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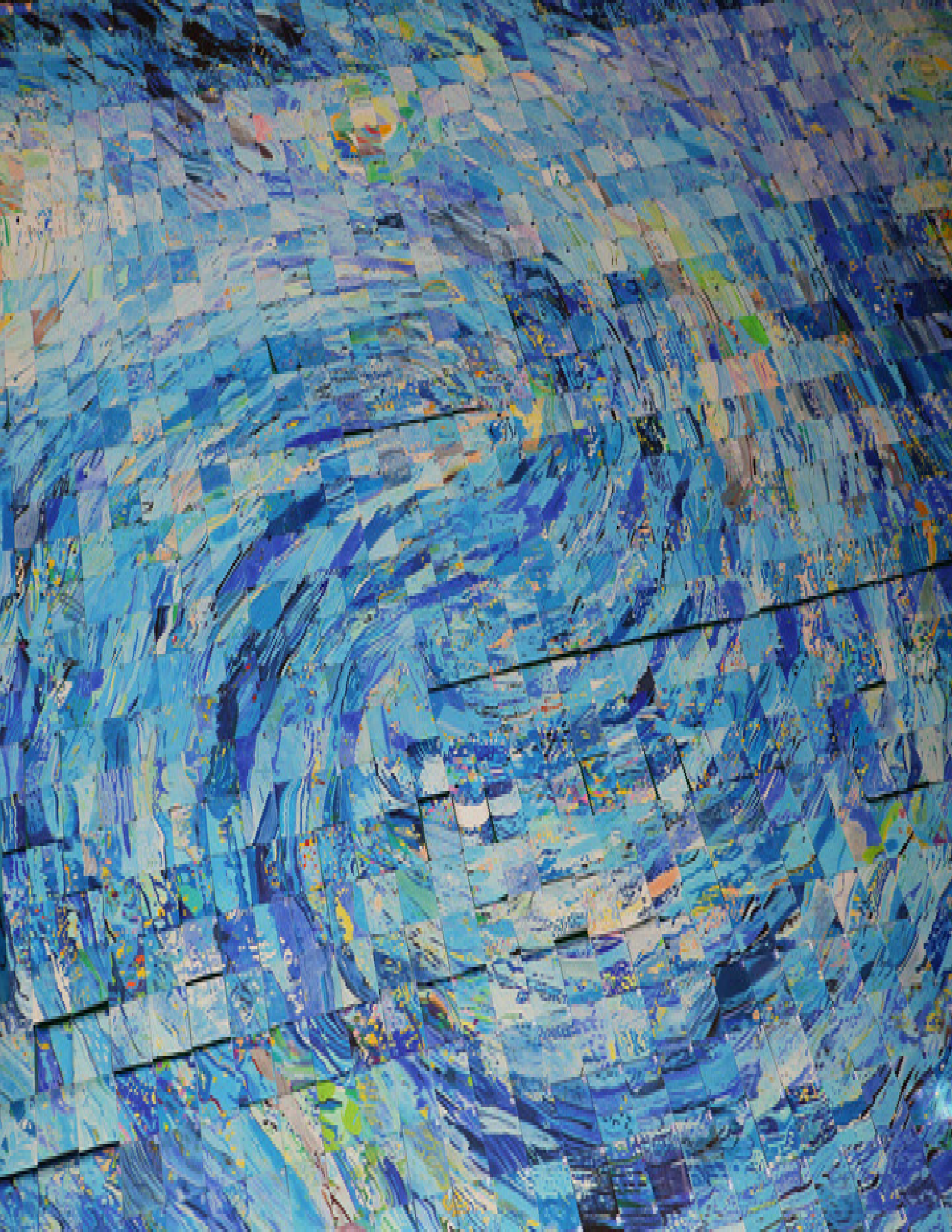

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REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Possible Unexplored Aspects of Covid-19 Pathogenesis: The Role of Carboxypeptidase A3

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). First reported in 2019, it has already caused more than 500 million cases worldwide. The problem of COVID-19 treatment is still relevant, and it is necessary to study in detail the pathogenesis of COVID-19, including the involvement of different immune cells and their mediators. There is increasing evidence of the important role of mast cells (MCs) and their specific protease carboxypeptidase A3 (CPA3) in the pathogenesis of COVID-19. MCs chymase and tryptase are already well studied, while CPA3 is of growing interest. The aim of this review is to study the CPA3 features and mechanisms of its participation in the pathogenesis of COVID-19 and some other infectious and non-infectious diseases.

Methods and Results: A literature search was carried out using Scopus, Web of Science, PubMed, Medline, and E-Library databases. Of the158 articles analyzed, 33 were included in the review. CPA3, expressed by MCs in various organs, including human lungs, plays a role in the pathogenesis of COVID-19 by indirectly causing pulmonary fibrosis, associating with levels of inflammatory cytokines and chemokines, and severity of COVID-19.(**International Journal of Biomedicine. 2022;12(2):179-182.**)

Key Words: COVID-19 • mast cells • carboxypeptidase A3

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Abbreviations

CCL, C-C motif chemokine ligand; **CPA3**, carboxypeptidase A3; **IL**, interleukin; **IP-10**, interferon-gamma-induced protein 10; **MCs**, mast cells.

Introduction

COVID-19 is a contagious disease caused by SARS-CoV-2. First reported in Wuhan, China, in December 2019, it has caused more than 500 million cases worldwide. Many aspects of COVID-19 pathogenesis have already been studied in detail, but the involvement of different immune cells and their mediators is of great interest now. There is increasing evidence of the important role of mast cells (MCs) and their specific protease carboxypeptidase A3 (CPA3) in the pathogenesis of COVID-19. MCs are important cells of innate and adaptive immunity, which play a considerable role in inflammation, allergic reactions, autoimmune diseases, parasitic infections, and tissue homeostasis. Upon activation, MCs release granules containing various mediators, including specific proteases: chymase, tryptase, and CPA3. MCs chymase and tryptase are already well studied, while CPA3 is of growing interest. There is evidence that CPA3 participates in the pathogenesis of cancer and inflammatory diseases of the gastrointestinal tract, as

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well as respiratory, cardiovascular, musculoskeletal and other systems. CPA3 features and mechanisms of its participation in the pathogenesis of COVID-19 and some other infectious and non-infectious diseases will be considered in this review.

CPA3 as an important component of the mast cell secretome

CPA3, as well as chymase and tryptase, is an important component of the mast cell secretome. Human MCs are classified according to their protease content and distribution in tissues: mucosal-type secreting tryptase is mainly found in the mucous membrane; the serosal-type secreting tryptase, chymase, and CPA3 are localized in the skin and lungs.[1] There are detailed studies about the structure and functions of chymase and tryptase, but other components of MC granules, such as cathepsin G, renin, matrix metalloproteinase 9, active caspase 3 and CPA3, are less studied.[1] At the same time, CPA3, being a numerous component of the mast cell secretome, plays an important biological role.[2-4] In humans, CPA3 is usually co-expressed in MCs expressing both tryptase and chymase,[5] but also in only tryptase-positive MCs associated with allergic diseases.[6] CPA3 is strongly associated with negatively charged proteoglycans, especially with heparin in the secretory granules of MCs.(5) CPA3 is released not only from MCs, but also from basophils, which are found in the blood and migrate to inflamed tissues. [7] CPA3 is a zinc-binding metallocarboxypeptidase of the family M14, similar to pancreatic carboxypeptidase, which cleaves C-terminal amino acid residues from proteins and peptides.[8] The M14 family consists of 4 subfamilies: the A/B subfamily (M14A), the N/E subfamily (M14B), the bacterial peptidoglycan hydrolyzing enzymes subfamily (M14C), and the complex cytosolic carboxypeptidases CCPs/Nna1-like subfamily (M14D).[9] CPA3 belongs to the M14A subfamily. Thus, CPA3 is an integral part of the mast cell secretome, being released upon its activation, having a complex structure and mechanism of action.

CPA3 in the development of infectious and non-infectious diseases

The involvement of CPA3 in the pathogenesis of many infectious and non-infectious diseases has been demonstrated. CPA3 plays a role in the development of atherosclerosis, participating in the formation of atherosclerotic plaques due to the degradation of saraphotoxin, and neurotensin, decreasing their biological activity.[10] CPA3 is also involved in the pathogenesis of cancer and inflammatory diseases of the gastrointestinal tract, as well as the respiratory, cardiovascular, and musculoskeletal systems, and can be considered as a diagnostic marker and pharmacological target.[11-14] CPA3 expression may be associated with eosinophilic esophagitis,[15,16] colon cancer[17], and type 2 eosinophilic asthma.[18-20]

CPA3 participation in the remodeling of the extracellular matrix has also been described. The CPA3-chymase complex affects fibroblasts, increasing their mitotic activity, and can also participate in the formation of collagen fibrils, which modify procollagen molecules.[5] These aspects are indirectly confirmed by studies on modeling adhesive processes in the abdominal cavity in laboratory animals, as well as in the study on lungs and kidneys with chronic inflammation or fibrosis.[3]

CPA3 may also play a protective role against parasitic infections. *Ascaris suum* produce a CPA3 inhibitor, which improves the parasite's survival in the host's organism.[21] The Strongyloides stercoralis invasion leads to a CPA3 increase in patients' blood serum.[22] CPA3 is also capable of deactivating snake venom (saraphotoxin), destroying it and increasing the survival of mice in experimental models.[23]

The CPA3 transcript is detected in human mastocytosis, as well as in MCs infiltrating various human tumors,[24] which makes it a potentially useful biomarker for detecting neoplastic MCs. Thaiwong T. et al.[25] studied lymphatic nodes of 78 dogs with a previously confirmed diagnosis of cutaneous metastatic mast cell tumors at the Universidade de São Paulo and the Michigan State University Veterinary Diagnostic Laboratory. The authors found that CPA3 expression levels were positively associated with the diagnosis of HN2 (early metastases) or HN3 (obvious metastases). It was found that the CPA3 messenger RNA (mRNA) expression was significantly different in lymph nodes diagnosed with HN0 (without signs of metastasis), compared with nodes diagnosed with HN2 (P<0.001) or HN3 (P=0.040), as well as in lymph nodes diagnosed with HN1 (pre-metastases), compared with HN2 (P<0.001) or HN3 (P=0.026). Significantly increased levels of CPA3 mRNA expression were found in HN3 lymph nodes, compared to HN2 (P=0.033). Thus, it can be concluded that CPA3 can participate in the pathogenesis of tumors and metastasis.

The role of CPA3 in the pathogenesis of COVID-19

There is growing evidence of CPA3 involvement in the pathogenesis of COVID-19. This involvement can be explained by the abundant CPA3 expression by MCs in various organs, including human lungs,[26] which are one of the main sources of MCs.[3] There is evidence that neurotensin, kinetensin, neurotransmitter N, angiotensin I and endothelin-1, identified substrates for CPA3, are associated with pulmonary fibrosis,[27] which is often observed in patients with COVID-19.[28] In addition, CPA3 limits the biological effects of endothelin-1, causing an impact on lung parenchyma and systemic blood flow. CPA3 has indirect vasodilating and bronchodilatory effects, as it converts leukotriene C4 to leukotriene F4, reducing the formation of leukotrienes D4 and E4 with more powerful broncho- and vasoconstrictive effects.[29] In addition, serum CPA3 proved to be a good biomarker for detecting patients with severe COVID-19.[30]

Soria-Castro R. et al.[30] analyzed levels of histamine, CPA3, serotonin, and heparin in blood serum of 21 patients with mild and moderate COVID-19, 41 patients with severe COVID-19, and 10 patients from the control group. They revealed increased CPA3 levels (P<0.05) and decreased serotonin levels (P<0.01) in patients with COVID-19, compared with the control group. Histamine and heparin levels did not change in patients with COVID-19. Moreover, the level of CPA3 was significantly increased in patients with severe COVID-19, (P<0.01) compared with mild or moderate disease. In addition, the study demonstrated a significant correlation between CPA3 and markers associated with inflammation: the level of circulating neutrophils (P=0.0447) and C-reactive protein (P=0.00703). CPA3 was also associated

with the assessment of the severity of the disease in the rapid assessment of organ failure associated with sepsis (qSOFA - quick Sepsis-related Organ Failure Assessment) (P =0.00862). Thus, altered CPA3 levels in COVID-19 patients may indicate the involvement of MCs in the pathogenesis of COVID-19, and this protease can be considered a potential biomarker during COVID-19.

Gebremeskel S. et al.[31] also studied levels of chymase, β-tryptase, and CPA3 in the blood serum of 19 patients with SARS-CoV-2 and 20 uninfected from the control group to find out whether MC activation was associated with SARS-CoV-2 inflammation. Significantly higher levels of inflammatory mediators were detected in the serum of patients with SARS-CoV-2 than in the uninfected control group, including CCL2 (P<0.0001), CCL3 (P<0.0001), CCL4 (P<0.0001), IL-6 (P<0.0001), and IL-8 (P<0.0001), IP-10 (P<0.0001), VEGF (P<0.0001), TNF-alpha (P<0.0001), and interferon-γ (P<0.0001). The authors also found significantly elevated levels of chymase (P<0.0001), β-tryptase (P<0.01), and CPA3 (P<0.0001) in the serum of patients with SARS-CoV-2, which proves the presence of systemic activation of MCs. Also, protease levels positively correlated with the levels of many inflammatory cytokines and chemokines associated with the severity of COVID-19 disease, including IP-10, CCL2, and CCL4. The established links show that MC activation and CPA3 activity are associated with inflammation in COVID-19 and are features of its pathogenesis that deserve special attention.

Thus, CPA3, expressed by MCs in various organs, including human lungs, can affect the lung parenchyma and blood flow, mediate vasodilating and bronchodilating effects, can be indirectly associated with pulmonary fibrosis in COVID-19, with levels of inflammatory cytokines and chemokines, and with severity of COVID-19.

Conclusion

Analyzing all information given above, we can conclude that specific protease CPA3 contained in MCs and released upon their activation is an important characteristic of MC protease phenotype. CPA3 participates in the development of infectious and non-infectious diseases, including disorders of the gastrointestinal tract, respiratory, cardiovascular, and musculoskeletal systems, as well as parasitic infections, pathogenesis of tumors and metastasis, fibrosis, inflammation, atherosclerosis, and other disorders. There is increasing evidence that CPA3 plays a role in the pathogenesis of COVID-19 by indirectly causing pulmonary fibrosis, associating with levels of inflammatory cytokines and chemokines, and severity of COVID-19. As CPA3 is poorly studied at this moment, it is necessary to continue further studies to discover the possibilities of its use as a diagnostic marker and a pharmacological target in the treatment of various pathological conditions.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Mast Cells as the Target of the Biological Effects of Molecular Hydrogen in the Specific Tissue Microenvironment

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Abstract

Mast cells (MCs) as key players in the development of both physiological and pathological processes in the organism can form a specific tissue microenvironment. Having a rich secretion of biologically active substances, MCs can secrete tryptase and/or chymase and thereby participate in the regulation of processes such as inflammation, neoangiogenesis, allergic reactions, and oncogenesis. Reactive oxygen intermediates (ROI) play an essential role in regulation of MC degranulation, shown in vitro and in vivo models. Application of molecular hydrogen as a substance with antioxidant characteristics pathogenically appears to be an important mechanism decreasing MC secretory activity, and, as a consequence, a novel option to reduce an inflammatory background in the specific tissue microenvironment.(**International Journal of Biomedicine. 2022;12(2):183-187.**)

Key Words: mast cell • tryptase • chymase • specific tissue microenvironment • molecular hydrogen

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Abbreviations

MC, mast cell; **ROI**, reactive oxygen intermediates; **UCP**, uncoupling protein; **Drp**, dynamin-related protein

The state of the specific tissue microenvironment represented by the vessels, cellular component, and extracellular matrix plays a key role in forming the abnormal focus. Each organ has specific cellular complexes of tissues that use proper regulatory mechanisms to support the local homeostasis. Mast cells (MCs) actively participate in the management of cellular cooperations, monitoring the majority of the key parameters of the cellular microenvironment.[1,2] The unique character of MCs involves an extraordinary combination of the sensor apparatus adapted to informationally significant signals of the integrative-buffer metabolic medium, on the one hand, and the polyfunctional effector apparatus represented by the secretome, on the other hand. The existent modifications of the tissue microenvironment are registered by MCs with the help of a wide range of receptors, including surface IgG receptors, toll-like receptors, C-type lectin receptors, retinoic acid-inducible gene-I (RIG-I)-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors, siglecs, G-protein-coupled receptors, lipid mediator receptors, alarmin receptors, leukocyte immunoglobulin-like receptors, cytokine receptors, integrins, tetraspanins, nuclear receptors, and many others.[3,4]

Adequately responding to challenges of the tissue microenvironment, MCs with high selectiveness secrete a rich arsenal of biologically active substances. This arsenal may be divided into pre-formed mediators and mediators newly synthesized in the process of activating MCs. Previously

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accumulated products of secretome are represented by a wide range of biogenic substances: biogenic amines (histamine, serotonin, dopamine, polyamines), proteases (chymase, tryptase, carboxypeptidase A, cathepsin G, granzyme B, metalloproteinase), enzymes (kinogenase, heparanase, angiogenin, active caspase-3), including enzymes of lysosomes (β-hexosaminidase, β-glucuronidase, β-D-galactosidase, aryl sulfatase A, cathepsins), proteoglycans (heparin, chondroitin sulfate), cytokines (TNF, IL-4, IL-15)), chemokines (RANTES, eotaxin, IL-8, MCP-1 and others, growth factors (TGF-β, bFGF, VEGF, NGF, SCF), as well as numerous regulatory peptides (corticoliberin, endorphin, endothelin-1, P substance, vasoactive intestinal peptide, angiogenin, bradykinin, leptin, renin, somatostatin, etc.). Products of re-synthesis include cell-derived cytokines, growth factors, mitogens (TNF, interleukins; EGF, bFGF/FGF- 2, GM- CSF, IFN-γ, NGF, PDGF, SCF, TGF- β1, VEGF/VPF, and others), MC-derived chemokines, and various lipid metabolites, namely prostaglandins and leukotrienes.[5-7]

With the help of secretome components, MCs are closely integrated into the genesis of adaptive and pathological conditions, representing not only an informative marker of the disease progression, but also a prospective therapeutic target. Specific MC proteases (tryptase and chymase) are of great significance. Secretory pathways of proteases and other secretome components represent various options of the substance secretion, with high selectiveness, into the extracellular matrix.[2,8,9]

The accumulated experimental data on the biogenesis and effects of MC tryptase allow considering it a multi-functional mediator with specific molecular-cellular mechanisms. The tryptase attracts a special interest in the pathogenesis of an allergy and inflammation under pathologies of the various body systems, including cardiovascular, respiratory, digestive, nervous, musculoskeletal systems and the skin, in the realization of carcinogenesis, and in the study of the tissue adaptive mechanisms under various ambient conditions, including the microgravic environment.[10] The immune-morphological study of the tryptase biological effects offers novel potentials for diagnostics of pathological conditions and monitoring of the performed therapy, as well as in the search for new pharmacological solutions to the treatment.

