normal RC system morphology, the operator should also recognize their anatomical variations. This study presented two cases of mandibular premolars with RC configuration Vertucci type II and III after a clinical diagnosis of irreversible pulpitis. RC preparation in two cases was performed using the crown-down technique. After following the standard irrigation protocol, the RCs were obturated using the warm vertical condensation technique. Knowledge of RC system variations, with their preparation and 3D obturation, is the appropriate qualification for ET. (International Journal of Biomedicine. 2023;13(2):346-349.)

Keywords: root canal • crown-down • vertical condensation • gutta-percha • mandibular premolars

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Abbreviations

ET, endodontic treatment; RC, root canal; WL, working length.

Introduction

An appropriate diagnosis, treatment plan, correct debridement, disinfection, and root canal (RC) system obturation are needed for successful treatment.⁽¹⁾ The RC preparation must be done without procedural errors while respecting the root canal's working length (WL) and maintaining its natural path.⁽²⁾ This can be achieved with the correct selection of instruments in terms of their size and design.^(3,4) Also, for a successful RC treatment the operator must have knowledge of internal RC morphology to locate

all RCs and properly clean, shape, and obturate the RC space in three dimensions.⁽⁵⁾ Despite all of this knowledge, RC preparation and obturation are still challenging due to the variety and complexity of RCs caused by different genetics, ethnicity, gender, and age, as well as the existence of lateral or accessory canals and isthmuses.⁽⁶⁾

Vertucci has identified eight different RC types, depending on their number and configuration.⁽⁷⁾ Due to this variability in RC morphology, endodontic treatment (ET) of lower premolars is a challenge for operators.⁽⁸⁾ Based on various population studies, the RC morphologies of the mandibular premolars mainly vary between Vertucci types I-VII.^(9,10)

Vertucci type II performs as two separate canals that leave the pulp chamber but join to form one canal at the exiting point; in Vertucci type III, one canal leaves the pulp chamber,

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divides into two within the root, and then merges to exit in one canal.⁽⁷⁾ These two RC configurations in lower premolars require proper preparation and obturation techniques to achieve 3D and hermetic obturation.

These two case reports describe the endodontic management of the second mandibular premolars with Vertucci type II and III, which were prepared with the crown-down technique and obturated with the vertical gutta-percha condensation technique.

Case Presentation

Case 1: A 50-year-old male patient came to the private dentistry clinic “Dr. Berisha” in Peja complaining that he had a toothache, followed by a sharp pain upon thermal stimulus, with lingering pain (often 30 seconds or longer after stimulus removal), spontaneity (unprovoked pain) and the frequency of referred pain increasing during the night.

The percussion, palpation, and thermal vitality test were negative. During RC exploration, the patient felt the pain and then was injected with infiltrative anesthetic Septanest 1:100.000 (4% Articain Hydrochloride with 1:100000 Epinephrine) (Septodont, Saint-Maur-des-Fosses Cedex, France) using Carpo syringe. The retroalveolar radiography revealed an intact periapical tissue with Vertucci type II configuration of the RC. Tooth 45 (lower right second premolar) had a deep cavity. This tooth was diagnosed with symptomatic irreversible pulpitis.

Before the treatment, the patient was informed that his clinical information and radiographic images may be reported in the journal. Therefore, the authors certify that they have obtained patient consent, and the patient was informed that his name and initials would not be published. ET was performed with a rubber dam. After caries were removed, access to the cavity was prepared, and two RC orifices were localized. Loupes with 3.0 magnification (Univet Loupes Spa, Rezzato, Italy) were used for canal localization. The RCs were explored with sizes 06, 08, and 10 K-files (DiaDent, France). WL was set at 1mm from the apical foramen. The pulp tissue was removed, and the RCs were prepared with E-flex gold rotary files (Eighteeth Medical Technology, Changzhou, China) using the crown-down technique. During shaping and cleaning, the operators used 17% EDTA gel (Cerkamed, Stalowa Wola, Poland). After opening the pathway with hand files 06, 08, and 10 K-Files (DiaDent, France), the operators used E-flex gold rotary file size 19/04 followed by size 20/04. Each canal's orifice was expanded with E-flex gold rotary file size 17/08 and finished with E-flex gold rotary file size 25/04. Apical enlargement was performed with an E-flex gold rotary file size up to 25/04. During the ET, the operators used the endodontic handpiece E-connect S (Eighteeth Medical Technology, Changzhou, China) at a rotation of 350rpm and torque 2.5Ncm, introducing the instrument passively into the RC. The RCs were irrigated following Marcus Haapasalo's protocol and using Irriflex, a flexible RC irrigation needle (Produits Dentaires SA, Vevey, Switzerland). During the shaping and cleaning of each canal, the operators performed irrigation with 2 ml of 5.25% sodium hypochlorite activated

with an ultrasonic device Ultra X (Eighteeth Medical Technology, Changzhou, China) for 30 seconds in each canal. After shaping and cleaning, the operators continued with 3 ml 5.25% NaOCl (Cerkamed, Stalowa Wola, Poland) with 30 seconds activation and 1ml EDTA 17% for 1 minute activated with Ultra X. They repeated the process with 2 ml 5.25% NaOCl in each canal with activation for 30 seconds and 1ml 17% EDTA with 30 seconds of ultrasonic activation. The drying was performed with Paper points.

The RC-WL was verified with radiography, followed by obturation of the RCs with vertical gutta-percha condensation using Fast Pack and Fast Fill (Eighteeth Medical Technology, Changzhou, China). The gutta-percha size was 25/04 (DiaDent, France) and was used in combination with Sealapex sealer (Kerr Corporation, Orange, CA, USA). ET was completed in one visit (Figure 1A-F).

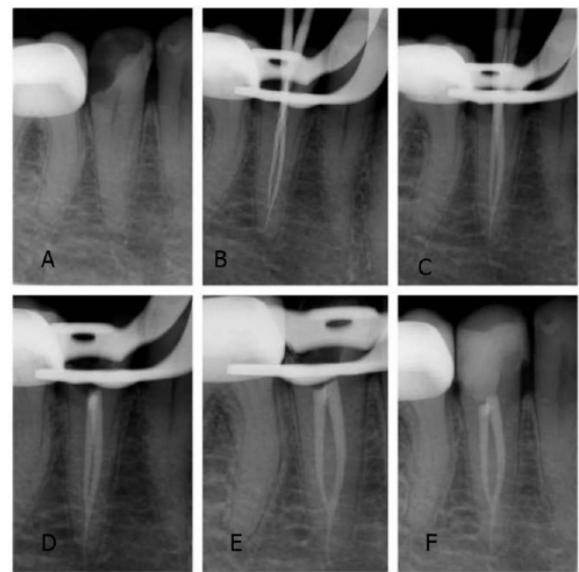


Fig. 1. A. First diagnostic radiography; B. Working length determination; C. Verification of working length; D. Radiography after root canal obturation; E. Radiographic image from another view; F. Coronal filling.