The biological significance of the chymase depends on the mechanisms of degranulation and is characterized by selective effects and the cellular and non-cellular components of the specific tissue microenvironment. The chymase is known to be involved in the mechanisms of allergy and inflammation, angiogenesis and oncogenesis, remodeling of the extracellular matrix of the connective tissue, and modifications of the organ histoarchitectonics. The number of chymase-positive MCs in the intra-organ population, mechanisms of secretome biogenesis, and degranulation represent the informative criteria for interpreting the internal organ status. The chymase takes an active part in the signaling molecular-cellular integrative mechanisms of the specific tissue microenvironment. The analysis of chymase-positive MCs gives new opportunities for understanding physiological and pathological reactions in various body systems, including cardiovascular, respiratory, digestive, musculoskeletal systems, the skin, and others.

The chymase is now of special importance relating to the fundamental oncological problems. This circumstance determines the need to further study specific MC proteases in basic and clinical research. Direct or indirect chymase effects in relation to the smooth muscle tonus of the cardiovascular and respiratory organs; penetration of vessels of the microcirculation; immunocompetent cells; cells of the fibroblastic programmed differentiation; the secretory epithelium; regulation of the cell division, growth, differentiation and apoptosis; modulation of cytokine, chemokine and growth factor activity; and remodeling of the extracellular matrix of the specific tissue microenvironment allow significantly expanding the informative value of the histologic study relating to a specific internal organ/tissue.

Therefore, further study of the role of MC proteases in the biology of the specific tissue microenvironment will significantly expand current views about the functional potentials of their organ-specific populations, giving unique opportunities to diagnose and evaluate the efficiency of the therapeutic protocols and to find breakthrough pharmacological solutions in the targeted therapy. Nowadays, there is a great deal of experimental data about the essential role of the reactive oxygen intermediates (ROI) in the regulation of MC degranulation on models in vitro and in vivo. ROI, which participate in intracellular signaling, stimulate certain anti-inflammatory MC mediators. There are several sources of ROI in MCs, including the electron transport chain of mitochondria, dehydrogenases in the matrix, p66shc protein in the intermembrane space and monoamine oxidases in the outer mitochondrial membrane, xanthine oxidase, cyclooxygenases, myeloperoxidase. NADPH-oxidases (NOX-enzymes) and lipoxygenases generate ROI in response to the hormone, growth factor, and cytokine effects. Most of these enzymes form superoxide ($O_2^{\bullet-}$), which later reacts to form peroxide (H_2O_2), giving a hydroxyl-radical (•OH).

High concentrations of ROI are known to impair DNA, RNA, lipids, and proteins; their low concentrations act as significant mediators participating in cell growth regulation, adhesion, differentiation, apoptosis, and other functions.[11,12] Results of numerous studies confirm that MC degranulation is caused by the chemical agents (salts of mercury and gold, substance 48/80, Ca^{2+} ionophores, etc.), as well as physiological stimuli (antigens, neurotrophic growth factor, P substance, and others), and is accompanied by the increased content of ROI in the cytosol.[13]

ROIs participate in the formation and further support activity of the intracellular complex regulating the level of Ca^{2+} in the cytoplasm.[13] The model of ovalbumin-induced food allergy demonstrated the participation of ROI formed on the basis of the PI3K-dependent path in the intensification of Ca^{2+} mobilization.[14] MC activation by the factors inducing phagocytosis may be also accompanied by ROI production.

Application of molecular hydrogen as a substance with antioxidant characteristics pathogenically appears to be an important mechanism for decreasing MC secretory activity, and, as a consequence, a novel option to reduce an inflammatory

background in the specific tissue microenvironment. In particular, it has been demonstrated that inhibition of ROI accumulation in the murine MCs prevented FcεRI-dependent degranulation and secretion of leukotrienes and cytokines.[13,15]

The protein of the mitochondria inner membrane UCP2 regulating ROI production was found to be able to inhibit the MC activation.[16] UCP2 is included in the family of uncoupling proteins; its title member, UCP1, results in the thermoregulatory uncoupling of oxidative phosphorylation in the mitochondria of brown adipose tissues. Recent studies have demonstrated that UCP2 catalyzes the transport of malate, oxaloacetate, and aspartate in exchange for phosphate through the inner mitochondrial membrane.[17] Export of C4 leukotrienes from mitochondria results in inhibition of oxidation of the Krebs cycle substrates; this significantly modifies the metabolism in mitochondria and, as a result, essentially decreases ROI production. Moreover, UCP2 not only neutralizes ROI but prevents its formation. As demonstrated, it may also have an impact on the dynamics of the increased concentration of calcium ions necessary for MC degranulation.[18]

In addition to inhibition of ROI production, uncouplers may modulate the Ca^{2+}-dependent signaling inhibiting Ca^{2+} accumulation in mitochondria, and decrease the excessive ROI generation by the mitochondria respiratory chain.[19-21]

The process of MC degranulation is known to be accompanied by the transfer of mitochondria towards the plasma membrane and their Drp1 mediated fragmentation.[22] Inhibition of Drp1 activity or its expression suppresses fragmentation of mitochondria and their transfer towards the plasma membrane, decreasing MC degranulation and TNF secretion.[22] As it is known, fragmentation of mitochondria in cells is able to be induced by the mitochondrial ROI effect;[23,24] this, in turn, may contribute to the regulation of MC activation.

ROI may cause reverse post-translational modifications of proteins participating in the intracellular signaling. For instance, some of the proteins are composed of functionally significant cysteine residues that may be exposed to oxidation. Thus, H_2O_2 can oxidize sulfhydryl groups (–SH) by forming sulfenic acid (–SOH); the latter may react with glutathione by forming the disulfide linkage (protein-SSG), with neighboring thiol groups with the formation the disulfide linkage (–SS–), or with amides with the formation of sulfanilamides. Sulfenic acid (–SOH) may be modified by exposure to further oxidation to sulfinic ($–SO_2H$) and then sulfonic ($Cys–SO_3H$) acids. Each of these modifications is able to change protein activity, thereby affecting its function in the signal transmission path.[25]

The increase of cytoplasmic concentration of calcium ions that are the key elements in mechanisms of degranulation appears to be one of the major events mediated by ROI impact and modification of the cellular redox status.[26,27] In particular, mitochondrial ROI may play an important role in Ca^{2+} mobilization.[28,29] It should be noted that modification of the Ca^{2+} intracellular concentration, in turn, impacts ROI generation.[29]

Phosphatases SHP-1, SHP-2, and PTEN participating in MC activation contain cysteine residues in their catalytic center and serve as one of the possible targets for ROI.[29,30] As shown, phosphatase inhibition under the H_2O_2 effect and/or with the help of pervanadate causes phosphorylation of tyrosine residues of β- and γ-subunits of FcεRI, calcium influx, and MC degranulation.[31]

All isoforms contain zinc finger domains and a high concentration of cysteine residues located in the regulatory region, as well as free sulfhydryl groups in the catalytic site. Moreover, redox-dependence of protein kinase C may be related to oxidative activation of phospholipase C[32,33] and mobilization of Ca^{2+}, and to phosphorylation of tyrosine residues by the redox-sensitive kinases of the Src family.[33] Therefore, ROI-dependent activation of protein kinase C is considered to be one of the mechanisms regulating MC activation.

In addition, linker for activation of T cells (LAT) (tansmembrane adapter protein associated with T cell activation) may also be the target for ROI; interaction with it enables induction of the FcεRI-dependent path of MC activation.

It is known that MARK-signaling with mitogen-activated protein kinases also depends on ROI.[34] As reported, transcriptional factors activated by the FcεRI-dependent path are redox-sensitive, including NF-κB and AP-1.[35] Thus, ROI may be of great significance in the regulation of the FcεRI-signaling cascade for MC degranulation. There is also a range of other potential targets sensitive to ROI impact. Research studies relating to the impact of ROI on various paths of MC activation, especially the FcεRI-dependent way, give striking perspectives for developing medical preparations based on antioxidants and inhibitors of ROI production and introducing them into clinical practice.

Molecular hydrogen, with its antioxidant characteristics being widely discussed nowadays, may serve as such an agent.[36-38] Molecular hydrogen may be applied for the effective treatment of pathological conditions associated with MCs, first of all, allergic conditions. Molecular hydrogen may be applied in various ways and acts as a blocker of the secretory MC activity, restricting their potential to the formation of the anti-inflammatory background in the specific tissue microenvironment; it may also be beneficial in the therapy of the diseases of various inflammatory and allergic geneses.

Therefore, MCs are closely involved in the effects of molecular hydrogen at the level of the specific tissue microenvironment, providing its anti-allergic, anti-inflammatory, anti-apoptotic, immuno-modulating, and vasotropic effects, as well as effects remodeling the extracellular matrix.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Insight about Different Physical Therapy Techniques for Management of Hypertrophic Scars

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Abstract

The most common complication of burn injuries is scar formation, which causes structural and cosmetic impairment. Between 1 and 3 months after an injury, hypertrophic scarring develops. Hypertrophic scarring caused by surgical operations, trauma, and particularly burns is a source of worry for patients and a difficult problem for practitioners. Burn scars can cause aesthetic and functional problems, resulting in limitations in activities of daily living. They can substantially impact one's quality of life, both functionally and cosmetically. Despite using many therapeutic techniques, scar control remains a significant concern. Pharmacological and physical therapy, as well as surgical procedures, are used with varying degrees of success and efficacy. New treatments for scar prevention are currently making their way into normal practice, due to the implementation of novel procedures for treating hypertrophic scars and keloids, as are new insights into the pathophysiology of excessive scarring. The purpose of this study was to review different physical therapy modalities that might be effective for the management of hypertrophic scars. (**International Journal of Biomedicine. 2022;12(2):188-192.**)

Key Words: hypertrophic scar • pressure garment therapy • shock wave • laser

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Abbreviations

ECM, extracellular matrix; **ESWT**, extracorporeal shock wave therapy; **HTS**, hypertrophic scars; **ROM**, range of motion

Introduction

Scar formation is a result of the wound healing process that occurs when body tissues are damaged by a physical injury. Hypertrophic scars (HTS) and keloids are pathological scars caused by abnormal responses to trauma and can be itchy, raised, painful, rigid, and cause severe functional and cosmetic disability.(1)

HTS is a dermal form of the fibroproliferative disorder that often develops following thermal or traumatic injury to the deep regions of the skin (deep dermis) and is characterized by excessive deposition and alterations in the morphology of the collagen and other ECM proteins. Unlike keloids, which progress beyond the original area of injury, HTSs remain within the boundary of the initial injury. HTSs occur at the site of injury resulting in cosmetic disfigurement, and can cause functional problems that often recur despite surgical attempts to remove or improve the scars. When HTSs are present in mobile skin regions, they can cause contractions that limit joint mobility. These difficulties can lead to psychological and social issues for burn survivors.(1-3)

Wound healing can be divided into 4 stages: hemostasis phase, inflammation phase, proliferation phase, and tissue remodeling phase. In these 4 stages, there are complicated interactions within a complex network of profibrotic and antifibrotic molecules, such as growth factors, proteolytic

enzymes, and extracellular matrix (ECM) proteins. Each molecule plays its part in the different phases of the wound healing process. As soon as the injury occurs, the hemostasis process begins, and the bleeding is controlled by the aggregation of platelets at the injury site. The subsequent formation of the fibrin clot helps stop the bleeding and provides a scaffold for the attachment and proliferation of the cells. Growth factors and cytokines are secreted by the inflammatory cells, and they share in initiating the proliferative phase of wound healing; after that, angiogenesis and collagen synthesis, followed by tissue remodeling, complete the stages of the wound healing process.[1] The delicate balance of deposition and degradation of ECM protein will be disrupted if either excessive production of the collagen, proteoglycans, and fibronectin by fibroblasts or deficient degradation and remodeling of ECM occurs. HTSs occur when the inflammatory response to injury is prolonged, leading to the pathological characteristics of HTS, including increased vascularization, hypercellularity, and excessive collagen deposition.[1,2]

Risk factors of HTS

Burn injuries, especially large and/or deep burn wounds, elevate the risk of pathological scar development because of the prolonged duration of the inflammatory phase. Indeed, it has been shown that a burn wound that heals in less than 10 days has a 4% risk of developing into HTS, whereas a burn wound that takes 21 days or more to heal has a 70% or greater risk. Infection like that in ear-pierce holes is prone to repeated infections, which lead to prolonged inflammation in the reticular dermis, increasing the risk of developing a scar. Local mechanical tension in location on the body increases formation of scarring such as (anterior chest-scapular region–sternum), whereas they seldom occur in areas where the tension of the skin is rare. Some hypertrophic scar patients have a familial history of pathological scarring, which suggests that these scars can be driven by genetic factors. Patients with black skin are 15 times more likely than patients with white skin to develop pathological scars.[4]

Signs and symptoms of HTS

There is hard and thickened raised tissue over the wound site. Skin color over the wound site from pink to red to purple appears most commonly on the upper trunk of one's body – back, chest, shoulders, upper arms, and the skin that covers joints. The scar usually develops from 1 to 2 months after injury and may cause irritation, itching, tenderness and/or pain sensation. The scar on the skin over a joint may limit your joint's normal movement.[5]

HTS types

Even a normal scar passes through a period when it is immature, and that means it is pink and often with a healing ridge (edema plus collagen synthesis). HTS during the immature stage will be pink and slightly raised, firm but not hard, and can be itchy sometimes. It begins soon after injury and takes months to resolve, typically peaking a few weeks after injury. A mature scar is a transition from an immature scar to a mature scar, and the visual marker is the resolution of erythema at this point; the inflammatory cells, endothelial cells and most of the fibroblasts have undergone apoptosis, and the epithelium looks completely normal. Mature scars are flat without erythema, the scar is stable without symptoms, and scar pigmentation is similar to surrounding skin, although they can be paler or slightly darker.[6]

HTS prevention

Following surgery or accidental trauma, the priority should always be to prevent the abnormal formation of the scar. In the case of surgical procedures, scar prevention measures should be initiated through or before the operation. In surgery, the position and the length of the incision line should be carefully considered and, if possible, should always be along the skin tension lines. Excessive scar formation can be prevented by reducing inflammation and providing rapid wound closure, for example, early debridement of dead tissue to help wound healing, reducing the risk of infection through rinsing and cleansing, and best dressings to provide moist wound healing and/or early surgical wound coverage. During surgery, the surgeon should ensure that excessive tension on the wound edges is avoided.[6,7]

The 3 major components of scar prevention immediately after wound closure are: decreased tension, hydration/taping/occlusion, and pressure garments. Wounds that have high tension on edges, like those perpendicular to skin tension lines and those in sternal regions, have a higher risk of developing excessive scar formation, which can be decreased using post-surgical taping for a period of 3 months.[6]