Case 2: A 30-year-old female patient came to the private dentistry clinic “Dr. Berisha” in Peja complaining of a toothache, followed by sharp pain, spontaneity (unprovoked pain), and the frequency of referred pain increasing during the night.

The percussion, palpation, and thermal vitality test were negative. Tooth 45 was under a porcelain crown. The porcelain crown was removed. During RC exploration, the patient felt the pain and then was injected with infiltrative anesthetic Septanest 1:100.000 (4% Articain Hydrochloride with 1:100000 Epinephrine) (Septodont, Saint-Maur-des-Fosses Cedex, France).

The retroalveolar radiography revealed an intact periapical tissue with Vertucci type III configuration of the RC. This tooth was diagnosed with symptomatic irreversible pulpitis. Endodontic treatment was performed with a rubber dam.

After the removal of the old filling and caries, access to the cavity was prepared, and one RC orifice was localized. It was divided into two canals within the root and then merged to exit in one canal. Loupes with 3.0 magnification (Univet Loupes Spa, Rezzato, Italy) were used for canal localization. The RCs were explored with sizes 06, 08, and 10 K-files (DiaDent, France). WL was set at 1mm from the apical foramen. The pulp tissue was removed, and the RCs were prepared with E-flex gold rotary files (Eighteenth Medical Technology, Changzhou, China) using the crown-down technique. During shaping and cleaning, the operators used 17% EDTA gel (Cerkamed, Stalowa Wola, Poland). After opening the pathway with hand files 06, 08, and 10 K-Files (DiaDent, France), the operators used E-flex gold rotary file size 19/04 followed by size 20/04. The canal's orifice was expanded with E-flex gold rotary file size 17/08 and finished with E-flex gold rotary file size 30/04. During the ET, the operators used the endodontic handpiece E-connect S (Eighteenth Medical Technology, Changzhou, China) at a rotation of 350 rpm and torque 2.5 Ncm, introducing the instrument passively into the RC. The RCs were irrigated using Irriflex, a flexible RC irrigation needle (Produits Dentaires SA, Vevey, Switzerland).

The operators performed irrigation with 2ml of 5.25% sodium hypochlorite (Cerkamed, Stalowa Wola, Poland) activated with an ultrasonic device Ultra X (Eighteenth Medical Technology, Changzhou, China) for 30 seconds in each canal. After shaping and cleaning, the operators continued with 3ml 5.25% NaOCl with 30 seconds activation and 1ml EDTA 17% for 1 minute activated with Ultra X. They repeated the process with 2 ml 5.25% NaOCl in each canal with activation for 30 seconds and 1ml 17% EDTA with 30 seconds of ultrasonic activation. The drying was performed with Paper points. The RC-WL was verified with radiography, followed by obturation of the RCs with vertical gutta-percha condensation using Fast Pack and Fast Fill (Eighteenth Medical Technology, Changzhou, China). Gutta-percha size was 30/04 (DiaDent, France), and the sealer Sealapex (Kerr Corporation, Orange, CA, USA) was used for obturation. After the ET of tooth 45 was completed, a new porcelain crown was made (Figure 2 A-F).

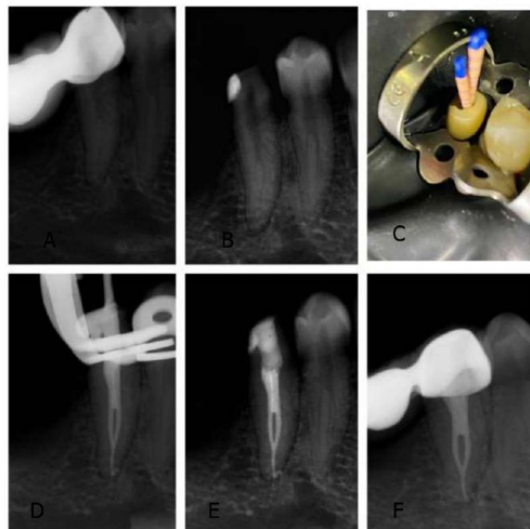


Fig. 2. A. Initial radiography; B. After crown removal; C. Root canal obturation; D. Radiography after obturation; E. Coronal seal; F. Porcelain crown.

Discussion

RC preparation is the critical step for RC obturation therapy, including RC cleaning and formation. Before RC preparation, the location, length, shape, and number of RC orifices need to be determined.⁽¹²⁾

Therefore, the upgraded knowledge of RC anatomy, combined with contemporary endodontic instruments, preparation and obturation techniques, and magnifications, may improve the success of ET.⁽¹³⁾

Mandibular premolars are also considered among teeth that are challenging in RC preparation and obturation. Previous studies have reported that the incidence of the first mandibular premolar is most commonly one root with one canal and ranges from 75.8%–97.9% cases, whereas two or more apical foramina were found in 21.1%–24.2 % cases with different morphology according to Vertucci types.^(14,15) Further, it has been reported that the second premolar usually has one root with a single RC system that ranges from 65% to 100% of cases, but many studies have also reported that the single root with two canals was observed in 1%–11% of cases, with a single apical foramen in 91.8%, or two or more apical foramina in 8.2% of cases.^(14,15) The choice of RC preparation technique depends on the design and shape of the instruments and RC system anatomy.⁽¹⁶⁾ The crown-down technique has been reported to be superior to other preparation techniques for curved RCs since this technique ensures access to the full WL, with minimal apical transportation from the natural path or reduction of the apical blockage and disables extrusion of canal content beyond apical foramen.⁽¹⁷⁾ According to obturation techniques, the vertical compaction technique ensures the adaptation of plasticized obturating material to the RC abnormalities, accessory canals, and isthmuses and produces consistently dense, dimensionally stable RC fillings more effectively than with cold gutta-percha.^(18,5) After heat application, gutta-percha can move apically and laterally through compaction pressure, ensuring three-dimensional obturation of RC space.⁽¹⁹⁾ Thus, poor cleaning and shaping and short or beyond apex obturated RC may cause the failure of ET, thus resulting in periapical lesions.^(20,21)

In our two case reports, the crown-down technique was carried out using E-flex gold rotary files produced by Eighteenth Medical company with handpiece E-connect S at a rotation of 350 rpm, resulting in adequate preparation of RCs without procedural errors, which, after irrigation with sodium hypochlorite 5.25% and EDTA, were obturated with vertical gutta-percha condensation using Fast Pack and Fast Fill. The results were very satisfactory, but they need to be followed up to determine if the preparation and obturation technique is successful.