Moisturizing creams and moisture-retentive dressings have been shown to be beneficial for itching scars and can decrease their size and the pain or discomfort connected with scars. Studies have shown that in the time following wound healing, water still evaporates more rapidly during the formation of scar tissue scar tissue. Dehydration encourages the production of cytokines, leading to much collagen deposition by fibroblasts, which results in scar formation. So, these products may help to prevent excessive scar formation by restoring the water barrier through occlusion and hydration of the skin and need to be used as soon as the wound is healed.[6,8]

For patients with scars that spread, e.g., after burns, pressure garments may be used prophylactically in wounds that take >2–3 weeks to heal spontaneously, with the device being applied as soon as the wound is closed, and the patient can tolerate the pressure.[6,7]

General preventive measures for all types of scars include avoiding exposure to sunlight and the continued use of sunscreens with a high to maximum sun protection factor (>50) until the scar has matured. Randomized studies in animals and humans have shown that ultraviolet radiation increases scar pigmentation and worsens the clinical appearance of scars. The general rule: scars should always be re-evaluated (4–8 weeks) after surgery to determine whether additional scar management interventions are required or whether preventive therapy can be terminated.[7]

Assessment of scar

Japan Scar Workshop (JSW), a scar assessment scale, consists of 2 tables. One table is used to determine whether the scar is a typical mature scar, a hypertrophic scar, or a keloid. This grading system helps the user to select the most appropriate treatment method for the scar. Another table is used to judge the response to treatment and for follow-up. The 2 tables contain

sample images of each hypertrophic scar subjective item; this allows the user to evaluate each item without hesitation. The classification table consists of 2 parts risk factors and present symptoms, and the risk factors consist of 6 items (each of which has 2-3 categories): human race, familial tendency, number of scars, region of the scar, age at onset and causes. The present symptoms consist of 6 items (each of which has 2-3 categories): the size of the scar (cm^2), vertical growth, horizontal growth, the shape of the scar, erythema around the scars and subjective symptoms. Thus, the total number of items is 12. The categories in each item are weighted (0-1-2). The minimum and the maximum number of points in the classification table are 0 and 25, respectively. The classification of the scar score is as the following: The scar score ranging between 0-5 will have mature characteristics. From 6-15 or 16-25, the scar is a hypertrophic scar or keloid scar, respectively. The evaluation table consists of 6 items: induration, elevation, redness of the scar, erythema around the scar, spontaneous and pressing pain, and itch. Each item has 4 categories: namely, none, weak, mild, and strong; these categories are weighted (0-1-2-3). The lower and upper limits of the number of points in the evaluation table are thus 0 and 18, respectively. When the symptoms improve, the total score decreases.[8,9]

Physiotherapy techniques for management of post-burn HTS

Physical therapy treatment aims to influence the scar maturation process and therefore enhance the physical and mechanical properties of the scar. Also, it prevents adhesions, thus improving tissue strength and gliding.[10]

Pressure garment therapy (PGT)

Pressure garments are considered the favored therapeutic strategy for both the prevention and treatment of HTS. Currently, PGT is the general conservative management for preventing HTS formation.[11]

The precise mechanisms of the PGT action are not precisely understood. However, the most acceptable mechanisms are those that apply mechanical pressure by pressure garments, which leads to limiting the supply of blood, oxygen, and nutrients to the scar area, therefore, reducing fibroblast activity and the levels of collagen production more rapidly than the process of scar maturation. Consequently, mechanical pressure facilitates scar maturation, thinning and softening of scar tissues, reducing erythema of the scar, and minimizing itch and pain associated with HTS.[12,13]

Pressure garments should be used immediately when the healing tissue can tolerate the pressure and worn for at least 23 hours and/or 1 day for a period of 6 to 12 months or until the scar matures.[12]

Commonly, the amount of pressure between 15–40 mmHg is the recommended pressure level as being more effective and safer. Thus, pressure higher than 40mmHg is more likely to produce many side effects such as blistering, paraesthesia, abnormal bone growth, and limb necrosis. Alternatively, the pressure >15 mmHg is more likely to have no effect on scar tissue.[12]

However, PGT has some disadvantages that limit its effectiveness, such as the occurrence of complications, including overheating, pruritus, blistering, swelling, wound maceration, abnormal bone growth, poor compliance due to severe patient discomfort, the difficulty of applying a pressure garment evenly on the scar area, particularly in concave areas and flexor joints.[13-15]

Silicone therapy

Silicone sheets and silicone gels are considered non-invasive, first-line therapeutic strategies for both prophylaxis and treatment of hypertrophic scars. They are soft and semi-occlusive gel sheets that are durable and easy to handle. Silicone sheets should be worn for 12–24 hours each day for 3 to 6 months, commencing 2 weeks after wound healing. To avoid side effects such as rashes and infections, they should be washed daily with mild soap and water. Silicone gel is applied in fluid form to the skin, and when it dries forms a silicone sheet that is transpicuous, soft, and impermeable to fluids. Silicone gel should be applied twice a day.[7]

Silicone gels are easy to apply and suitable for use on visible areas such as the face and hands. On the contrary, silicone sheets are not suitable for use on visible areas, large areas of skin, and mobile body parts such as the joints. This justifies increased patient preference for and compliance with silicone gels.[17,16]

The major mechanisms of action of silicone therapy are occlusion and hydration of the stratum corneum. An increase of transepidermal water evaporation after wound healing results in dehydration of keratinocytes; after that, the keratinocytes release cytokines to fibroblast activation and increase the level of collagen production. Silicone products decrease water loss from the skin; therefore, hydration of the keratinocytes will be increased, resulting in reducing the stimulation of keratinocytes and producing cytokines; thus, fibroblasts will not be activated.[7,17,18]

Massage therapy

Massage therapy is a conventional therapeutic strategy for the treatment of HTSs. Manual or mechanical scar massage should be used in combination with silicone and pressure therapy when possible.[18] Scar massage is used to improve scar pliability, ROM, and soften scar tissue by mechanical disruption of adhering fibrotic scar tissue and also to reduce pain and itching associated with scarring, according to the gate control theory of Melzack and Wall. To be effective, massage therapy should be applied daily.[15]

Extracorporeal shock wave therapy (ESWT)

ESWT is a novel, non-invasive type of physical therapy for HTS treatment.[19] The exact mechanism underlying the positive effect of ESWT is still unclear. However, it is believed that suppressed epithelial-mesenchymal transition might be responsible for the anti-scarring effects of ESWT. On a histological level, after ESWT exposure, the levels of collagen type I, TGF-β1, α-SMA, fibronectin, and TWIST1 were considerably reduced in the HTS. However, E-cadherin was increased. Also, mechanical disruption of tissue by cavitation shock waves induces microtrauma in scar tissue, which results in scar remodeling. Furthermore, shock waves affect pain receptor physiology. Consequently, ESWT (1) enhances the HTS appearance and symptoms, and (2) ameliorates ROM, demonstrated by an increase in passive ROM. ESWT is considered safe, easy to apply, and tolerable by patients; it has

a low incidence of associated side effects, is cost-efficient, and can be used in an outpatient setting.[20,21]

Laser therapy

Many different laser types are effective for the HTS treatment.[14] The most prominent is the 585-nm pulsed dye laser (PDL). Another prominent laser type for the HTS treatment is the 1064-nm Nd:YAG laser. The recommended energies are 6.0J/cm^2 to 7.5J/cm^2 (7-mm spot) or 4.5 J/cm^2 to 5.5J/cm^2 (10-mm spot) and 14J/cm^2 (5-mm spot). To obtain the best outcomes, 2 to 6 sessions of treatment are recommended, and one every 3 to 4 weeks.

The mechanism underlying the therapeutic effect of laser therapy is that the laser vaporizes the blockage inside the vessel and decreases the vascularity of scar tissues. Thus, a reduction in vascularity decreases inflammatory cytokine or growth factor levels in the tissue, suppressing the formation of scars.[22] The most common side effects of laser therapy include hyperpigmentation, hypopigmentation, blistering and postoperative purpura.[11]

Kinesio Taping

Kinesio Tape is a therapeutic, highly elastic tape made from cotton. It is self-adhesive, thus it adheres to the skin. The mechanisms underlying the positive effect of Kinesio Taping on scars include that it eliminates multidirectional forces and tension of the scar, helps to avoid exacerbation of the inflammatory response during wound healing, aids in the softening of scar tissue and decreasing formation of adhesions, and yields low load for a prolonged period of stress on scar tissue, all of which in turn improve scar tissue remodeling.[23] Therefore, using Kinesio Tape improves the pliability, vascularity, height, thickness, cosmetic appearance, pigmentation, and pain relief.[24] Kinesio Tape should be worn 24 hours/day and reapplied every 3-5 days. And the period between applications should be increased once the scar responds and matures. Kinesio Taping is considered a low-cost and comfortable technique for treating widespread hypertrophic scars, and it is easy to use for health care providers and patients. Also, it can be applied to regular and irregular body parts like the neck and face.[23]

Conclusion

Hypertrophic scarring is a significant issue with long-term functional, cosmetic, and psychological consequences for the patient. HTS has a complex etiology. The depth of the wound is one component that has a significant influence. Burns that are only on the surface heal faster and leave fewer scars. Additional components of healing come into play when the depth of the wound increases and affects structures deeper than the epidermis. Pressure, silicone, massage, extracorporeal shock wave, laser therapy, and Kinesio Taping are all useful and approved non-invasive physiotherapeutic treatments for individuals with HTS.

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Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Updates on Hirsutism: A Narrative Review

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Abstract

Hirsutism is described as an abnormal amount of hair development in females in a male-like way. Excessive hair growth is frequently associated with severe emotional discomfort. Hirsutism is a frequent presentation to dermatologists, as women seek both aesthetic and therapeutic treatment options for their condition. Hirsutism is caused by an excess of androgens, most frequently from the ovary or adrenal glands. Hirsutism is frequently linked with metabolic disorders such as polycystic ovary syndrome (PCOS); it can also occur idiopathically, as a side effect of medicine, or, rarely, due to a serious underlying condition. This article discusses the diagnosis and treatment of hirsutism concisely.(**International Journal of Biomedicine. 2022;12(2):193-198.**)

Key Words: polycystic ovary syndrome • hirsutism • treatment

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Abbreviations

CAH, congenital adrenal hyperplasia; **DHEA-S**, dehydroepiandrosterone sulfate; **FSH**, follicle-stimulating hormone; **GnRH**, gonadotropin-releasing hormone; **LH**, luteinizing hormone; **NCAH**, nonclassic CAH; **OCPs**, oral contraceptive pills; **PCOS**, polycystic ovary syndrome; **SHBG**, sex hormone–binding globulin.

Introduction

Hirsutism is the abnormal development of dense, black hair in locations where women's hair development is generally modest or non-existent. Men's body hair pattern development is more common in androgen-stimulated areas, including the face, chest, and areolae.(1) In women, hirsutism generates severe anxiety and low self-esteem. Although it is a benign disorder in and of itself, it is frequently a symptom of a more serious underlying endocrine issue. In the majority of cases, hirsutism is a harmless disease that is mostly of aesthetic importance. However, hirsutism might be a signal of an ovarian or adrenal tumor if it is accompanied by masculinizing signs or symptoms, especially if they appear after puberty. Fortunately, these conditions are uncommon. Hypertrichosis, which is an increase in body hair that is not restricted to androgen-dependent regions, must be separated from hirsutism. Hypertrichosis is characterized as abnormal hair development in areas other than androgen-dependent parts, whether terminal or vellus hairs. Hypertrichosis is a condition that can be either congenital or acquired. Acquired hypertrichosis can occur as a side effect of some medications, such as phenytoin, penicillamine, L-thyroxine, and others, or as a result of systemic disorders, including hypothyroidism and malnutrition. Depending on the amount of hair, hirsutism can be classed as I (hirsutism) or IV (virilization).(2) The most essential factor in determining the diagnosis is a change in hair growth type and rate. Recently, camera equipment and computer software were used to establish a procedure for evaluating hirsutism. There is a considerable variation in hair

structure and growth rate between hirsute and non-hirsute women, as evidenced by digital imaging of hair development.[3] Following a comprehensive clinical assessment, laboratory work and radiology are performed to verify or exclude underlying reasons. Hair removal and pharmacological therapy of any associated etiology are among the first steps in management, which also involve patient education and support.

Hirsutism affects around 5% to 10% of women in the childbearing period.[4,5] Excess hair is a cosmetic problem for women, and it may have a negative impact on self-esteem.[6] Hair growth that is normal or acceptable is determined by a female's ancestry, family, cultural, and social hair volume, and distribution norms. Females of the Mediterranean region have a fair quantity of face and body hair, while Asian females have very little. Hirsutism is clinically rated using the Ferriman-Gallwey (FG) scale, with an FG score of 8 or above indicating the person is hirsute.[4]

PATHOPHYSIOLOGY

Numerous variables, including growth factors, mediators, and reproductive hormones, influence hair development. Hair growth patterns have been demonstrated to be affected by thyroid and growth hormones. Sex hormones, especially androgens, influence the kind of hair that develops and distributes throughout the body. Vellus follicles in certain regions grow into terminal hair when testosterone levels rise throughout adolescence.[8] This is determined by the degree and length of androgen exposure, local 5-alpha-reductase function, and the inherent hair follicle susceptibility to androgen action.[9] In most androgen-sensitive areas, high androgen levels result in enhanced terminal hair growth (e.g., regions of the upper lip/chin/chest/back). Despite the fact that androgen excess is at the basis of the majority of hirsutism instances, hair development and androgen volumes are only slightly linked.[7] Hirsutism is produced by hair follicles that are more responsive to normal or unusually high androgen levels.[10] As a result, excessive hair growth is frequently seen in people with endocrine problems defined by hyperandrogenism (ovaries or adrenal gland abnormalities). Three stages are postulated for the physiologic process of androgenic action: (1) Adrenal and ovarian production of androgens; (2) Transport of androgen in the blood by carrier proteins (most notably SHBG); (3) Androgen receptor binding and intracellular modification.

In brief, hirsutism could be induced by central androgen overproduction, increased peripheral androgen conversion, reduced metabolism, and increased receptor binding. Circulating testosterone must be transformed into dihydrotestosterone, a more powerful follicle-active derivative, in order to have an enhancing effect on the hair follicle. This conversion is carried out by the 5-alpha-reductase enzyme, which is present in the hair follicle. Due to individual differences in hair follicle androgen reactivity, the degree of hirsutism does not correspond to the amount of elevated serum androgens. Testosterone promotes hair development by enhancing the size and pigmentation of hair. Estrogens work against testosterone, decreasing hair development and resulting in finer, lighter hairs. Hair growth is unaffected by progesterone. SHBG regulates the quantity of available testosterone (the physiologically active androgen, which induces hair development following conversion to dihydrotestosterone). Low SHBG concentrations increase free testosterone bioavailability. SHBG levels decline as a result of the following: External androgens, PCOS, Cushing syndrome, Hyperinsulinemia, Overweight, Hypothyroidism, Elevated prolactin, and Overproduction of growth hormone. SHBG concentrations, on the other hand, rise in response to greater estrogen levels, such as those seen during oral contraceptive medication. As a result of the elevated SHBG levels, circulating testosterone activity is reduced.[9]

Polycystic ovary syndrome

PCOS is a prevalent cause of hirsutism that usually appears throughout puberty. Menstrual abnormalities or infertility are symptoms of PCOS, as are insulin resistance (metabolic syndrome or diabetes), indicators of increased androgen (such as hirsutism and acne), and laboratory proof of excess androgens. PCOS can be diagnosed without polycystic ovaries. Although the cause is unclear, it is believed that the etiology of PCOS is complex. PCOS develops whenever the ovaries are prompted to generate high androgen amounts. Increased LH secretion or hyperinsulinemia can induce this. Hyperinsulinemia increases GnRH pulse frequency, LH over FSH dominance, and androgen synthesis, while decreasing SHBG levels.[11] This disease is thought to be caused by gonadotropin-dependent functional ovarian hyperandrogenism, but a modest, functional adrenocorticotropic-dependent increase in androgens has been seen in several patients. PCOS can appear with a solitary DHEA-S increase in rare situations.[12]

Idiopathic hirsutism

Idiopathic hirsutism refers to hirsutism that arises for no apparent reason, such as in women who have regular menses, normal androgen levels, and no other symptoms that suggest other causes of hirsutism.[4] Whereas this phrase is often used, it can be deceptive, particularly if idiopathic hirsutism is diagnosed using routine laboratory testing, which does not usually show androgen excess.[7] Idiopathic hirsutism is believed to be caused by a small increase in androgens secreted from the ovary or adrenal gland, enhanced activity of 5-alpha reductase in the hair follicle, or androgen receptor anomalies.[10]

Other conditions of excess androgen

HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome is a set of genetic diseases characterized by significant insulin and glucose metabolic abnormalities as well as symptoms of hyperandrogenism. It is distinct from PCOS.[13] SAHA (seborrhea, acne, hirsutism, and acanthosis nigricans) syndrome is a clinical spectrum linked to high androgen levels. These symptoms can occur as a result of the HAIR-AN syndrome or a different source of elevated androgens, such as ovarian, adrenal, hyperprolactinemic, or idiopathic sources. Exogenous variables such as androgenic medicines (Progestins, Diazoxide, Minoxidil, Phenytoin, Danazol, Glucocorticoids, Anabolic steroids, Testosterone, Cyclosporine) and NCAH can also cause hirsutism. Premature pubarche, hirsutism, and menstrual abnormalities are all symptoms of NCAH, which is caused by a 21-hydroxylase

insufficiency.[14-16] Adrenal or ovarian androgen-secreting tumors, both benign and malignant, are uncommon causes of hirsutism. In such circumstances, hirsutism may appear suddenly or progress quickly, and it may be accompanied by virilization symptoms such as a deeper voice, greater muscular mass, clitoromegaly, and increased libido.[13]

CLINICAL AND INSTRUMENTAL ASSESSMENT OF HIRSUTISM

Women's excessive hair that exceeds regionally acceptable levels could be just as unpleasant as hair loss on the scalp. Hirsutism can manifest itself in a variety of ways. It commonly arises during adolescence in women with familial hirsutism, for example. In individuals with PCOS and CAH, hirsutism typically develops over time. When an androgen-secreting tumor forms, hirsutism emerges suddenly. Obtaining a proper menstrual history is critical since women with high androgen will experience irregular menstrual periods. Menstrual irregularities are typical in women with PCOS. Idiopathic hirsutism can affect women who have regular periods. When collecting a history, ethnicity should also be considered. In comparison to many Asian women, who have very little hair, women of Mediterranean heritage have more body hair, on average. Finally, a review of hirsutism, infertility, and obesity in the family, as well as medication usage, should be conducted.[10]

Excess terminal hair in a male pattern characterizes a woman with hirsutism; however, hirsutism might be difficult to detect in women with blond hair. The FC model, a quantitative way of evaluating hair growth, permits assessing hirsutism severity by analyzing hair growth in nine main anatomical areas: Moustache, temple, and beard regions on the face; Central chest; Areolae; Linea alba; Back (upper); Lower back; External genitalia; Buttock area; Inner thighs.[1] Other signs include Acanthosisnigricans; Obesity; Virility and Cushing's symptoms; Alopecia and acne.[17]

The purpose of biochemical testing of hirsute women is to find those with significantly high androgen levels, which might indicate the presence of androgen-secreting tumors. After ruling out hereditary and drug-induced causes of hirsutism, androgen excess hirsutism must be considered. The serum DHEA-S and total testosterone values are often acquired to exclude adrenal and ovarian cancers. Baseline testosterone (total or free) evaluation, as well as DHEA-S, may typically identify if additional testing is required. These hormones may reveal the cause of increased androgen secretion.[17]

Regarding serum testosterone, it is debated whether total testosterone or free testosterone is a more efficient screening test. Total testosterone testing is less costly and perhaps easier to interpret. Free testosterone, on the other hand, may be a more sensitive sign of hormonal imbalances. Tests for testosterone levels should be done first thing in the morning. The highest acceptable limit for testosterone in plasma differs per laboratory; however, it often falls between 70ng/dL and 90ng/dL. It's also worth noting that testosterone levels change by about 25% during different parts of the menstrual cycle. Because hirsutism is caused by dihydrotestosterone, the much more powerful testosterone metabolite, there is no direct association between testosterone levels and the degree of hirsutism. Most women with anovulation and hirsutism have increased free serum testosterone(>80 ng/dL). A tumor workup is recommended for most individuals with total testosterone >200 ng/dL (>100 ng/dL in post-menopause). Pelvic and ultrasound examinations are generally sufficient to confirm PCOS in this workup. An adrenal computed tomography scan is conducted if the test findings are negative.[17]

The DHEA-S level is high in certain hirsute people. Moderate increases point to hirsutism being caused by the adrenal glands. Normal DHEA-S levels and high testosterone levels suggest that the excess androgen is produced by the ovaries rather than the adrenals. Most individuals with a DHEA-S greater than 700 mcg/dL (400 mcg/dL in post-menopause) should have a tumor workup. Adrenal hyperplasia, rather than the exceedingly uncommon adrenal carcinomas, causes a rise in this level.

Consider the following additional tests if a woman has significant or fast progressing hirsutism or indications of virilism (e.g., irregular or missed cycle, acne, deep voice, androgenetic alopecia, increased muscle mass circumfluous, enhanced libido, clitoral enlargement):

•Androstenedione in the blood: Androstenedione is produced by the adrenal glands or the ovaries, and its levels are frequently increased in hyperandrogenic individuals. A serum androstenedione level of 100 ng/dL indicates an ovarian or adrenal tumor.

•LH and FSH: LH is often increased while FSH is decreased in women with PCOS, resulting in high LH/FSH ratios (>2).

•17-Hydroxyprogesterone: Morning 17-hydroxyprogesterone levels are measured as a screening test for late-onset CAH. DHEA-S and 17-ketosteroids levels are normal or slightly increased. Precursors of testosterone and cortisol are at an all-time high. Patients with PCOS have slightly increased urinary 17-ketosteroid levels. The most prevalent defect linked with CAH is a 21-hydroxylase deficiency, which is diagnosed by a 17-hydroxyprogesterone level >800 ng/dL.

•A dexamethasone suppression test is required for an intermediate 17-hydroxyprogesterone level (200-800 ng/dL); however, this level is normal in many females with adult 21-hydroxylase insufficiency, and corticotropin stimulation could lead to overdiagnosis of the illness.

•Prolactin level: PCOS is more common in oligomenorrheic patients. Sensitivity and specificity problems affect LH, FSH, and prolactin tests. Testing almost never improves the patient's result.

•Urine cortisol testing: If Cushing's syndrome is suspected, urine cortisol testing for 24 hours is required.[18]

Imaging investigations of the adrenal and ovarian organs may be necessary for patients with suspected PCOS or a potential tumor.[18] A biopsy of a hirsute area will reveal terminal hairs; however, a biopsy is not really required for diagnosis.[13]

TREATMENT

When an underlying problem is discovered, it is critical to treat it. PCOS is seen in the majority of individuals with

severe hirsutism, and the most essential therapies are to reduce the chances of endometrial hyperplasia and cardiovascular diseases. Hirsutism management is only essential if the extra hair is cosmetically bothersome to the patient. Simple hair bleaching is an economical alternative to hair removal that works effectively when hirsutism is not severe. Bleaches lighten the hair color, making it less apparent.[19] The patient should be informed about cosmetic and pharmacological therapy, as well as the possibility of long-term treatment and the negative effects of certain medications or surgeries. At the start of treatment, the patient should have realistic expectations. Patients should be informed that, rather than completely eliminating hair follicles, pharmacologic treatment will most likely result in reduced hair development, and hair removal will be less frequent. Obtaining an FG score at baseline and, if feasible, at each visit should be used to track therapy response. The patient's opinion of improvement is most likely the most relevant consequence. During therapy, no recommendations for monitoring testosterone are required. Therapy could be maintained as long as the patient chooses during the reproductive years but should be stopped if pregnancy is planned.[18]

Hair removal

Depilation

Depilatories are products that remove hair from the skin's surface. Shaving and applying thioglycolic acid are examples of depilatory procedures. Shaving eliminates all hairs; however, it is followed by the return of anagen hairs, generating scratching as they grow in. Shaving does not appear to accelerate or coarsen future hair development. The majority of women, on the other hand, avoid shaving facial hair. Chemical depilation could be the ideal option for treating big regions in people who can't afford more expensive procedures like electrolysis or laser epilation. Chemical depilatories work by diminishing the sulfide bonds contained in hairs, which helps to detach the hair from its follicle. Irritant responses and folliculitis are possible side effects.[4]

Temporary epilation

Epilation is the removal of unbroken hair from the root. Tweezing or plucking is a standard procedure. Irritation, hair follicle damage, folliculitis, hyperpigmentation, and scarring are all possible side effects of this approach. Waxing is the cutaneous application of melted wax. It is peeled away from the skin as it cools and hardens, eliminating any attached hair. This procedure is inconvenient and can lead to folliculitis. Waxing on a regular basis may cause hair shrinkage and, in the long run, a permanent reduction in the quantity of hair. Natural sugars, which have long been utilized in regions of the Middle East, are gaining popularity as a substitute for waxes. They epilate like waxes but with less abrasion. Threading is a method of removing hair from the root using cotton threads, which is popular in several Arab nations. Home epilating devices that use a rotational or frictional approach to remove hair are available. Traumatic folliculitis can be caused by any method. In the past, radiation treatment was a common way of hair removal. It has, however, lost popularity and is no longer acceptable.[4]

Permanent epilation

Hair is destroyed via electrolysis, thermolysis, or a combination of the two methods, which use a tiny, flexible electrical wire that generates electricity when inserted into the hair. Thermolysis is a quick form of electrolysis that employs a high-frequency alternating current rather than a direct galvanic current. Thermolysis and electrolysis are slow procedures that may be used on any skin or hair color, but they require several sessions. These methods can cause pseudofolliculitis, folliculitis, and postinflammatory pigmentation in the skin, which can be painful. Lasers have the ability to treat greater areas more quickly than thermolysis or electrolysis. They contain skin-cooling systems that help to keep the epidermis from being destroyed throughout the operation. The color of one's skin and hair may frequently indicate whether or not a laser should be utilized. On fair-skinned persons, lasers are more effective on dark hairs. The laser, which only targets the melanin, does not compete with darker hairs in lighter skinned people. In dark-skinned people, a new procedure that gives hairs greater energy over longer periods of time may be safe and effective. Multiple sessions are required for long-term hair elimination. Laser treatment can cause folliculitis, pseudofolliculitis, pain, and pigmentary changes. It's still unclear if lasers are more successful than more traditional procedures for permanent hair removal. They are unquestionably more expensive.[18]

Pharmacologic treatment

Typically, pharmacologic therapies for hirsutism are chosen depending on the underlying etiology. Medications (antiandrogens) are frequently used together with cosmetic hair reduction procedures. Because androgens rebound to their previous levels when these medicines are stopped, they must be taken continually. These drugs are not recommended during pregnancy, because there is a danger of feminizing a male fetus. These substances can be used alone or in combination.[18]

Oral contraceptives

Oral contraceptives are frequently the first line of defense in ovarian hyperandrogenism and idiopathic hirsutism. Oral contraceptives also aid in enhancing antihirsutism benefits and preventing the negative effects of spironolactone and other antiandrogen-therapy-induced menstrual period irregularities. The combinations of estrogen and progestin in OCPs are generally thought to be safe and cost-effective. The capacity of progestin to decrease LH production and, as a result, ovarian androgen production, is its mode of action. Estrogen raises SHBG levels, which lowers free testosterone and other androgens bound to it. OCPs also work by interfering with the production of adrenal androgens. A combination of ethinyl estradiol (0.03 to 0.035 mg) plus a progestin (CPA or drospirenone) with low androgenic or antiandrogenic characteristics is commonly used to start OCPs.[19]

Antiandrogenic drugs

Many antiandrogenic medications are used off label, to treat hirsute women. Spironolactone, a competitive inhibitor of 5-alpha reductase and androgen receptors, has been shown to be useful in the treatment of hirsutism. When initiating antiandrogen treatment, reliable contraception should be utilized in women of reproductive age. Spironolactone is commonly used in doses of 100mg to 200mg per day to treat hirsutism. Potential side effects include polyuria,

postural hypotension, irregular cycle, hyperkalemia, and liver problems. Spironolactone has been shown to be tumorigenic in animal experiments; however, this has not been shown in people.[18]

Cyproterone inhibits androgen receptors and 5-alphareductase activity in a competitive manner. It can be taken with an oral contraceptive pill for only the first 10 days of the cycle (50 or 100 mg dosage) or in a low dose with a combination oral contraceptive pill (Diane-35). Tiredness, emotional changes, risk of venous thrombosis, and lower libido are among the side effects, which are comparable to the risks associated with oral contraceptives. Importantly, there is a possibility of feminizing a male fetus in women of reproductive age; thus, reliable contraception must be utilized.[10,20]

Flutamide, an experimental antiandrogen, has shown promise in the treatment of hirsute women. Flutamide is a competitive, nonsteroidal androgen receptor inhibitor. It has a high chance of causing hepatotoxicity.[4,18]

Finasteride 1mg is rarely used to treat hirsutism (off label). It suppresses dihydrotestosterone levels by inhibiting type II 5-alphareductase. Hepatotoxicity, gastrointestinal disturbances, reduced desire, and male fetus feminization are the risk factors (pregnancy category X). As with all antiandrogens, dependable contraception is recommended for all women of reproductive age. The treatment of hirsutism with dutasteride, a type I and II 5-alpha-reductase inhibitor, has still not been explored (pregnancy category X).[4]

Insulin-sensitizing agents

Metformin, like other insulin sensitizers, is less successful than antiandrogens at decreasing hirsutism. On the other hand, it is beneficial in inducing ovulation in polycystic ovarian syndrome patients. Metformin (Glucophage) lowers insulin levels, which lowers ovarian testosterone levels by inhibiting ovarian insulin receptors in a competitive manner. This medication is useful in treating hirsutism in PCOS patients. Gastrointestinal discomfort is a typical side effect, and lactic acidosis is a significant but uncommon complication.[18,21]

Other drugs

Cimetidine and ketoconazole are two more antiandrogen medications. Cimetidine is ineffective for the treatment of hirsutism, while ketoconazole is linked with a considerable risk of hepatotoxicity, as well as various medication interactions.[18]

In individuals with severe hyperandrogenism, GnRH is only used if antiandrogen and oral contraceptive medications have failed. They inhibit luteinizing hormone release and ovarian androgen production. Because GnRH analogs lead to menopausal-level estrogen decline, these medications are administered intramuscularly every month, generally with an estrogen-progestin supplement. Menopausal symptoms, such as vaginal atrophy, hot flushes, and osteoporosis, are among the side effects. Because these medicines totally block ovulation, many gynecologists do not recommend further contraception in women of reproductive years. GnRH analogs, on the other hand, are not authorized as a contraceptive and are classified as pregnancy category X.[18]

In women with typical 21-hydroxylase insufficiency, steroids are frequently required for a long time. They sustain ovulatory cycles while suppressing adrenal androgen production and controlling hirsutism. In females with NCCAH 21-hydroxylase deficiency, trials comparing glucocorticoids to antiandrogens and OCPs have indicated that glucocorticoids are more successful in reducing adrenal androgens but less efficient in treating hirsutism. According to the Endocrine Society, glucocorticoids should not be used to treat hirsutism in women who do not have a classic or nonclassic type of CAH caused by a 21-hydroxylase deficiency. Glucocorticoids are recommended for women with NCCAH who do not react to or cannot take OCPs or antiandrogens, or who want to induce ovulation.[18]

Topical, surgical treatment, and lifestyle

Eflornithine is a topical cream that serves as a development suppressor rather than a depilatory. Ornithine decarboxylase, a hair development enzyme, is inhibited by this drug. It's for ladies who wish to get rid of their undesirable facial hair. Application twice a day for at least 4-8 weeks is mandatory before the result is realized.[4,18] When ovarian or adrenal tumors are confirmed to be the cause of excessive body hair, the tumor may usually be removed. Many tumors, however, are cancerous and lethal.[18]

Although many hirsute females are obese, the link between fatty tissue and hair development is unknown. Clinically, for obese hirsute women with monthly abnormalities, losing weight may control menses and decrease hirsutism.[12,23]

In conclusion, treating hirsutism sometimes necessitates an interdisciplinary approach. Frequently, many specialties are involved in this process. The purpose of this multifaceted approach is to treat not only cosmetic problems via medical treatment and hair removal, but also the female's self-image anxieties and emotional stress caused by excessive body hair.