Conclusion

Based on the results of two reported cases, it may be concluded that the operator's knowledge of RC anatomy, and visualization of it using radiography, combined with adequate preparation (crown-down) and obturation (warm vertical condensation) technique, will lead to the correct diagnosis and successful RC treatment.

Competing Interests

The authors declare that they have no competing interests.

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Septic Arthritis of the Knee in a Neonate: A Case Report

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Abstract

We present a case of septic arthritis of the knee in an 18-day-old female patient diagnosed by ultrasound. The final diagnosis of septic arthritis of the left knee was confirmed by a microbiology examination, which found *Pseudomonas aeruginosa*. The patient was treated with arthroscopic drainage and appropriate antibiotic therapy. (International Journal of Biomedicine. 2023;13(2):350-352.)

Keywords: septic arthritis • *Pseudomonas aeruginosa* • arthroscopic drainage • antibiotic therapy

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Introduction

Septic arthritis (SA) is an inflammation of joints caused by an infection. The incidence of SA in children ranges between 5 and 12 cases per 100,000 persons.⁽¹⁾ The highest incident rates are seen among children aged between zero and four years old.⁽²⁾ SA is 1.4 to 1.7 times more common in males than in females.⁽²⁻⁴⁾ The pathogens implicated in pediatric joint infections commonly include methicillin-sensitive *Staphylococcus aureus*^(5,6) and *Kingella kingae*^(5,6-9) and, more rarely methicillin-resistant *Staphylococcus aureus*,^(10,11)

group A *Streptococcus*, group B *Streptococcus*,^(12,13) *Escherichia coli*,^(12,13) and *Streptococcus pneumoniae*.⁽¹⁴⁾ *Pseudomonas aeruginosa* is a very rare causative organism, mostly in immunocompromised individuals.⁽¹⁵⁾ The symptoms and signs of SA include fever, swelling, pain, and impaired range of movement.⁽¹⁶⁻¹⁸⁾ In patients with SA, a diagnostic puncture of the joint typically finds purulent fluid with between 50,000 and 150,000 cells/ μ L of predominantly neutrophils.⁽¹⁹⁾ Without proper treatment, SA could lead to joint destruction, growth failure, permanent dysfunction, or deformity of the limbs in many children affected.^(20,21)

Case Presentation

We present an 18-day-old female patient who was born by normal delivery done by a midwife in one of the villages in Sudan. Her mother felt swelling and fever in the baby's left leg and came to the hospital in the emergency department. The GP, after examination, referred the patient to a pediatrician. The examination showed redness, a fever of 38°C, pain, and limited mobility in the left knee joint. A general blood test revealed elevated levels of white blood cells of 19.000 μ L and an erythrocyte sedimentation rate of 70 mm/h. The x-ray showed a normal bone and joint space, no periosteal reaction or lytic bone lesion, and no evidence of osteomyelitis (Figure 1). After that, the clinician requested a left knee ultrasound which showed enlargement of the hypoechoic area in the joint capsule, indicating fluid retention (suprapatellar bursal complex joint effusion) with internal turbidity and synovial thickening; ligament, tendons, and subcutaneous tissue were normal (Figures 2 and 3). The pediatrician directly referred the patient to an orthopedic surgeon who diagnosed the late stage of the process. Arthroscopy and partial removal of the synovium with drainage was performed, and an intraoperative sample taken. The final diagnosis of SA of the left knee was confirmed by a microbiology examination, which found *Pseudomonas aeruginosa*. The patient was prescribed appropriate antibiotic therapy.



Fig. 1. Lower limb x-ray. Soft tissue is swelling around the left leg, especially around the knee joint. No periosteal reaction or lytic bone lesion, and no evidence of osteomyelitis.

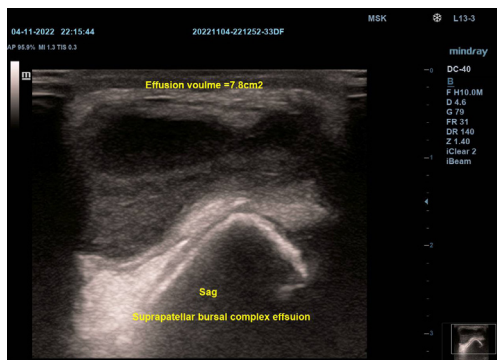


Fig. 2. Knee ultrasound, sagittal scan. Enlargement of the hypoechoic area in the joint capsule, indicating fluid retention (suprapatellar bursal complex joint effusion).

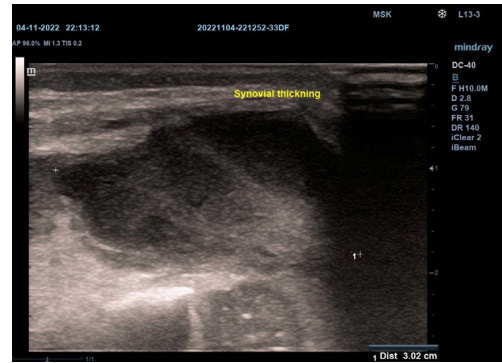


Fig. 3. Knee ultrasound, sagittal scan. Synovial thickening.

Discussion

SA is a dangerous condition that needs emergency treatment. Patient management involves joint drainage and empiric antibiotic therapy initially, which can later be specified according to test results.^(22,23) Several studies⁽²⁴⁻²⁶⁾ suggest that two weeks of targeted systemic antibiotic therapy after surgical drainage may be sufficient. The suggested duration for parenteral antibiotic treatment ranges from 3 days up to 6 weeks, resulting from several mainly observational studies with a relatively poor level of evidence.^(27,28) Several pediatric textbooks recommend at least 4–6 weeks of treatment.^(29,30) In general, antibiotics should be administered intravenously for 2 weeks and then orally for another 2 weeks. Then oral therapy should be stopped depending on the results of C-reactive protein (CRP) and white blood cell (WBC) count.⁽³¹⁾ Delays in identification and treatment might result in major side effects, such as osteonecrosis and joint degeneration. On the other hand, SA discovered in time is no cause for alarm. Surgical drainage is recommended for early-diagnosed neonatal SA. A conservative approach may be more efficient for patients whose diagnosis and treatment have been delayed for more than 2 weeks. However, there is still a controversy regarding the management of SA in neonates. Early diagnosis and proper treatment of SA are essential to obtain good outcomes and avoid sequelae.

Informed Consent

The patient's parents consented to use the data from this case for publication.

Competing Interests

The authors declare that they have no competing interests.

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Pituitary Adenoma Presenting with True Bitemporal Hemianopsia: A Case Report

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Abstract

Pituitary adenomas grow from the anterior pituitary gland and can cause compression of surrounding structures, including the optic chiasm, leading to visual field defects such as bitemporal hemianopia, visual acuity or color perception reduction. In this case report, we present the case of a 45-year-old male patient who presented with long-lasting headaches and visual disturbances, including diplopia and a decline in visual acuity. Despite being initially diagnosed with migraine by a neurologist, a thorough ophthalmological examination revealed visual field defects consistent with true bitemporal hemianopsia. MRI results confirmed the presence of sellar mass, and the patient was referred to the neurosurgery department for immediate intervention. The patient underwent surgical removal of the mass, which was identified as a pituitary adenoma upon histological examination. Three months later, the patient experienced significant improvements in subjective and objective visual acuity, with no diplopia or scotomas in the visual field. In conclusion, this case report highlights the importance of considering pituitary adenoma as a potential cause of visual disturbances, even in the absence of bitemporal hemianopsia. Thorough ophthalmological examination, including visual field testing, can aid in the early detection of chiasmal involvement and prompt referral for further investigation. Early intervention, including surgical removal of the tumor, can prevent other complications and significantly improve visual acuity and overall quality of life for patients. (*International Journal of Biomedicine*. 2023;13(2):353-356.)

Keywords: pituitary adenoma • sellar mass • bitemporal hemianopsia

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Introduction

Pituitary adenomas account for approximately 15% of all brain tumors. They are the most common cause of sellar tumors after the third decade of life, representing 90% of all sellar masses.^(1,2) Visual impairment is primarily due to suprasellar tumor extension with optic chiasm compression, which leads to visual field defects such as classical bitemporal hemianopia, visual acuity or color perception reduction,

and optic nerve atrophy.⁽³⁾ Pituitary adenomas, which grow upward from the pituitary stalk, compress the chiasm from below, preferentially involving the inferior, nasal, and macular nerve fibers. Vision loss due to compression first affects the supertemporal visual fields, then inferotemporal, inferonasal, and finally, superonasal fields.⁽⁴⁾ There is clinical significance to the detection of visual field loss in pituitary disease, and capturing peripheral loss is important to the early diagnosis of chiasmal involvement.⁽⁵⁾ Even though Putri et al.,⁽⁶⁾ have

described bitemporal hemianopsia as the most common visual field defect of chiasmal compression, in a study involving 115 patients with pituitary macroadenoma conducted by Lee et al.,⁽⁷⁾ 89 of them presented with a visual field defect but only one of them presented with true bitemporal hemianopsia. Even though bitemporal and mixed defects are the most common, bitemporal hemianopsia is extremely rare in patients with macroadenoma, according to this study.

Case Presentation

We present a case of a 45-year-old male patient, with the main complaints being long-lasting headaches and visual disturbances, mainly in the form of double vision (diplopia) over the past three months. He has been receiving continuous treatment for headaches using analgesics but still experiences persistent headaches with no signs of relief. Initial consultations with a family doctor were unsuccessful, and he is recommended to see a neurologist, who diagnoses him with migraine and starts the treatment. The patient came to our clinic for a check-up after being referred by his previous doctor. His complaints of diplopia were continuing. Furthermore, he was experiencing a decline in visual acuity in the last two weeks. He also reported seeing shadows and was in an unstable mental state, anxious and unfocused.

At examination: The patient's right eye visual acuity is 20/32 without correction, while the left eye visual acuity is 20/63. Intraocular pressures measure 12mmHg for the right eye and 13mmHg for the left eye, respectively. No pathological changes were observed in the anterior segment; the cornea was transparent, the pupil reacted well to light in the right eye, and there was a relatively mild afferent pupillary defect in the left eye, with no change in the iris. Sub-atrophy of the optic nerve head was observed in the posterior segment, more pronounced in the left eye. The macula showed no pathological changes, and neither did the retinal vessels. We performed visual field testing (perimetry), and the results showed defects in the visual field, specifically a true bitemporal hemianopsia (Figure 1). Concretely, a temporal scotoma with the nasal portion preserved was observed in the right eye. A temporal scotoma and defects with partial involvement of the upper and lower nasal quadrant were perceived in the left eye. After this thorough examination, the patient's symptoms prompted a referral to the radiology clinic for further investigation. An MRI of the head was ordered, and the results indicated the presence of a sellar mass (Figure 2).

Given the severity of the situation, the patient was referred to the neurosurgery department for immediate intervention. A team of experienced neurosurgeons evaluated the patient's case and determined that it was necessary to remove the surgical mass to prevent further complications. The patient underwent a surgical procedure, which involved careful removal of the sellar mass. Following the procedure, the patient was closely monitored and received appropriate care by a multidisciplinary team of healthcare professionals involved in the patient's care to ensure that the patient received the best possible treatment to maximize the chances of a successful outcome and recovery. Postoperative MRI findings are illustrated in Figure 3.

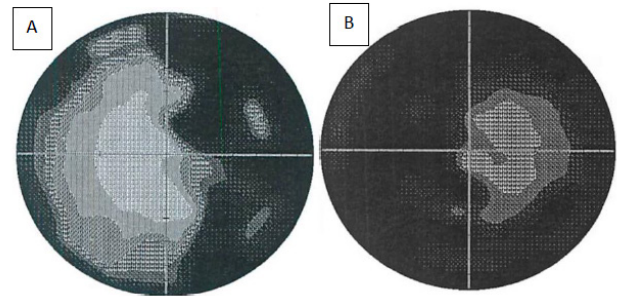


Fig. 1. Visual acuity measured through perimetry at first examination in the clinic. A) Right eye; B) Left eye