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Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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An Overview of Diagnosis and Treatment of Melasma

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Abstract

Melasma is a persistent hyperpigmentation condition that is most commonly seen in middle-aged women. The malar, central, and mandibular parts of the face are most affected by this condition, Factors that contribute to melasma's etiology are still a mystery. Multifactorial causes are now widely accepted in these cases. In order to diagnose melasma and its variants, distinguish them from other pigmented illnesses, and keep track of treatment progress and side effects, several diagnostic tools are employed. The limited response and high recurrence rate make treating melasma difficult. Melasma can be treated with a variety of methods, including topical medicines, laser treatment, and injections. The objective of this article is to offer a concise overview of melasma diagnosis and management.Melasma treatment is a cosmetic challenge. Chemical, physical, and laser therapy are all options. (**International Journal of Biomedicine. 2022;12(2):199-203.**)

Key Words: hyperpigmentation • melasma • diagnosis • dermoscopy • treatment

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Introduction

Cosmetically, facial pigmentations are essential, and they have a significant influence on patients' quality of life. Melasma is the most frequently acquired pigmented condition, characterized by homogeneous hyperpigmented patches with asymmetric contours on the face, most typically in middle-aged women with dark skin. The use of oral contraceptives, exposure to the sun, and pregnancy are all aggravating factors.[1-3] Hydroquinone, retinoid, azelaic acid, and glycolic acid are some of the topical medicines now often utilized to treat hyperpigmentation. Irritation is one of these chemicals' most prevalent side effects.[4-6]

Causes of Facial Hyperpigmentation

Frequently, the reasons for facial hyperpigmentation are unclear. Genetic and racial variables both have a role. Increased pigmentation is more common in those with dark skin. UV exposure, endocrine factors, pharmaceuticals (e.g., phenytoin, phototoxic medications), and other diseases (e.g., autoimmune thyroid *diseases*) can all exacerbate melasma, and are also implicated in other melanoses to a lesser extent. Cosmetics and Addisonian pigmentation may contribute to the development of face melanosis.[6]

Hypermelanos is due to genetic and environmental causes:

- •Ephelides (freckles), Peutz–Jeghers syndrome, Naevus of Ota
- •Lentigines, juvenile acanthosis nigricans, café au lait macules, melasma

Acquired hypermelanosis:

- •Metabolic: Liver disease, porphyria and haemochromatosis
- •Endocrine: Melasma and Addison's disease
- •Chemical: Minocycline pigmentation, Berloque dermatitis
- •Post inflammatory hyperpigmentation: Lichen planus, erythema dyschromicum
- •Tumors: Acanthosis nigricans with adenocarcinoma, malignant and metastatic melanoma.[7]

Diagnostic Approach to Facial Hyperpigmentation

History and physical examination

The patient's history and physical examination provide insight into the etiology of hyperpigmentation. The history should include information on the lesion's start, as certain illnesses (e.g., neurofibromatosis) are present from birth, whereas others (e.g., ephelides) emerge during infancy or (e.g., melasma) occur during pregnancy. The presence of systemic symptoms may imply hyperthyroidism or Addison's disease. A study of drug usage, dietary supplements, and exposure to plants and UV light can assist in determining if hyperpigmentation is a side effect of a medicine or a phototoxic response. Neurofibromatosis, ephelides, and lentigines can be diagnosed based on the size and number of lesions. The border, color, and nature of a lesion aid in differentiating melanoma from benign lesions, whereas the pattern of skin changes assists in identifying melasma and acanthosis nigricans[8] (Table 1).

Clinical Features of Melasma

Patches ranging from light to dark brown with uneven boundaries typically occur on the facial area.(Figures 1-7) Melanosis occurs in three typical patterns:

(1) Centrofacial (the most frequent), including the forehead, cheeks, nose, upper lip (save the philtrum and nasolabial creases), and chin.

(2) Malar, which affects the cheekbones and nose.

(3) Mandibular, running parallel to the jaw.

The extensor portion of the forearms and the middle area of upper chest are less often used locations.[6,9]

Wood's light

A Wood's lamp may aid in diagnosing certain conditions. When examined with a Wood's light, epidermal melanosis intensifies and becomes more confined.[10] Lamp evaluations are typically reserved for Fitzpatrick skin types I–IV. They are ineffective on those with skin type VI and are only marginally effective on persons with skin type V. Generally, the Wood's light is incapable of distinguishing between epidermal and dermal pigments in mixed hypermelanosis in all skin types.[3,6,9]

Dermoscopy

Dermoscopy is a systematic approach for evaluating the colors and structural components of the skin.[11] There have been developed scoring methods that evaluate asymmetry, border, color, and dermatoscopic features. The development of computerized image analysis is assisting in differentiating benign melanocytic lesions from melanoma.[12-15] Dermoscopy has exceeded conventional evaluation methods in distinguishing melasma from other sources of face hyperpigmentation, measuring its depth, monitoring treatment efficacy, and detecting early consequences, such as atrophy and telangiectasia.[16,17]

Histopathology

A biopsy can conclusively determine the site of the hypermelanosis; however, it is seldom necessary for

Table 1.

Clinical features of often occurring face hyperpigmented disorders.[2,8]

	Color	Age of onset	Medication related	Sun exposure
Melasma	Brown cheek, forehead and upper lip macular lesions	Adulthood	Pregnancy, oral contraceptive pills	Enhanced by exposure to sunlight
Post inflammatory hyperpigmentation	Macular brown discoloration at the location of inflammation	Any age	Irrelevant	Injuries caused by physical or chemical agents or dermatoses
Ephelides	Multiple small red, tan, or brown macules, on sun-exposed regions	Early life	Irrelevant	Increased number and pigmentation
Lentigines	Multiple small tan, brown, or black macules on sun-exposed parts	Early life	Irrelevant	Enhanced in sun exposed regions
Photoallergic/phototoxic reaction	Diffuse inflammation followed by hyperpigmentation in sun-exposed regions	Any age	Irrelevant	Sun exposure coupled with the problematic medications
Café au lait macules	Multiple small macules with smooth or asymmetrical, but distinct edges	Congenital or during childhood	Irrelevant	Uninvolved
Hemochromatosis	Hyperpigmentation in a diffuse slate-gray or bronze hue	Adulthood	Irrelevant	Uninvolved
Poikiloderma of Civatte	Reticulate dark pigmentation Lateral and low neck	Adulthood	cosmetics, hormones	Increased by sun exposure
Erythromelanosis follicularis faciei et colli	Affecting follicles Pre auricular, maxillary areas. Symmetric pigmentation	Adulthood	Irrelevant	Uninvolved

this purpose; rather, it is used to ascertain an origin that is unknown.[18-20] Three types of hypermelanosis are defined based on the melanin distribution (as determined by lesion color, its enhancement under Wood's light, and pathology):

1. Brown hypermelanosis: A condition in which the basal and suprabasal layers have an excess of melanin, and the pigmentation is exacerbated when exposed to the Wood's lamp.
2. Blue hypermelanosis: A condition in which the dermis has an excess of melanin and the pigmentation is not exacerbated by Wood's light.
3. Mixed hypermelanosis: Melanin levels in the epidermis and dermis are elevated.[21]

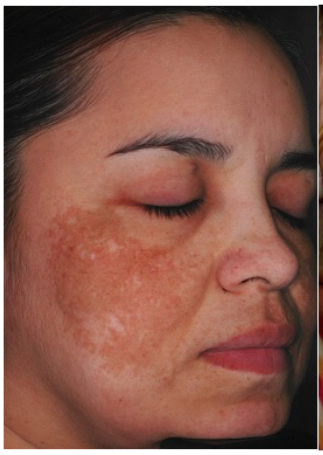

Fig.1. *Melasma on the cheek.* [6]

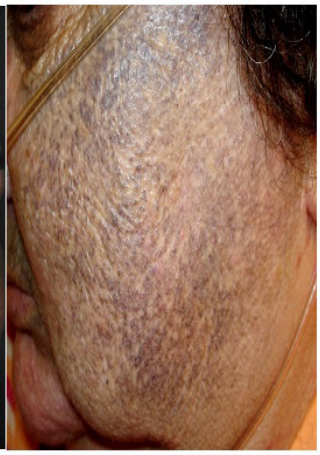

Fig.2. *Minocycline hyperpigmentation* [6]

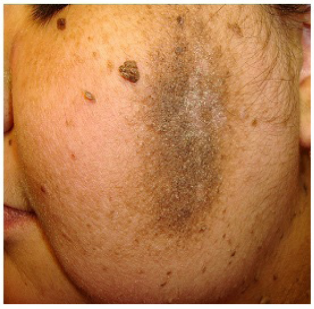

Fig.3. *Acanthosis nigricans*[6]

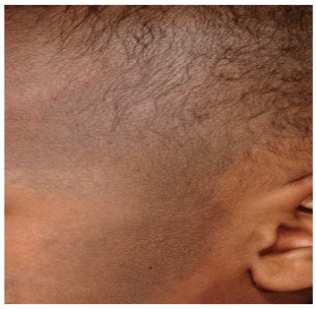

Fig.4. *Nevus of Ota*[22]

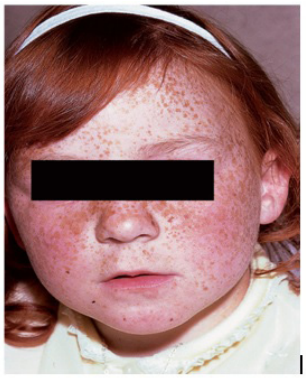

Fig.5. *Freckles* [22]

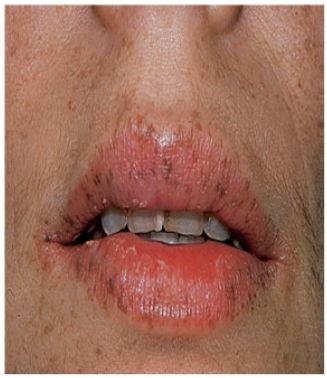

Fig.6. *Multiple lentigines (Peutz-Jeghers syndrome)*[9]

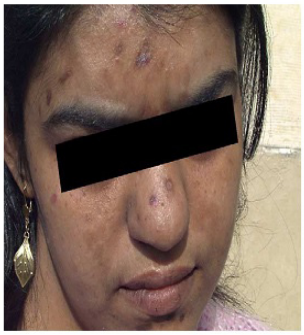

Fig.7. *Post-inflammatory hyperpigmentation* [23]

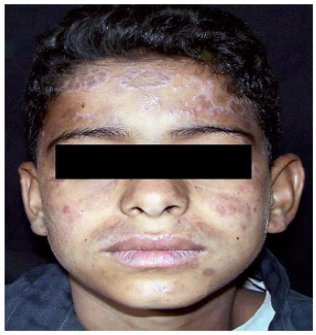

Fig.8. *Actinic lichen planus* [1]

Treatment of Facial Hyperpigmentation

Topical medications that influence pigment synthesis, such as broad-spectrum sunblock and camouflage, are typically used as first-line treatment. Chemical peels are widely used as a second-line treatment; however, they should be used with caution in those with darker skin. While laser and light therapy are possibly beneficial for individuals who have failed to respond to conventional treatment methods, they also entail a high risk of aggravating the condition.[6]

General Instructions

All patients should avoid prolonged exposure to the sun. They should dress appropriately and protect themselves with broad-spectrum sunscreen. If the use of drugs or cosmetics has resulted in facial hyperpigmentation, they must be discontinued. If melasma develops during pregnancy, it is important to apply sunscreen, with the condition improving by the end of gestation.[2]

Sunscreens

Patients with facial hyperpigmentation should apply a broad-spectrum UVA and UVB-protective sunscreen with an SPF not less than 30 and a physical block, such as titanium dioxide or zinc oxide, and should repeat regularly. Additionally, patients should be advised to wear protective caps and clothes when outside and to avoid sunlight if feasible.[18]

Treatment of Melasma

Hydroquinone 3% or 4%, glycolic acid 10% peel, azelaic acid 20% cream, and retinoids (e.g., tretinoin 0.05% or 0.1 % cream; adapalene 0.1% or 0.3 % gel) all exhibit some efficacy.[24,25] Combination treatments are more successful at bleaching than monotherapies, and usually physicians begin therapy with one of these formulae applied once daily (at night), followed by maintenance with 2% hydroquinone[26] (Table 2).

Numerous modest studies indicate that laser treatment or a combination of strong pulsed-light therapy and hydroquinone with sunscreen may be useful in treating dermal or refractory/mixed-type melasmas.[27] Melasma caused by pregnancy or oral contraceptive usage often resolves several months after birth or discontinuation of medication; therefore, cautious waiting should be urged wherever possible.[28]

Table 2.

The most often used depigmenting formulas in melisma.[26]

Name of Formula	Active ingredients
Kligman's formula	Hydroquinone 5% Tretinoin 0.05%-0.1% Dexamethasone or betamethasone valerate 0.1% in hydroalcoholic base in the form a cream or an ointment
Pathak's formula	Hydroquinone 2% Tretinoin 0.05%-0.1% in hydroalcoholic base in a cream or ointment base
Westhorf's formula	N-acetylcysteine 3% Hydroquinone 2% Hydrocortisone 1% in ointment base

According to Lee et al.,[29] topical trans-4-tranexamic acid, a plasmin inhibitor, effectively inhibits UV-induced pigmentation. This procedure involves injecting 0.05ml–0.1ml of highly diluted tranexamic acid or a single product intradermally or subcutaneously into areas of the body with medical or cosmetic issues. It may be able to treat dermal-type melasma in addition to mixed-type melasma by injecting tranexamic acid intradermally.[29,30]

Conclusion

Melasma treatment is a cosmetic challenge. Chemical, physical, and laser therapy are all options. Melasma due to pregnancy or contraception usage may disappear spontaneously over time in patients with light skin.

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Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Hand Sanitizers Containing Alcohol and their Effects on the Skin during the COVID-19 Pandemic

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Abstract

COVID-19 is a contagious disease with a high case fatality and morbidity rate associated with a pandemic outbreak. Transmission of SARS-CoV-2 infection can take place via airborne transmission, droplet, or direct contact. Implementing effective preventive measures remains the most important option available for dealing with SARS-CoV-2. The effectiveness of surface disinfectants, sanitizers, and personal protective equipment is totally based on three factors—strength, composition, and material—to determine whether or not preventive measures will be effective. Coronavirus transmission is disrupted by the use of an alcohol-based sanitizer containing 62% to 95% alcohol, which can denature viral proteins. However, hand sanitizers and disinfectants used on a regular daily basis may harm the skin's surface. In this study, we cover the importance of selecting the right disinfectant, the proper method of hand sanitization, and how to minimize the harmful effects on the skin while enhancing the inhibitory activity that could be a viable prescription for fighting COVID-19.(**International Journal of Biomedicine. 2022;12(2):204-208.**)

Key Words: SARS-CoV-2 • alcohol-based hand sanitizers • contact dermatitis

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Abbreviations

ABHS, alcohol-based hand sanitizers; **ACD**, allergic contact dermatitis; **HS**, hand sanitizer; **ICD**, irritant contact dermatitis.

Introduction

The World Health Organization (WHO) has declared a global emergency in response to a current, unexpected COVID-19 outbreak in China.[1,2] SARS-CoV-2, a single-stranded RNA virus, has the largest genomic configuration among all RNA viruses, with a genome size of 26-32Kb.[3] In the case of coronavirus, the bat is the most likely primary host to transmit viral infections, which is why it is called a zoonotic virus. Vaccination and antiviral drug therapy are the first lines of defense in the fight against viral infections, according to the WHO. The inability to obtain effective antiviral drug therapy against COVID-19 is now exacerbating the situation to a greater extent.

Infection prevention and control, according to WHO guidelines, is an evidence-based and practical approach to prevent avoidable infections from harming patients and health workers. According to statistics,[4] infection control programs contributed to significant reductions of healthcare-associated infections by 30%.

Because of the worldwide crisis that the COVID-19 pandemic has created, infection prevention and control are the most important remaining opportunities for preventing the outbreak of viral infection. The transmission of SARS-CoV-2 infection can take place through airborne and droplet transmissions, or direct contact. To reduce the spread of COVID-19, it is necessary to implement appropriate preventive measures, such as the habitual applications of

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effectual rubs, hand sanitizers, and soaps, as well as special defending tools (appropriate face/eye guards, a proper grown, and fitted masks). The alcohol-based sanitizer dissolves the lipid's membrane, denatures the proteins of microorganisms, and inactivates them. But at the same time, the regular use of hand disinfectants may hurt the skin of the hands.

The goal of this review was to investigate the efficiency of alcohol-based hand sanitizers (ABHS) against the coronavirus infection in humans, as well as unpleasant influences and commendations to reduce the negative impact of the hand sanitizer (HS) on the skin.

This review was carried out in accordance with the PRISMA checklist.[5] It was based on prior research published in English databases such as PubMed and Google Scholar in scientific articles published between 2000 and 2021 with the keywords "hand washing," "skin impact," "alcohol," and "hand sanitizers." The information extracted from each study included the study's primary characteristics, such as study design, year of publication, and the primary author's name. We selected articles from a large number of reports in accordance with the effectiveness of HS and the possible consequences of using them during the current COVID-19 pandemic.