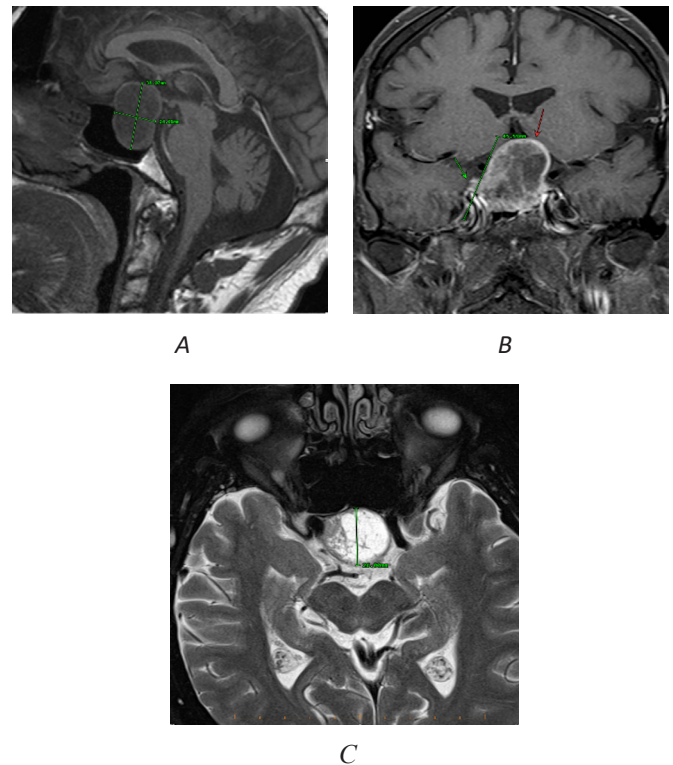


Fig. 2. Preoperative MRI findings A) MRI findings of a large sellar and suprasellar solid-cystic macroadenoma; B) Peripheral contrast-enhancement of the solid component on the right. The optic chiasm is cranially displaced and compressed (red arrow). Bilateral anterior cerebral artery (ACA) and anterior communicating artery (ACoA) are also located craniodorsally, but no signs of infiltration exist. On the right, suspicion of infiltration of the cavernous sinus, as we have a solid mass up to the lateral intercarotid line (green arrow); C) The pituitary fossa is enlarged to 25 mm (anteroposterior).

The material removed was examined by a pathologist at London Pathology Service (London, United Kingdom). Histology showed an epithelial neoplasm, arranged in the sheets, papillary and acinar structures, and containing a rich vascular network. The individual tumor cells with round monomorphic nuclei and well-defined amphophilic or eosinophilic nuclei were observed. No mitotic figures were seen and necrosis was absent, as was fibrosis. The appearances as those of pituitary adenoma (Figure 4).

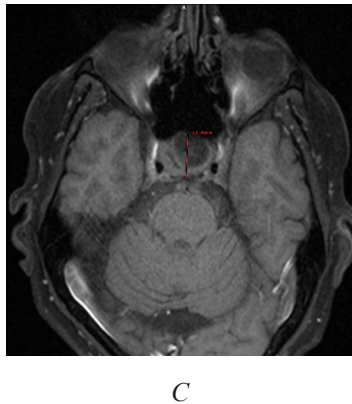
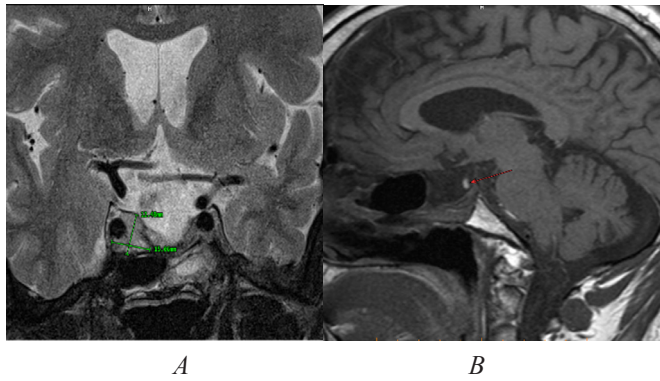


Fig. 3. Post-operative MRI findings after extirpation of pituitary macroadenoma by the transsphenoidal route. A) Solid remnant about 15 x 12 mm near the right ACL extending to the lateral intercarotid line. The suprasellar space, including the optic chiasm, is completely free. The signal from the internal carotid arteries is normal; B) T1 hyperintensity of the neurohypophysis appears parasagittal to the left (red arrow); C) Pituitary fossa remains enlarged in all directions.

Fig. 4. Sellar region lesion – Pituitary adenoma.

Three months later, the patient returned to our clinic with an improved overall state. Clinically, the patient had multiple improvements in subjective and objective visual acuity and other ophthalmological examinations. We observed an improvement in visual acuity of 20/22 in the right eye and 20/32 in the left eye, with no diplopia or scotomas in the visual field (Figure 5). The pupils reacted well to light in both eyes, and there was no longer an afferent pupillary defect in the left eye. Sub-atrophy of the optic nerve head was still persistent in the left eye in the fundus photograph. The patient was discharged and scheduled for follow-up visits.

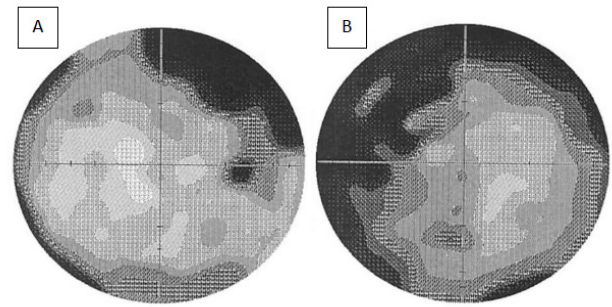


Fig. 5. Visual acuity measured through perimetry postoperatively in the clinic. A) Right eye; B) Left eye

Discussion

There have been studies that discovered different pathologies through visual field defects. Deleu et al. found a Rathke “pouch” cyst in a 71-year-old patient who presented at the ophthalmology department for the first time for a decrease in the temporal visual field of the left eye. Rathke cleft cysts (RCCs) are benign remnants of the Rathke’s pouch, the anlage of the anterior pituitary during embryogenesis. RCCs usually are asymptomatic and are found incidentally, hence the name incidentaloma. RCCs become symptomatic only when they grow massive and compress the optic chiasm. In a case by Deleu et al., 11 days after the operation, the patient described significant improvements in her visual field.⁽⁸⁾

Other rare tumors, such as rhabdomyosarcoma, have been described to be found in the intrasellar region and thus cause bitemporal hemianopsia. Stein et al.⁽⁹⁾ describe a case of embryonal rhabdomyosarcoma in a 34-year-old patient who had been treated for two years for decreased visual acuity and intraocular pressure and, five months before presentation, complained of bitemporal hemianopsia.

Furthermore, Azarpira et al.⁽¹⁰⁾ described a 50-year-old patient who presented with headaches and visual disturbances for two months, and on ophthalmologic examination, bitemporal hemianopsia was detected. The MRI studies showed an intra-sellar mass. Following the initial diagnosis of pituitary adenoma, she was operated on, and histological examination showed a coexisting gangliocytoma with pituitary adenoma and RCC.

Gupta et al.⁽¹¹⁾ presented a case of a pregnant woman with right eye hemianopsia. Her visual acuity on the right eye at 37 weeks was 20/40, and 20/200 two weeks later. She gave birth 2 days afterward and returned for review 2 weeks after giving birth with a visual acuity of 20/30 in the right eye. An MRI conducted a few days later revealed a largely symmetrical pituitary macroadenoma with chiasmal compression. The reason for the change in the visual acuity may be related to the enlargement of the adenoma during pregnancy, but no imaging was obtained before.

Our patient, similar to other cases reported in the literature, was wandering through different clinicians and three months with complaints of headaches and later diplopia. Initially, he was diagnosed with migraine, which did not

respond to therapy. After being surgically treated through endoscopic surgery, a method that has emerged as a first-line of treatment,⁽¹²⁾ his visual field defects and visual acuity got better. Such visual field improvements following endoscopic surgery have been reported in various studies^(13,14) and are consistent with our findings. Even after surgery, minimal defects in the upper temporal fields were still present in our patient. This happened because of the upward compression of the tumor in the optic chiasm.⁽¹³⁾

Conclusion

This case report highlights the importance of performing a thorough ophthalmological examination in patients presenting with headaches and visual disturbances, even if they have already been diagnosed with a neurological condition such as migraine. In this case, perimetry or visual field testing was crucial in detecting visual field defects, which led to further investigations and diagnosis of a sellar mass. Timely diagnosis and intervention, in this case, prevented further complications, and the patient made a good recovery after surgical removal of the mass. This report emphasizes the importance of a multidisciplinary approach in the management of patients with pituitary adenomas, involving ophthalmologists, neurologists, neurosurgeons, and pathologists.

Competing Interests

The authors declare that they have no competing interests.

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The Persistence of COVID-19-Related Pancytopenia as A Possible Sign of Hairy Cell Leukemia: A Case Report

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Abstract

COVID-19 is known to cause many hematological abnormalities, such as thrombocytopenia, leucopenia, and lymphopenia. Pancytopenia, a decrease in all peripheral blood cell lines, is a rare complication not commonly seen in patients with COVID-19. We report a case of a patient who experienced COVID-19 infection with mild clinical symptoms like fever, fatigue, and muscle and bone aches. The laboratory examinations revealed pancytopenia, mainly neutropenia, thrombocytopenia, mild anemia, and relative lymphocytosis, which persisted after infection resolution. The splenomegaly in abdominal echography and the characteristics of lymphocyte elements in peripheral blood smear examination raised suspicion of the presence of hairy cell leukemia (HCL); therefore, the patient was further examined with a bone marrow biopsy, which confirmed the diagnosis. The persistence of pancytopenia after recovery from COVID-19 infection, especially in patients with splenomegaly, should raise suspicion of another hematological coexistence diagnosis like HCL. (**International Journal of Biomedicine. 2023;13(2):357-360.**)

Keywords: COVID-19 • hairy cell leukemia • pancytopenia

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Abbreviations

HCL, hairy cell leukemia; RBC, red blood cells; WBC, white blood cells.

Introduction

A novel coronavirus, SARS-CoV-2 [an enveloped, positive sense, single-stranded RNA virus], causing COVID-19, has produced an ongoing pandemic in many countries.⁽¹⁾ Although the main target of the virus is the human respiratory system, it can affect other systems of the organism, especially the hematopoietic system and hemostasis.⁽²⁻⁴⁾

COVID-19 is known to cause many hematological abnormalities, such as thrombocytopenia, leucopenia, and lymphopenia.⁽⁵⁾ Pancytopenia, a decrease in all peripheral blood cell lines, is a rare complication not commonly seen in patients with COVID-19. The common causes of

pancytopenia are drug-induced bone marrow toxicity, tumors, autoimmune processes, and viral infections, including human immunodeficiency virus, parvovirus B19, cytomegalovirus, or Epstein-Barr virus.⁽⁶⁾ In the case of a viral infection, the etiological mechanism is bone marrow aplasia, which is caused by various mechanisms. In COVID-19 infection, in rare cases, persisting pancytopenia may reveal a coexisting hematological disorder.^(7,8)

Case Presentation

A 53-year-old woman without previously known diseases was diagnosed with COVID-19 infection in

September 2021 after an investigation for clinical signs of fever, fatigue, myalgia, and arthralgia. A blood test detected leucopenia (WBC - $3.47 \times 10^3/\mu\text{L}$) with moderate neutropenia (neutrophils - $0.63 \times 10^3/\mu\text{L}$), lymphocytosis (lymphocytes - $2.57 \times 10^3/\mu\text{L}$), thrombocytopenia (platelets - $64 \times 10^3/\mu\text{L}$), and mild anemia (RBC $3.81 \times 10^6/\mu\text{L}$, Hb - 10.4 g/dl). Blood iron (168.1 $\mu\text{g/ml}$) and ferritin (14.45 ng/ml) were normal, while the D-dimer value was slightly elevated (762 ng/ml). After the COVID-19 infection was resolved, the pancytopenia, especially leucopenia with neutropenia and thrombocytopenia, persisted; therefore, a peripheral blood smear examination and an abdominal echography were performed. In the peripheral blood smear, we detected a clonal group of lymphocytes that presented fine cytoplasmic extensions (Figure 1), while abdominal echography revealed the presence of splenomegaly. In these conditions, a diagnosis of HCL was suspected.

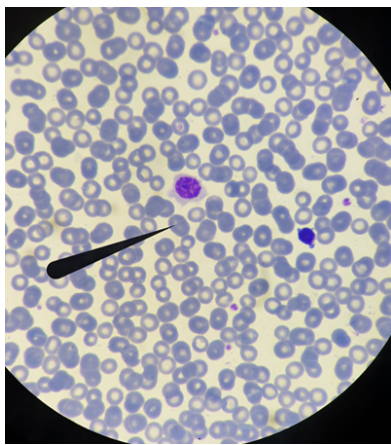


Fig 1. Hairy cell leukemia, cytological features. Peripheral blood smear showing characteristic lymphoid cells with pale blue or blue-gray cytoplasm and hair-like cytoplasmic projections. The nucleus is often eccentrically placed and is oval or indented, with loose, spongy chromatin.

The patient preferred to have further examinations to establish the diagnosis and undergo treatment at Pisa University Hospital. By this time (November 2021), neutropenia with lymphocytosis and thrombocytopenia persisted in the blood test.

The patient has received the osteo-medullary biopsy in which discreetly cellulated preparations were characterized by an infiltration of lymphoid elements equal to 70%, about half of which presented thin membrane protrusions compatible with the “hairy” phenotype. Normal hematopoietic lines were markedly reduced.

In the analysis of immunophenotype, the clonal lymphocytes were positive for pan-B-cell markers CD19, CD20, and positive for specific markers of hairy cell phenotype CD103, CD11c, and CD25. Considering these results, the diagnosis of HCL was established.