SARS-CoV-2 Infection

SARS-CoV-2 belongs to the genus Betacoronavirus, which are enclosed, affirmative single-strand RNA viruses with similar morphology.[6] Lipid solvent, involving ether and ethanol, can deactivate these viruses (75%). The SARS-CoV-2 infection causes coughing, breathlessness, and fever. Surfaces of inanimate and animate items could be responsible for SARS-CoV-2 transmission, but the main mode of transmission is human to human. SARS-CoV-2 transmission occurs through droplets, coughing, contaminated hands and surfaces, and other means.[6,7]

COVID-19 & Preventive Care

Among the most important precautionary measures are the use of personal preventive equipment (face and eye protectors and well-fitting masks), good hand hygiene, and effective surface disinfectants. However, frequent handwashing with water and soap, or disinfecting hands with alcohol-based sanitizers that were recommended by WHO, can help to reduce the risks of COVID-19 transmission from an infected person to others. WHO has issued instructions to local manufacturers concerning HS production.[8]

Types, Mechanism of Action, and Recommended Concentrations of ABHS

The WHO recommends two preparations containing ethanol or isopropyl alcohol. The first contains isopropyl alcohol (99.8%), and the second - ethanol (96%). Alcohol's sanitizer/biocidal power varies with its type and strength. Alcohol's biocidal action is interrupted by microbial exterior protein coagulation; a 90% alcohol concentration instantly coagulates the microbial proteins.[8] Adding various inorganic or organic acids, including phosphoric acid, fumaric acid, peroxyacetic acid, L-lactic acid, citric acid, or lactic acid, to an alcohol-based disinfectant can increase its efficacy against various viruses. As a result of the inadequate dissolution of organic substances into free ions, their lipophilic character and capacity to permeate the infected biomolecules, along with their propensity to cause hazardous effects, rise.[9] Isopropyl alcohol (70%-72%) and ethanol (60%-70%) are preferred as disinfectants/biocidal agents because of their predetermined concentration ranges. Disinfectant/biocidal agents used in hand sanitizers have been shown to improve considerably the effectiveness of hand hygiene.[9]

Skin Anatomy and Physiology

The three skin layers are the superficial epidermis, the middle dermis, and the innermost hypodermis. Additionally, the skin protects the body from mechanical trauma and water loss while also protecting against microbial invasion. The stratum corneum, the outermost layer of the epidermis, serves as the primary barrier between the body and the environment. The epidermis is composed of keratinized, stratified squamous epithelium.[10] The squamous cell layer is involved in the transfer of certain substances in and out of the body. The stratum corneum is the cornified layer of the skin consisting of 15–30 sheets of corneocytes (cornified keratinocytes).[10] As lamellar bodies are exocytosed during keratinocyte terminal differentiation, these lipids will occupy the cellular components between the corneocytes and help maintain the epidermal protective barrier.[11] The squamous cell layer also contains cells called Langerhans cells. These cells attach themselves to antigens that invade damaged skin and alert the immune system to their presence.[12,13] The epidermis also contains melanocytes producing melatonin, which colors skin and shields it from the sun's UV rays.

As well as acting as a barrier to harmful microbes, the skin is home to numerous helpful bacteria, notably *Aureus epidermis*, *Staphylococcus*, *Micrococcus* spp., *Mycobacterium* spp., and *Corynebacterium* spp.[14] By competing for nutrition or boosting the skin's defense system, these bacteria may provide colonization resistance against harmful microorganisms. Under normal circumstances, microbes are not harmful. However, they could become virulent if the skin flora distribution is interrupted, such as by frequent hand washing or lengthy use of topical agents.[15,16] In order to decrease the occurrence of infection, the microbial equilibrium is reached and preserved through continuous skin regeneration. The process takes around 28 days, beginning with the process of mitosis of the basal epithelium and ending with a desquamating stage. When keratinocytes are leached off from the epidermis, the microorganisms that colonize the surface of the skin are removed. Bacterial invasion is kept to a minimum as microbial populations grow in a balanced manner.

Impact of Alcohol-Based Sanitizer on the Skin

When using alcohol-based hand sanitizers regularly, specific safety methods should be considered. The sebaceous glands on the skin's surface secrete oil that is comprised of free fatty acids, particularly lauric and sapienic acids, which have intrinsic antiviral action.[17] ABHS that is regularly used

may remove natural oil from the surface of the skin, leading to a reduction in antiviral activity and dehydration of the skin. In addition, skin dehydration is typified by cracked cuticles, which could provide pathogens with effortless access to deep skin layers, resulting in the promotion of microbial infection. Aside from that, the potential for fire hazards associated with ABHS is an inescapable associated factor for the skin. Additionally, ABHS is known to cause allergic reactions. Irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) are the two most frequent skin responses associated with ABHS use. ICD symptoms can range from mild to severe, with manifestations such as dryness, pruritus, erythema, and bleeding being common. ACD symptoms can be mild and localized or severe and widespread, depending on the severity of the disorder.[18]

There are a number of possible causes for adverse reactions to alcohol-based preparations, including sensitivity to a contaminant, carbonyl compounds, or other excipients such as perfumes, benzyl alcohols, parabens, or benzalkonium chloride.[19] HS and other hand hygiene products can sometimes damage the skin through such effects as neutralization of the endothelium proteins and changes in intercellular lipids, as well as a decrease in corneocyte cohesiveness and water-binding ability of stratum corneum.[20] The most severe problem is the degradation of the lipid shield, specifically with prolonged exposure to liposome cleansers and lipid-dissolving alcohols, which may infiltrate deep skin layers and modify the epidermal ecology, culminating in more recurrent migration by microbes.[21]

Ethanol Impact on the Skin

The well-known disinfectant ethanol is used in a variety of applications. Because of a scarcity of current research, it is still unclear whether it could be a cause of skin cancers as a consequence of penetrating the skin layers.[22] Contact of the epidermis with an ethanol-based hand disinfectant is related to a low level of systematic toxicity.[23]

As a result of the wide range of individual reactions and tolerance levels to ethanol, determining the level of a toxic dose of ethanol-based hand rub can be difficult. When ethanol is applied to immature skin in infants and young children, it can cause reactions as well as systemic toxic effects.[24] When ethanol comes into contact with the skin or eyes, it can cause irritation and an allergic reaction, while extended contact can cause cracking or dehydration of the skin, as well as peeling, redness, and itching.[25]

The study by Kramer et al.[26] was designed to assess dermal ethanol incorporation through disinfection and surgical hand hygiene, as well as to quantify absorption levels in human subjects. According to the findings of this study, dermal absorption of ethanol-based hand sanitizers was below toxic levels in humans and could therefore be safe.

Isopropyl Alcohol Impact on the Skin

A dose of roughly 0.5–1 ml/kg of 70% isopropyl alcohol-based sanitizers is considered toxic, but the exact amount varies according to the individual's tolerance level to the substance.[27] An acute toxic response to dermal exposure will occur at a concentration of LD50 >2000 mg/kg. Isopropanol absorption through the skin can cause skin irritation, and extended and recurrent experience can result in dryness, redness, itching, and rash on the skin.[28]

Augmented Risks of Other Viral Infections

Sanitizers have long been used around the world as a disinfectant to improve hand hygiene. Extreme uses of alcohol-based sanitizers as a coronavirus protective method increase the permeability of the skin, deprive the skin of water and oil, and cause irritation and roughness of the skin. Damage to and dehydration of the skin creates a breeding ground for various disease-rooting bacteria, increasing the risk of viral entrance into the skin and, as a result, indirectly increasing the risk of infection through skin dysfunctions.[25]

Antimicrobial Fight Originated by Frequent Hand Sanitizer Application

In the era of the coronavirus pandemic, frequent use of hand sanitizers has become mandatory as a preventive measure.[25] But there is also the risk that microbes that have been repeatedly exposed to disinfectants or other genotoxic chemicals are more likely to develop mutations as a result of natural selection, making them more resistant to the effects of these sanitizers. Antimicrobial resistance increases the workload of already overburdened healthcare professionals.[29]

Recommendations to Prevent or Minimize the Cutaneous Adverse Effects Several methods, either individually or in combination, can be used to prevent or minimize the negative effects of sanitizers, including choosing products that contain fewer irritants, humidifying the skin after sanitizing hands, and refraining from engaging in behaviors that might aggravate or cause skin irritations.[30-32] Instead of using alcohol or certain antiseptic soaps, ABHS including emollients or humectants could be applied to avoid the drying and irritant effects of these products.[32] The healthcare and elderly employees who frequently dress in fitted gloves are more susceptible to having dehydrated, irritated skin than the general public. As a result, lotions comprising oils, fats, or humectants should be used by those at high risk of developing skin conditions. It is important to increase the amount of emollient when living in a cold, dry climate.[33] Ethanol has the lowest alcohol-based effect on skin irritation.[34]

Conclusion

Hand hygiene is one of the significant, imperative and infection control measures against the transmission of SARS-CoV-2. As a result of the speedy efficiency and activity in destroying coronaviruses, ABHS is becoming increasingly popular. Choosing ABHS with some of the proper alcohol and using appropriate hand hygiene procedures during hand washing is critical to ensuring that all microbes are successfully eradicated with the least impact on the skin. The use of emollients in conjunction with sanitizers may help to reduce skin irritation. The irritant contact dermatitis and allergic contact dermatitis associated with ABHS, on the other

hand, are the decisive harmful effects that need to be explored in greater depth.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

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Gender Differences in 10-Year Mortality in Patients with Coronary Artery Disease with Elevated Lipoprotein(a): In Search of Invisible Guardian Angel

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Abstract

The objective of our study was to examine the contribution of elevated Lp(a) to 10-year mortality in 140 patients with coronary artery disease (CAD), depending on gender, baseline lipid levels, ApoA-I, ApoB, and the *ApoA1*, *ApoE*, and *ApoB* gene polymorphisms.

***Methods and Results*:** The study included 140 patients (75 men and 65 women) of the Uzbek population, hospitalized with diagnosis "CAD, unstable angina (IIB class, E. Braunwald et al., 1989)" in the period between January 2009 and February 2011. The endpoints at 10-year follow-up were death from cardiovascular causes (fatal myocardial infarction and sudden cardiac death). In the studied cohort of CAD patients, Lp(a) distribution was skewed to the right, the median was 16.9 mg/dL, and the mean was 34.3 mg/dL. At the same time, Lp(a) value greater than the 75th percentile was >41 mg/dL. In this regard, we compared the baseline values in 2 groups of patients: Group 1 (Lp(a)<41mg/dL) and Group 2 (Lp(a)>41 mg/dL). The 10-year cardiovascular mortality rate was higher significantly in Group 2 (RR=3.67; 95% CI: 1.67-8.11; *P*=0.0013). Cardiovascular mortality was significantly higher (*P*<0.001) in men of Group 2 than in men of Group 1 (RR=5.31; 95% CI: 2.03-13.88; *P*<0.001), while in women, the 10-year mortality in the compared groups did not differ significantly. Cardiovascular mortality was lower in patients with ApoA-I≥140 mg/dL, the majority of whom were women. The *ApoA1* A-carriers had significantly higher RR for cardiovascular mortality than non-A-carriers (*P*=0.0445).

Conclusion: In the absence of targeted Lp(a) therapy, long exposure to a level of Lp(a) of >41mg/dL is a factor that increases 10-year mortality in CAD patients. (**International Journal of Biomedicine. 2022;12(2):209-217.**)

Highlights

- 10-year cardiovascular mortality was higher in CAD patients with Lp(a) >41 mg/dL.
- However, the increase in mortality due to high Lp(a) was statistically significant in men than in women.
- The ApoA-I level was higher in women than in men.
- Cardiovascular mortality was lower in patients with ApoA-I ≥140 mg/dL, most of whom were women.

Key Words: lipoprotein(a) • apolipoprotein • coronary artery disease

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Abbreviations

ASCVD, atherosclerotic cardiovascular disease; **Apo**, apolipoprotein; **CI**, confidence interval; **CAD**, coronary artery disease; **CM**, cardiovascular mortality; **DM**. diabetes mellitus; **DBP**, diastolic blood pressure; **HDL-C**, high-density lipoprotein cholesterol; **HR**, hazard ratio; **hsCRP**, high-sensitive C-reactive protein; **LDL-C**, low-density lipoprotein cholesterol; **Lp(a)**, lipoprotein(a); **MI**, myocardial infarction; **NS**, not significant; **Non-HDL-C**, non-high-density lipoprotein cholesterol; **RR**, relative risk; **SBP**, systolic blood pressure; **TC**, total cholesterol; **TG**, triglycerides.

Introduction

Recognition of the leading role of the high levels of low-density lipoprotein cholesterol (LDL-C) in the onset and progression of atherosclerotic cardiovascular disease (ASCVD) is a key provision of the current recommendations of the European Society of Cardiology and the European Society of Atherosclerosis.[1] However, despite the effectiveness of step-down, lipid-lowering therapy in achieving the target level of LDL-C, patients with coronary artery disease (CAD) still have a sufficiently high residual risk of cardiovascular complications, which dictates the necessity of searching for new markers that affect the clinical course and prognosis of the disease.

The results of numerous epidemiological, clinical, experimental and genetic studies[2-5] have led to the official "inauguration" of Lp(a) as a risk factor for ASCVD in Recommendations of American College of Cardiology/ American Heart Association 2018 and the European Society of Cardiology/European Atherosclerosis Society 2019.[6,7] However, unlike LDL-C, therapeutic strategies for lowering Lp(a) for primary and secondary prevention of ASCVD are still under development and their impact on disease outcomes, including "hard" endpoints, also requires further study.

The objective of our study was to examine the contribution of elevated Lp(a) to 10-year mortality in 140 CAD patients, depending on gender, baseline lipid levels, ApoA-I, ApoB, and the *ApoA1*, *ApoE*, and *ApoB* gene polymorphisms.

Materials and Methods

Study subjects

A total of 140 patients (75 men and 65 women) of the Uzbek population, hospitalized with diagnosis "CAD, unstable angina (IIB class, E. Braunwald et al., 1989)" (main group) in the period between January 2009 and February 2011, were randomized in the prospective longitudinal study "Development of methods of differentiated pharmacotherapy and risk stratification in CAD patients, taking into account genetic polymorphism."

The exclusion criteria for the main group were MI in the previous 3 months, type 2 diabetes requiring insulin therapy, arterial hypertension grade II-III, atrial fibrillation, life-threatening ventricular arrhythmias, chronic obstructive pulmonary disease, chronic heart failure above functional class I (NYHA), chronic renal and hepatic insufficiency, preceding long-term use of lipid-lowering drugs, premenopausal hormone therapy.

The comparison group for assessing the prevalence of genetic polymorphisms consisted of 58 healthy individuals without diagnostic signs of CAD (exercise stress testing), comparable to patients by gender and age, without burdened family CAD history.

Basic therapy included double antiplatelet therapy (aspirin and clopidogrel; with aspirin intolerance - only clopidogrel, with clopidogrel intolerance - only aspirin); beta-blockers (bisoprolol in 100% of cases) in individually selected doses; ACE inhibitors (in 90% of cases); long-acting nitrates (in 60% of cases). Atorvastatin were prescribed for all patients at a dose of 20-40-80 mg/day in order to achieve target LDL-C level of <70 mg/dL. The lipid-lowering effect was assessed in 3 and 6 months after the initiation of therapy. Further control examinations were carried out at least 1-2 times a year. In the event of discontinuation of therapy, recommendations were given to resume taking atorvastatin. In case of repeated destabilization, coronary angiography was performed with hospitalization. The endpoints at 10-year follow-up were death from cardiovascular causes.

Functional and biochemical tests

All patients underwent the following examinations: 12-lead ECG, Echocardiography (EchoCG), ultrasound examination of the carotid arteries, 24-hour Holter monitoring, treadmill test, coronary angiography (in case of repeated destabilization with hospitalization), and blood tests.

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.

hsCRP in the blood was determined by a highly sensitive method of latex immunoturbidimetry on biochemical automatic analyzer Daytona. The blood levels of Apo-I and ApoB were determined on biochemical autoanalyzer Daytona by immunoturbidimetry, using monospecific antibodies to human ApoB and ApoA-I. The level of Lp(a) in the blood serum was determined by the latex immunoturbidimetry method using automatic biochemical analyzer Daytona and original commercially available kits.

Isolation of DNA and Genotyping of *ApoA1, ApoB,* and *ApoE* polymorphisms

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).

The *ApoA1* (G-75A), *ApoB* (-516C/T), and *ApoE* (ε2/ε3/ε4) gene polymorphisms were identified by the PCR-RFLP method. PCR was performed on the GeneAmp®9700 thermocycler (Applied Biosystems Inc., Foster City, CA, USA). The reaction mixture (20 mL) for PCR contained of 10 ng of genomic DNA, 20 pmol of each primer, 0.5 mM of each dNTP, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris·HCl (pH 8.8 at 25°C), 2% DMSO, and 1.0U of Taq DNA polymerase. Digested fragments of each gene were size-fractionated in 3% UltraPure™ Agarose (Thermo Scientific™ Wilmington, DE, USA) gel with ethidium bromide staining, and visualized on UV transilluminator.