In December 2021, the patient started therapy for HCL with cladribine followed by rituximab (a cycle of cladribine injection for five days and one injection a week of rituximab for four weeks), and the response has been very good. Two

months after treatment completion (March 2022), the blood elements were significantly improved: WBC - $5.44 \times 10^3/\mu\text{L}$, neutrophils - $3.54 \times 10^3/\mu\text{L}$, lymphocytes - $1.39 \times 10^3/\mu\text{L}$, RBC - $3.89 \times 10^6/\mu\text{L}$, Hb - 12.6 g/dl, and platelets - $216 \times 10^3/\mu\text{L}$. In the control abdominal ultrasound, the spleen was within normal limits. By this time, in the bone marrow cytology, the lymphoid line was reduced to 9% without hairy phenotype, while the analysis of immunophenotype resulted in a 4% lymphocytic population and a clonal of 0.1% of total cellularity with the phenotype of hairy cell (CD103/CD25/CD 11c) suggestive for MDR (minimal residual disease). The patient is in remission with MDR, in good clinical and hematological condition, and under periodical controls.

Discussion

As previously mentioned, the most common cytopenia referred to in COVID-19 infection is leucopenia with lymphopenia and thrombocytopenia. Lymphopenia is found in many COVID-19 patients, about 80%-85%, with a higher proportion in severe cases.^(9,10) The presence of lymphopenia is explained mainly by two mechanisms: First, the COVID-19 virus invades human cells by binding to the angiotensin-converting enzyme 2 receptor (ACE-2), which is found primarily in the lungs, heart, and gastrointestinal tract but also expressed on the surface of lymphocytes. Consequently, the virus may bind directly to these cells and cause lysis. The second mechanism is related to the fact that virus infection induces the production and release of a number of pro-inflammatory cytokines, such as IL-6, IL-10, IL-1 β , IL-2, TNF- α , and IFN- γ , which cause a very strong immune response to SARS-CoV-2 infection, referred to as the “cytokine storm.”^(11,12) The cytokine storm, especially the strong activation of IL6, is shown to induce lymphocyte apoptosis, atrophy of lymphoid organs, and decrease lymphocyte regeneration.⁽¹³⁾ This exaggerated immune response also leads to an increase in neutrophil production in COVID-19 infection, especially in more severe cases complicated with a secondary bacterial infection.^(14,15)

Thrombocytopenia is another important laboratory feature in COVID-19 patients, encountered in 20%-55% of cases and related to the severity of the disease.⁽¹⁶⁾ The low platelet count is explained by different mechanisms, such as a decrease in platelet production either from SARS-CoV-2 infection myelosuppression or a strong inflammatory response,^(17,18) platelet consumption in microthrombi induced from endothelial damage due to the potent COVID-19 inflammatory response,⁽¹⁹⁾ or direct destruction of platelets by the immune system, as in an immune thrombocytopenic purpura-like state.⁽²⁰⁾ On the other hand, different studies have demonstrated that anemia is not common in COVID-19 infection, even in severe cases.^(21,22)

Our case report showed that typical laboratory signs of COVID-19 infection were mild leucopenia and thrombocytopenia. The presence of lymphocytosis and not lymphopenia, which is a prominent laboratory feature in this infection, the presence of neutropenia and anemia, the persistence of these blood alterations after infection resolution, and finally, the hairy-like projections in a peripheral

blood smear of lymphocytes associated with splenomegaly in abdominal echography raised a strong suspicion for a simultaneous diagnosis of HCL, a rare lymphoproliferative neoplasm characterized typically by pancytopenia associated with splenomegaly.⁽²³⁾ It is slightly more common in females with a median age of presentation of 55 years old.⁽²⁴⁾ Most cases present the *BRAF* V600 mutation in memory B cells, which activate the mitogen-activated protein kinase (MAPK) pathway, promoting growth, survival, and the differentiation of the clonal of hairy cells.^(25,26) The reticuloendothelial organs are infiltrated from the clonal of hairy cells, which morphologically are presented as small lymphocytes with fine hair-like cytoplasmic extensions, expressing immunophenotypically in addition to pan-B cell antigens, specific markers of CD11c, CD25, and CD103.⁽²⁷⁾

Hairy cells release cytokines, such as IL-6 and TNF- α , that prevent regular hematopoiesis and promote bone marrow fibrosis leading to pancytopenia⁽²⁸⁾ similar to COVID-19 infection. The clinical course of the disease is usually indolent, mainly presenting with weakness and fatigue (80%), infections, pancytopenia, and splenomegaly, similar to our case.^(24,29)

To our knowledge, few cases are reported in the literature to be diagnosed with HCL or reactivated HCL during COVID-19 infection,^(8,30,31) but a recent cohort study found an elevation of new cases diagnosed with HCL, revealed by splenomegaly and/or cytopenia in the situation of COVID-19, emphasizing the importance of investigating cytopenia when present at COVID-19 diagnosis.⁽³²⁾

In conclusion, persisting pancytopenia after COVID-19 infection may reveal that a coexisting hematological disorder probably exists; in particular, persisting neutropenia and thrombocytopenia associated with splenomegaly should raise suspicion of the presence of HCL, a rare form of leukemia characterized by these features.

Competing Interests

The authors declare that they have no competing interests.

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Presentation of Myopericytoma in the Lower Leg: A Case Report with a Brief Review of the Literature

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Abstract

Myopericytoma is a rare benign tumor of soft tissue that emerges from perivascular smooth tissue. Myopericytoma primarily derives from soft tissue and skin of the inferior extremities and is rarely found in internal organs. Considering the rare encounter with this neoplasia, it is often misdiagnosed as lipoma or atheroma. Our patient presents with a lump in the lateral region of the Achilles tendon on the right side. On inspection, a small, painful lump of approximately 5 mm × 5 mm is noticed without cutaneous changes. In histopathologic examination, branched blood vessels are detected with a characteristic “hemangiopericytoma look-alike” appearance surrounded by prolonged myoid cells. (**International Journal of Biomedicine. 2023;13(2):361-363.**)

Keywords: myopericytoma • myoid cells • lower extremities

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Introduction

Myopericytoma is a rare, benign, subcutaneous tumor that is characterized by the appearance of myoid-shaped cells that tend to form circular perivascular growth.⁽¹⁾ Although the origin of the tumor is from the hypodermis and soft tissue, there have been reports of myopericytoma occurring in internal organs such as the kidney and stomach or even as a primary intracranial myopericytoma. However, the frequency of the tumor in such organs is exceptionally low. These tumors are believed to originate from perivascular myoid cells and are usually diagnosed by histopathological analysis.⁽²⁻⁴⁾ When the neoplasia originates from the subcutaneous or soft tissue, the

most common location is the distal extremities, emphasizing the lower extremities. The tumor usually presents as a small, painless lump growing slowly. While myopericytoma is considered a benign tumor, it can cause discomfort and pain, particularly if it grows in a weight-bearing area or compresses surrounding tissues.^(5,6) Treatment of myopericytoma usually involves surgical removal. Given the rarity of this neoplasia, reporting case studies is essential to enhance our understanding of its clinical features, treatment options, and prognosis. In this report, we present a case of a 58-year-old female who presented with a painful lump on the lateral side of her right leg's Achilles tendon. The surgical removal of the lump confirmed the diagnosis of myopericytoma, and the patient completely recovered after the procedure. The purpose of this report is to increase awareness of this rare tumor and highlight the importance of considering it in the differential diagnosis of subcutaneous and soft tissue tumors.