The *ApoA1* G-75A SNP

A 433-bp fragment of the ApoA1 gene was amplified by using the following forward (5'–AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG–3' and reverse (5'–TTA GGG GAC ACC TAG CCC TCA GGA AGA GCA–3') primers.[8] The PCR was conducted according to the following cycling program: initial denaturing at 94°C for 4 minutes, then 35 cycles of denaturation for 30 seconds at 94°C, annealing at

55°C for 30 seconds, and elongation for 30 seconds at 72°C, and then a final elongation step of 72°C for 5 minutes.

Amplified products were digested with 10U of endonuclease MspI at 37°C overnight. The presence of the restriction site at positions -75(G allele) resulted in four fragments of 209 bp, 113 bp, 66 bp, and 45 bp. The absence of the restriction site at -75(A allele) resulted in three fragments of 209 bp, 179 bp, and 45 bp.

The *ApoB* -516C/T SNP

A 422 bp fragment of the ApoB (-516C/T) gene was amplified by using the following forward (5'–GCT GGG GTT TCT TGA AGA CA–3') and reverse (5'–CAA GCG TCT TCA GTG CTC TG–3') primers.[9] Amplification was performed by an initial denaturing at 94°C for 3 minutes, then by 35 cycles of denaturation for 30 seconds at 94°C, annealing at 63°C for 30 seconds, and elongation for 30 seconds at 72°C, and then by a final elongation step of 72°C for 5 minutes. The resulting 422-bp PCR product was then digested for 2 hours at 37°C with the restriction enzyme EarI, and resulted in the following genotype-specific fragments: homozygotes for the C variant, the uncut fragment of 422 bp; C/T heterozygotes, 3 fragments of 422, 306, and 116 bp; and homozygotes for the T variant, 2 fragments of 306 and 116 bp.

The *ApoE* ε2/ε3/ε4 polymorphism

A 227 bp fragment of the *ApoE* gene was amplified by using the following forward (5'-TCC AAG GAG CTG CAG GCG GCG CA-3') and reverse (5'-ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A-3') primers.[10,11] Amplification was performed by an initial denaturing at 94°C for 3 minutes, then by 40 cycles of denaturation for 30 seconds at 94°C, annealing at 68°C for 10 seconds, and elongation for 1 minute at 72°C, and then by a final elongation step of 72°C for 5 minutes. The resulting 227 bp PCR product was digested with *Hha* I (isoschizomer *Cfo* I) enzyme and the ApoE genotypes were determined as described previously.[11] Briefly, genotype ε2/ε2 (112 Cys and 158 Cys) identified by 91 bp and 81 bp fragments, ε3/ε3 (112 Cys and 158 Arg) by 91 bp and 48 bp, ε4/ε4 (112 Arg and 158 Arg) by 72 bp and 48 bp, ε2/ε3 by 91 bp, 81 bp and 48 bp, ε3/ε4 by 91 bp, 72 bp and 48 bp, and ε2/ε4 by 91 bp, 81 bp, 72 bp, and 48 bp fragments. The restriction also produced shorter fragments, which were not accounted for, because they were not informative for genotyping.

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. For descriptive analysis, results are presented as mean ± standard deviation (SD), median (Me), interquartile range (IQR; 25th to 75th percentiles). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. Kruskal-Wallis test was used to compare differences between 3 or more independent groups. The Wilcoxon criterion was used to compare the differences between the paired samples. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. The Cox proportional hazards regression analysis was applied to assess the effect of various variables on the risk of CVD development. Hazard ratio (HR), RR (relative risk), and 95% confidence interval (CI) were calculated. A probability value of $P<0.05$ was considered statistically significant.

The study protocol was approved by the Ethics Committees of the Republican Specialized Center of Cardiology and the State Committee on Science and Technology of the Republic of Uzbekistan.

Results

In the studied cohort of CAD patients, Lp(a) distribution was skewed to the right, the median was 16.9 mg/dL, and the mean was 34.3 mg/dL. At the same time, Lp(a) value greater than the 75th percentile was >41 mg/dL (Table 1), and did not differ significantly between men and women.

Table 1.

Baseline clinical, hemodynamic and biochemical parameters of CAD patients depending on Lp(a) level

Parameters	Total (n=140)	Group 1 Lp(a)<41mg/dL) (n=105)	Group 2 (Lp(a)>41mg/dL) (n=35)
Men/Women, n (%)	75/65 (53.6/46.4)	56/49 (53.3/46.7)	19/16 (54.3/45.7)
Age, years	54.8±8.5	53.9±8.9	56.3±8.1
Hypertension, n (%)	124 (88.6)	92 (87.6)	32 (91.4)
Current smoker, n (%)	11 (7.8)	8 (7.6)	3 (8.6)
DM, n (%)	31 (22.1)	24 (22.8)	7 (20.0)
History of MI, n (%)	21 (15.0)	13 (12.4)	8 (22.8)
Previous PTCA, n (%)	11 (7.8)	7 (6.7)	4 (11.4)
Heart rate, bpm	77.9±12.7	78.1±13.2	77.1±11.2
SBP, mmHg	134.1±19.5	133.3±18.8	136.7±21.5
DBP, mmHg	85.4±11.1	85.3±10.4	85.4±13.1
TC, mg/dL	228.4±43.4	229.3±44.7	225.6±39.5
LDL-C, mg/dL	141.1±37.0	138.9±38.1	147.6±33.2
HDL-C, mg/dL	39.3±8.5	38.8±8.5	40.7±8.4
TG, mg/dL	185.0 (129.5-277.5)	192.0 (131-300)	181.0 (120-239)
Non-HDL-C, mg/dL	190.0±44.1	193.5±48.1	179.7±27.1
ApoA-I, mg/dL	136.4±33.9	137.5±37.1	133.0±21.8
ApoB, mg/dL	101.0±23.3	100.0±21.8	104.3±27.3
ApoB/ApoA-I	0.78±0.26	0.77±0.25	0.81±0.25
Lp(a), mg/dL	16.9 (8.8-41.2)	12.1 (6.9-19.7)	85.9 (59.0-123.5)**
Glucose, mmol/L	5.0 (4.5-5.7)	5.0 (4.5-5.8)	4.8 (4.4-5.2)
hsCRP, mg/L	4.6 (2.2-9.0)	4.7 (1.9-9.0)	4.5 (2.9-8.7)
Endpoints, n (%)	20 (14.3)	9 (8.6)	11 (31.4)*
RR	-	RR=3.67; 95% CI: 1.67-8.11; P=0.0013	

** - P<0.01 between Groups 1 and 2; ** - P<0.001 between Groups 1 and 2*

In this regard, we compared the baseline values in 2 groups of patients: Group 1 (Lp(a)<41mg/dL) and Group 2 (Lp(a)>41mg/dL). The 2 groups of patients did not differ in gender, age, the severity of the clinical condition, or hemodynamic and biochemical parameters; however, despite the standard statin therapy, the 10-year cardiovascular mortality rate was significantly higher in Group 2 (P=0.0013) (Table 1).

In the cohort of CAD patients, men and women were represented nearly equally: 54% and 46%, respectively (Table 2). Men and women did not differ significantly in age, cardiovascular risk factors, or severity of the clinical condition. However, when comparing lipid parameters, the levels of HDL-C (P=0.021) and ApoA-I (P=0.016) were higher in women than in men. Mortality from cardiovascular causes (fatal myocardial infarction and sudden cardiac death) during 10-year follow-up was recorded in 14(18.7%) cases among men and in 6(9.2%) cases among women (P=0.15). However, gender differences were observed: cardiovascular mortality was significantly higher (P<0.001) in men of Group 2 (Subgroup 2[M]) than in men of Group 1 (Subgroup 1[M]), while in women, the 10-year mortality in the compared subgroups (Subgroup 2[W]) and Subgroup 1[W]) did not differ significantly. At the same time, in women in both subgroups, the level of ApoA-I was significantly higher than in men, although HDL-C did not differ significantly. When constructing a Cox regression model, among the most significant determinants (gender, MI, DM, Lp(a), ApoA-I) affecting mortality, only Lp(a) value reached a significant degree in the whole group and in men (Table 3).

Taking into account the identified differences in the level of ApoA-I among the surveyed patients (Tables 1 and 2) and the possible effect of the cluster of ApoA-I-related lipid parameters on 10-year mortality, we compared the main clinical and biochemical markers and endpoints in patient groups with ApoA-I levels above and below 140 mg/dL (Table 4). In our study, this value was the median of distribution among women who had lower mortality rates with high Lp(a) levels than in men who had median ApoA-I of 125 mg/dL. Patients with ApoA-I ≥140 mg/dL had additional bonuses in the form of high HDL-C (P<0.001), low ApoB/ApoA-I ratio (P<0.001), and reduced cardiovascular mortality (P=0.031). Unfortunately, only 55(39.3%) patients, the majority of whom (63.6%) were women, had ApoA-I≥140mg/dL (Table 4)**.**

The distribution of polymorphic markers of the *ApoE* (ε2/ε3/ε4), *ApoA1* (G-75A), and *ApoB* (-516C/T) genes in CAD patients and healthy people were in Hardy-Weinberg equilibrium. An analysis of the frequency distribution of alleles and genotypes of the *ApoA1* G-75A SNP showed that the carriage of the allele A was more predominant in the CAD patients than in the healthy people (P=0.0016). We did not observe significant gender differences in the distribution of the studied genetic polymorphisms (Table 5).

Table 2.

Baseline clinical, hemodynamic and biochemical parameters of CAD patients depending on gender and (Lp(a) level

Parameters	Men	Women	Men		Women	
			Subgroup 1[M] (Lp(a)<41mg/dL)	Subgroup 2[M] (Lp(a)>41mg/dL)	Subgroup 1[W] (Lp(a)<41mg/dL)	Subgroup 2[W] (Lp(a)>41mg/dL)
n (%)	75 (53.6)	65 (46.4)	56	19	49	16
Average age, years	52.5±8.8	57.4±7.6	51.7±8.9	55.3±7.4	56.9±7.6	57.6±8.9
Hypertension, n (%)	67 (89.3)	57 (87.7)	49 (87.5)	18 (94.7)	43 (87.8)	14 (87.5)
Current smoker, n (%)	8 (10.7)	3 (4.6)	7 (12.5)	1 (5.3)	1 (2.0)	2 (12.5)
Diabetes, n (%)	14 (18.7)	17 (26.2)	10 (17.8)	4 (21.1)	14 (28.6)	3 (18.8)
History of MI, n (%)	12 (16.0)	9 (13.8)	6 (10.7)	6 (31.6)*	7 (14.3)	2 (12.5)
Previous PTCA, n (%)	7 (9.3)	4 (6.2)	4 (7.1)	3 (15.8)	3 (6.1)	1 (6.25)
TC, mg/dL	221.9±41.2	235.9±44.9	223.3±43.2	217.8±35.1	236.3±45.9	234.9±43.4
LDL-C, mg/dL	139.3±35.5	143.1±38.8	137.2±36.8	145.7±31.4	140.9±39.8	149.8±36.3
HDL-C, mg/dL	37.7±8.8	41.0±7.7^	37.4±9.2	38.6±7.9	40.3±7.4	43.3±8.5
TG, mg/dL	186.0 (132.0-257.0)	184.0 (128.0-299.0)	199.5 (141-292)	145 (118-201)	184.0 (123-354)	218.0 (141-295)
Non-HDL-C, mg/dL	188.2±45.6	192.2±42.7	191.3±49.4	179.2±31.2	196.1±47.0	180.3±22.3
ApoA-I, mg/dL	128.0±25.2	145.9±39.9^	130.8±27.2	120.0±15.8	145.1±44.9[&]	148.4±17.5[#]
ApoB, mg/dL	97.9±21.9	104.7±24.5	95.4±17.9	105.2±30.2	105.1±24.8[&]	103.3±24.3
ApoB/ApoA-I	0.79±0.22	0.77±0.29	0.75±0.20	0.89±0.25	0.79±0.3	0.71±0.2[#]
Lp(a), mg/dL	19.5 (8.6-42.2)	14.7 (9.3-39.0)	15.6 (7.0-21.4)	90.4 (63-124)**	10.8 (6.3-17.0)	80.0 (58-123)**
Glucose, mmol/L	4.9 (4.5-5.8)	5.0 (4.5-5.5)	5.0 (4.5-5.9)	4.6 (4.3-5.1)	5.0 (4.5-5.8)	5.0 (4.6-5.3)
hsCRP, mg/L	4.7 (2.5-9.0)	4.5 (2.0-9.0)	4.8 (2.4-8.8)	4.5 (3.6-9.0)	4.0 (1.8-10.4)	4.5 (2.8-5.5)
Endpoints, n (%)	14 (18.7)	6 (9.2)	5 (8.9)	9 (47.4)**	4 (8.2)	2 (12.5)
RR	RR=2.02; 95% CI: 0.83-4.96; P=0.15		RR=5.31; 95% CI: 2.03-13.88; P<0.001		RR=1.53; 95% CI: 0.31-7.60; NS	

*^ - P<0.05 - between men and women; *, **- P<0.05, P<0.01- between Subgroup 1[M] and Subgroup 2[M], and between Subgroup 1[W] and Subgroup 2[W]; [&]- P<0.05 between Subgroup 1[W] and Subgroup 1[M]; [#]- P<0.01 - between Subgroup 2[W] and Subgroup 2[M].*

Table 3.

Relationship between the influence of some lipid and clinical parameters on CM using Cox's multiple regression analysis adjusted for age in the whole group and in men

Parameters	Total (n=140)		Men (n=75)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Male sex	1.18 (0.79-1.76)	0.41	-	-
Lp(a)	1.01 (1.00-1.01)	0.039	1.01 (1.00-1.02)	0.015
DM	1.23 (0.77-1.95)	0.38	1.23 (0.57-2.65)	0.59
MI	1.17 (0.79-1.71)	0.42	1.13 (0.65-1.95)	0.67
ApoA-I	0.99 (0.98-1.01)	0.64	1.00 (0.96-1.10)	0.46

CM- cardiovascular mortality

Table 4.

Baseline clinical and biochemical parameters of patients depending on ApoA-I level

Parameters	ApoA-I<140mg/dL (n=85)	ApoA-I≥140mg/dL (n=55)
Gender, Men /Women, n (%)	55/30 (64.7/35.3)	20/35 (36.4/63.6)
Average age, years	53.9±8.2	56.0±9.1
DM, n (%)	17 (20.0)	14 (25.4)
History of MI, n (%)	15 (17.6)	6 (10.9)
Previous PCI, n (%)	7 (8.2)	4 (7.3)
TC, mg/dL	227.9±46.9	229.2±37.7
TG, mg/dL	181 (123-239)	204 (163-2180)
Non-HDL-C, mg/dL	189.1±44.3	191.6±44.2
HDL-C, mg/dL	37.0±7.5	42.8±8.8**
LDL-C, mg/dL	142.9±39.0	138.3±33.8
ApoA-I, mg/dL	115.4±15.5	168.7±28.8**
ApoB, mg/dL	98.8±25.0	104.5±20.0
ApoB/ApoA-I	0.88±0.27	0.63±0.15**
Lp (a), mg/dL	17 (9-39)	16 (8-49)
hsCRP, mg/L	5.24 (2.6-9.7)	3.72 (2.0-6.4)
Endpoints, n (%)	17 (20.0)	3 (5.4)*
RR	RR=3.67; 95% CI: 1.13-11.9; *P*=0.031	

*,** - *P*=0.031, *P*<0.001 - between groups

The SNPs of the *ApoA1* (G-75A) and *ApoB* (-516C/*T)* genes did not affect significantly the level of lipids and apolipoproteins in the blood in the CAD patients. Only, the *ApoE* ε4-carriers had significantly higher ApoB concentration (*P*=0.047) and ApoB/ApoA-I ratio (*P*=0.001). In addition, ε4-carriers had a tendency to decrease the level of ApoA-I (*P*=0.056). The *ApoA1* A-carriers had significantly higher RR for CM than non-A-carriers (*P*=0.0445) (Table 6).

Since ApoA-I is the inverse regulator in ApoB/ApoA-I ratio, it was interesting to compare the lipids and apolipoproteins in the *ApoE* ε4-carriers depending on the *ApoA1* G-75A SNP (Table 7). Patients with combined carriage of the *ApoE* ε4 allele and the GG genotype *of* the *ApoA1* G-75A SNP of the promoter region of the *ApoA1* gene showed a significantly higher level (*P*=0.01) of ApoA-I in the absence of a significant increase in HDL-C than carriers of the ε4+A alleles. This may indicate a higher level of primarily monomeric forms of ApoA-I in them, present in the plasma in the form of so-called "lipid-poor" ApoA-I or pre-β1HDL.