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Case Presentation

A 58-year-old female presented at the Orthopedic Department of the General Hospital with a complaint of a painful lump on the lateral side of her right leg's Achilles tendon. She stated that she faced difficulty while walking because of the discomfort caused by the mass when it came in contact with her footwear. The physical examination revealed a well-defined lump of 5mm×5mm, with no cutaneous changes and no signs of inflammation. However, the lump was extremely painful when palpated. The patient had no history of previous surgeries but was regularly taking anti-hypertensive medications. She denied any family history of a similar condition.

The initial diagnosis was atheroma, lipoma, or epidermal cyst. Following preparations, the senior author performed surgical excision under local anesthesia. During the procedure, the tumor was located and removed, and it was found to be encapsulated with no visible signs of local invasion. The skin was closed using Nylon 3.0 suture. The material obtained during the surgery was sent for histopathologic examination.

The histopathology report revealed the presence of numerous branched blood vessels with a characteristic appearance that resembled a hemangiopericytoma, surrounded by elongated myoid cells (Figure 1). However, no cytologic atypia or mitosis was observed, and no signs of cell invasion were identified.

In conclusion, the patient's lump was diagnosed as a benign hemangiopericytoma-like tumor successfully removed through surgical excision. Although the patient did not have any medical history of such a condition, monitoring and following up with patients with similar symptoms is essential, as early diagnosis and treatment can lead to better outcomes.

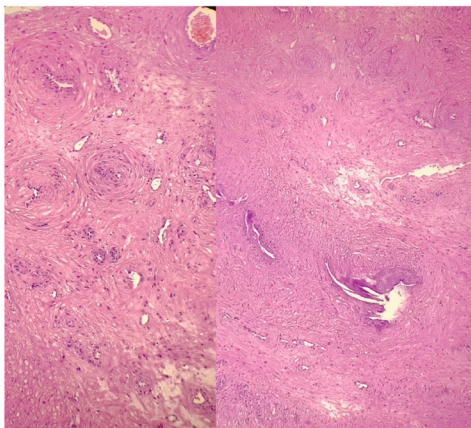


Fig.1. Increased vascular structure, prominent hemangiopericytoma, elongated myoid cells arranged in a concentric pattern, set in the collagenous stroma with mild edematous changes (H&E). x10 magnification; x4 magnification

Discussion

Myopericytoma is a rare and benign tumor of the skin and subcutaneous tissue, which was first described by

Requena et al. in 1996⁽⁷⁾ and then adjusted by Granter et al. in 1998,⁽⁸⁾ who identified its histological pattern as a “premature-type” of myofibromatosis, tumors with characteristics of hemangiopericytoma, glomus tumor, and tumors with a peculiar concentric perivascular proliferation of spindle cells. These studies suggest that the structure of the described tumors derives from a common origin. In 2002, the WHO included myopericytoma in the pericytic group in the Classification of Tumors of Soft Tissue and Bone.⁽⁹⁾

Although myopericytomas are slow-growing tumors that commonly arise in the lower extremities, they can also be found in the upper extremities, head and neck region, and trunk.^(10,11) While rare, myopericytomas have been reported in the region of the foot and ankle, with one case report of a myopericytoma presenting as a painful soft tissue mass in the plantar aspect of the first and second toes.⁽¹²⁾ When the neoplasia originates from the subcutaneous or soft tissue, the most common location is the distal extremities, emphasizing the lower extremities.^(5,13) However, there have been reports of myopericytoma in visceral organs such as the kidney and stomach, or even as a primary intracranial myopericytoma, although the frequency of the tumor in such organs is exceptionally low.⁽²⁻⁴⁾

Because myopericytoma is rare, it is often mistaken for lipoma or atheroma and can also be misdiagnosed as sarcoma.⁽¹⁴⁾ Our findings align with the description of myopericytomas in general, being well-circumscribed and nonencapsulated masses with spindle-shaped cells and a concentric perivascular growth pattern.

The incidence of the tumor is higher in middle age, but it can occur between the ages of 10 and 70, and there is no proof of hereditary linkage. While recurrence is very rare, it has been reported and is correlated with either incomplete removal or indistinct borders.⁽¹⁵⁾ Malignancy is also very rare, but it has been reported. In our case, none of the above-mentioned complications were noticed in the six-month and one-year follow-ups.

Surgical excision is the preferred treatment method, and several studies have shown good-to-excellent results with a low local recurrence rate.⁽¹⁶⁾ It is important to emphasize the need for close monitoring of patients after surgery to ensure complete removal and prevent a recurrence. Overall, the rarity of myopericytoma underscores the importance of careful clinical evaluation and histological examination to reach an accurate diagnosis and inform appropriate treatment decisions.

Competing Interests

The authors declare that they have no competing interests.

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[Corrigendum] Evaluations of Paranasal Sinus Disease Using Multidetector Computed Tomography in Taif City, Saudi Arabia

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Abstract

Corrigendum for 'Evaluations of Paranasal Sinus Disease Using Multidetector Computed Tomography in Taif City, Saudi Arabia' by: Alotaibi O, Osman H, Hadi Y, Alzamil Y, Alyahyawi A, Al-Enezi MS, Alafer F, Abanomy A, Khandaker MU, Almeshari M. International Journal of Biomedicine 12(4): 575-579. doi: 10.21103/Article12(4)_OA9. Following the publication of this article, the authors have realized that errors were made with the description of the listed affiliation addresses. Therefore, the author affiliations and addresses, in this paper should have appeared as follows: Osama Alotaibi^{1,2}, Hamid Osman³, Yasser Hadi⁴, Yasser Alzamil⁵, Amjad Alyahyawi^{5,6}, Mamdouh S. Al-Enezi⁵, Feras Alafer⁷, Ahmad Abanomy⁸, Mayeen Uddin Khandaker^{9,10}, and Meshari Almeshari⁵;

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