Discussion

Lp(a) is an independent risk factor for cardiovascular disease, and its inclusion in known risk scales (Framingham Risk Score and Reynolds Risk Score) improves the prediction of adverse cardiovascular events.[12,13] However, in contrast to the results for men (n=5161) in the framework of the JUPITER study, which showed a strong association of Lp(a) with the development of cardiovascular diseases (myocardial infarction, ischemic stroke, coronary revascularization, and cardiovascular death), including with low TC, the results of 3 cohort studies in women demonstrated its limited effect on cardiovascular risk.[14]

In Women's Health Study (WHS, n=24,558, a median of follow-up of 10.2 years), a case-cohort sample from the study of Women's Health Initiative (WHI, n=1,815, a median of follow-up of 9.9 years), and in women in JUPITER study (n=2569, an average of follow-up of 1.9 years, endpoints also included hospitalizations additionally due to angina destabilization), a high Lp(a) did not affect the development of endpoints independently, but only in combination with the level of TC >220 mg/dL.

Table 5.

Distribution of polymorphic markers of the ApoE (ε2/ε3/ε4), ApoA1 (G-75A), and ApoB (-516C/T) genes in CAD patients and healthy individuals in the Uzbek population

Genes	"Damaging" alleles	Healthy (n=58)	CAD patients (n=140)	Women (n=65)	Men (n=75)
ApoE (ε2/ε3/ε4)	ε4-carriers / vs not-ε4	7/51 ε4/ε2 -1 ε3/ε4 - 6	35/105 ε4/ε4 -1, ε4/ε2 -1, ε3/ε4 -33	14/51 ε3/ε4 -14	21/54 ε4/ε4 -1, ε4/ε2 -1, ε3/ε4 -19
RR		RR=2.07; 95% CI: 0.98-4.39; NS		RR=1.17; 95% CI: 0.84-1.62; NS	
ApoA1 (G-75A)	A-carriers / vs GG	9/49 GA-9	60/80 AA-2, GA-58	29/36 AA-1, GA-28	31/44 AA-1, GA-30
RR		RR=2.76; 95% CI: 1.47-5.19; *P*=0.0016		RR=0.96; 95% CI: 0.66-1.40; NS	
ApoB -516C/T	T- carriers / vs CC	25/33 TT-1, TC-24	48/92 TT-1, TC-47	19/46 TT-1,TC-18	29/46 TC-29
RR		RR=0.80; 95% CI: 0.55-1.16; NS		RR=1.32; 95% CI: 0.82-2.12; NS	

Table 6.

Lipid metabolism parameters in CAD patients depending on polymorphic markers of the ApoE (ε2/ε3/ε4), ApoA1 (G-75A), and ApoB (-516C/T) genes in the Uzbek population

Parameters	*ApoE* ε2/ε3/ε4		*ApoA1* G-75A		*ApoB* -516 C/T	
	ε4-carriers (n=35)	non-ε4 carriers (n=105)	A-carriers (n=61)	non-A carriers (n=79)	T-carriers (n=48)	non-T carriers (n=92)
TC, mg/dL	234.1±41.2	226.5±44.1	230.4±45.1	226.8±42.2	227.5±46.8	228.9±41.7
TG, mg/dL	192 (125.0-250.0)	184.0 (131.0-295.0)	201 (135-284)	183 (123-269)	182.5 (128-242)	196 (134-314)
Non-HDL-C, mg/dL	196.6±40.9	187.9±45.1	189.8±43.6	190.2±44.8	185.9±41.1	192.2±45.7
HDL-C, mg/dL	37.5±7.0	39.8±8.8	39.2±8.6	39.3±8.4	39.9±9.4	38.9±8.0
VLDL-C, mg/dL	38.0 (25.0-46.0)	39.0 (26.0-63.0)	41 (27-59)	37 (26-54)	37 (24-49)	40 (27-67)
LDL-C, mg/dL	148.1±37.0	138.8±36.9	138.0±38.2	143.5±36.1	148.8±39.7	137.1±35.1
ApoA-I, mg/dL	126.9±29.4	139.5±34.8	131.8±37.1	139.9±31.0	134.7±35.2	137.2±37.4
ApoB, mg/dL	107.8±27.3	98.8±21.4*	97.5±19.8	103.8±25.4	105.0±23.0	98.9±23.3
ApoB/ApoA-I	0.90±0.3	0.74±0.2**	0.78±0.26	0.77±0.25	0.82±0.27	0.76±0.25
Lp(a), mg/dL	25.7 (12.9-37.1)	15.9 (7.6-51.5)	19.7 (6.3-40.2)	16.0 (9.2-44.0)	17.1 (9.1-32.1)	16.9 (8.5-43.1)
Glucose, mmol/L	4.9 (4.3-5.2)	5.0 (4.5-5.8)	5.1 (4.6-6.1)	4.9 (4.3-5.5)	4.9 (4.3-5.2)	5.1 (4.6-5.8)
hsCRP, mg/L	5.3 (3.6-8.9)	4.5 (2.0-9.0)	4.5 (1.8-8.5)	4.8 (2.3-10.4)	5.0 (1.5-9.3)	4.3 (2.3-4.8)
Endpoints, n (%)	5 (14.3)	15 (14.3)	13 (21.3)	7 (8.9)^	7/ (14.6)	13 (14.1)
RR	RR=1.00; 95% CI: 0.39-2.55; NS		RR=2.4; 95%CI: 1.02-5.66; *P*=0.0445		RR=1.03; 95% CI: 0.44-2.42; NS	

*,** - *P*=0.047, *P*=0.001 - between ε4-carriers and non-ε4 carriers; ^- *P*<0.05 - between A-carriers and non-A-carriers

Table 7.

The levels of lipids and apolipoproteins in the ApoE ε4-carriers depending on the ApoA1 G-75A SNP

Genes, Parameters	*ApoE*, ε4 carriers (n=35)		*ApoE*, ε4 non-carriers (n=105)	
	ApoA1 (GA,AA) (n=15)	*ApoA1* (GG) (n=20)	*ApoA1* (GA,AA) (n=45)	*ApoA1* (GG) (n=60)
Men/Women, n (%)	7/8 (46.7/53.3)	14/6 (70/30)	24/21 (53.3/46.7)	30/30 (50/50)
TC, mg/dL	234.1±52.7	234.1±31.4	230.1±43.0	223.8±45.1
TG, mg/dL	184.0 (125.0-234.0)	195.0 (145-253.5)	204.0 (135-295)	235.1 (123.5-299.5)
Non-HDL-C, mg/dL	195.7±53.4	197.3±30.0	188.6±40.6	187.3±48.6
HDL-C, mg/dL	38.4±8,2	36.8±6.2	39.6±8.9	40.0±8.9
LDL-C, mg/dL	138.2±42.2	155.5±31.7	138.2±37.7	139.2±36.6
ApoA-I, mg/dL	112.7±22.5	137.7±29.9*	138.8±38.9	140.0±31.8
ApoB, mg/dL	100.3±22.2	113.5±29.9	96.3±19.3	100.6±22.9
ApoB/ApoA-I	0.94±0.33	0.86±0.30	0.73±0.21	0.75±0.23
Lp(a), mg/dL	19.8 (12.0-29.3)	27.9 (13.7-40.6)	19.7 (6.3-55.0)	14.4 (8.3-45.2)
Glucose, mmol/L	4.9 (4.2-5.1)	5.0 (4.7-5.6)	5.1 (4.8-6.8)	4.9 (4.3-5.4)
hsCRP, mg/L	3.6 (1.8-8.9)	5.5 (4.0-16.2)	4.5 (1.5-8.0)	3.9 (2.2-9.7)
Endpoints, n (%)	4 (26.7)	1 (5.0)	9 (20.0)	6 (10.0)
RR	RR=5.33; 95% CI: 0.66-42.9; NS		RR=2.00; 95% CI: 0.77-5.21; NS	

* - *P=0.01 – between ApoA1 (GA, AA) and ApoA1 (GG)*

Though women had a significant but small change in C-statistic (0.790–0.797; *P*=0.035), such as in the WHS test sample, this did not make a significant contribution to improving the risk-stratification scale.[14] WHS and WHI did not show a significant effect of hormone therapy or race on the risk of cardiovascular outcomes, and JUPITER did not include women taking postmenopausal hormone therapy. These results indicate a different impact of high Lp(a) on cardiovascular risk in men and women.

Also, some other studies, including Cardiovascular Health Study in the elderly (2375 women and 1597 men),[15] Stanford Five-City Project,[16] and Framingham Heart Study (n=3121),[17] confirmed the role of Lp(a) as an independent cardiovascular risk factor in men, in the absence of a significant association in women. In our study, in the cohort of 140 patients with coronary artery disease, 10-year mortality from cardiovascular causes in the group of patients with Lp(a) level >41mg/dl exceeded significantly (*P*=0.002) mortality in the group with Lp(a) <41 mg/dL (Table 1).

However, as we noted in the above studies, the increase in mortality with high Lp(a) was most significant in men (*P*<0.001), with no significant association in women. At the time of randomization in the study, men and women did not differ in average age or the severity of the clinical condition (Table 2); however, women had significantly higher levels of HDL-C (*P*<0.05) and ApoA-I (*P*<0.01). In addition, only ApoA-I concentration remained higher among women than in men, regardless of the Lp(a) level (>41mg/dL or <41mg/dL).

In recent years, despite the epidemiological evidence,[18,19] it is known that clinical studies have not confirmed the linear feedback of HDL-C level with the risk of developing CVD.[20,21] In addition, pharmacological interventions aimed at increasing HDL-C levels using niacin[22] and CETP inhibitors[23] have not demonstrated a positive effect on cardiovascular disease outcomes. Also, genetic studies using Mendelian randomization have not confirmed a causal nexus between HDL-C level and CAD development.[24,25] In connection with it, attention switched to the functional abilities of HDL-C,[26] primarily to HDL-C conditioned capacity of cholesterol efflux from cells, which turned out to be a strong predictor of cardiovascular events.[27]

ApoA-I is a key component of HDL and plays a major functional role in the ability of cholesterol efflux from cells,[28] which probably explains the inverse correlation between the level of ApoA-I/HDL-C and a decrease in the risk of atherosclerosis. Unfortunately, a recent Phase II randomized clinical trial using 5 weekly infusions of MDCO-216 (recombinant ApoA-I Milano) and 10 weekly infusions of CER-001 (recombinant wild-type ApoA-I, 10 weeks) did not confirm their positive influence on the regression of coronary atherosclerosis,[29,30] which was assessed by intravascular ultrasound sonography. However, the study period of 5-10 weeks may not have been long enough. A third ApoA-I product, CSL112 representing a reconstituted form of native ApoA-I from human plasma,[31] has the most favorable surrogate criteria, including an increase in the ability to promote cholesterol efflux from cells in CAD patients,[32,33] and is preparing currently for full-scale clinical randomized trials of Phase III.

Results of a recent genetic study using Mendelian randomization also have not found causal nexus between ApoA-I level and CAD development,[34,35] which coincides with the data of a Richardson et al. study,[36] conducted using data of UK BioBank, which has demonstrated once again that apoB is causally related to CAD. The association of polymorphisms of genes involved in Lipid metabolism was studied in several studies. [37-41] Our limited study of genetic polymorphisms [*ApoA1* (G-75A), *ApoE* (ε2/ε3/ε4), and *ApoB* (-516C/T)] found a connection between the *ApoE* ε4 allele with increased ApoB level and ApoB/ApoA-I ratio. However, CAD patients with combined carriage of the *ApoE* ε4 allele with the *ApoA1* GG genotype showed a higher level of ApoA-I. It is possible that in response to an increased level of ApoB and ApoB/ApoA-I in carriers of the ε4 allele, activation in the promoter region of *ApoA1* in patients with the GG genotype of the *ApoA1* G-75A SNP[38] contributes to an increase in the concentration of ApoA-I in plasma and atheroprotective effect.[42]

It is known that, along with an increase in the ability to promote cholesterol efflux from cells, HDL-C/ApoA-I has pleiotropic properties, including antioxidant, anti-inflammatory, and antithrombotic activity,[43] which perhaps help stabilize rather than reduce atherosclerotic plaques, improving cardiovascular outcomes.[31] In contrast, Lp(a) has oxidative, proinflammatory and prothrombotic properties underlying its proatherogenic action.[44,45]

Liu et al.[46] have found in a transgenic mouse model that under conditions of an increased Lp(a) level, an increase in ApoA-I level has a dominant effect on a decrease in susceptibility to atherosclerosis under various conditions, including those that are not associated with changes in plasma lipids.

At the same time, in women who have been taking statins for a long time (10 years) to lower LDL-C, long-term exposure to increased natural ApoA-I perhaps interferes with the proatherogenic effect of Lp(a). The results of a 10-year prospective SWAN study are interesting, in which an increase in total cholesterol, LDL cholesterol, and ApoB was found in 3302 women of various ethnicities a year after the onset of physiological menopause, but Lp(a) level did not change significantly.[47] Levels of HDL-C and ApoA-I increased, to the greatest extent, during the 1-year interval around physiological menopause, and 12 months after its onset, HDL-C level decreased (P=0.01), while the concentration of ApoA-I did not decrease, but remained increased.

There is no doubt that at the present stage, the problem of combating excess mortality from high Lp(a) is extremely acute. Many "weapons" will be needed in this way: from a targeted decrease in its concentration to the neutralization of the mechanism of action, risk stratification of patients, and differentiated choice of treatments.

Conclusion

The results of our study have demonstrated clearly that in the absence of targeted Lp(a) therapy, long exposure to a high level of Lp(a) is a factor that increases 10-year mortality in CAD patients. However, cardiovascular mortality was lower in patients with ApoA-I≥140mg/dL, the majority of whom were women.

Study Limitation

This study was limited to the framework of the genetic branch, which included the study of the ApoA, ApoB, and ApoE polymorphisms in 140 CAD patients that had not been studied previously in the Uzbek population. Therefore, some features in CAD courses in women with high Lp(a) deserve further in-depth study on a large clinical cohort.

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Competing Interests

The authors declare that they have no competing interests.

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Features in the Processes of Left Ventricular Remodeling Depending on the Degree of Renal Dysfunction in Patients with Chronic Heart Failure

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Abstract

Background: The aim of this research was to study the features of changes in the parameters of heart remodeling in patients with coronary heart disease (CHD) and chronic heart failure (CHF), depending on the degree of renal dysfunction (RD).

Methods and Results: The study included 150 CHD patients with NYHA functional class (FC) I-IIII of CHF. All examined patients were subdivided according to the level of eGFR: Group A included 81 patients with CHF FCI-III and eGFR>60 mL/min per 1.73 m^2; Group B included 69 patients with CHF FCI-III and eGFR≤60 mL/min per 1.73 m^2. It was found an increase in left ventricular (LV) mass in Group B by 11.4%, compared to Group A (*P*=0.000). Analysis of LV systolic function showed that in Group B, values of LF ejection fraction and fractional shortening were significantly lower than in Group A (47.64±0.61% vs. 52.7±0.28%, and 25.40±0.46% vs. 28.23±0.25%, respectively, *P*=0.000). Thus, in Group B, we found CHFrEF, compared to Group A with CHFpEF. Analysis of diastolic function revealed that in Group B, the E/A ratio was statistically higher than in Group A (1.12±0.05 vs. 0.81±0.04, respectively, *P*=0.000). At the same time, in Group B, values of IVRT and DT were significantly lower than in Group A (85.01±0.8 ms vs. 91.25±0.99 ms, and 177.8±2.1 ms vs. 197.5±2.07 ms, respectively, *P*=0.000). Thus, the signs of the impaired relaxation (Grade 1 diastolic dysfunction) and the pseudonormal filling pattern (Grade 2 diastolic dysfunction) were found in Group A and Group B, respectively.

Conclusion: RD in patients with CHF is an important factor in the significant deterioration of LV systolic and diastolic functions.(**International Journal of Biomedicine. 2022;12(2):218-221.**)

Key Words: chronic heart failure • left ventricular remodeling • renal dysfunction

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Abbreviations

CHF, chronic heart failure; **CHFrEF**, CHF with reduced ejection fraction; **CHFpEF**, CHF with preserved ejection fraction; **CHD**, heart coronary disease; **CKD**, chronic kidney disease; **DM**, diabetes mellitus; **DT**, deceleration time; **DD**, diastolic dysfunction; **eGFR**, estimated glomerular filtration rate; **EF**, ejection fraction; **FC**, functional class; **Fs**, fractional shortening; **IVST**, interventricular septal thickness; **IVRT**, isovolumic relaxation time; **LV**, left ventricle; **LVEF**, left ventricular ejection fraction; **Ld**, diastolic longitudinal displacement; **Ls,** longitudinal systolic displacement; **LVEDV**, left ventricular end-diastolic volume; **LVESV**, left ventricular end-systolic volume; **LVEDD**, left ventricular end-diastolic dimension; **LVESD**, left ventricular end-systolic dimension; **LVPWT**, left ventricular posterior wall thickness; **LA**, left atrium; **LVM**, left ventricular mass; **LVMI**, left ventricular mass index; **MI**, myocardial infarction; **RD**, renal dysfunction; **RWT**, relative wall thickness; **SV**, stroke volume.

Introduction

Despite significant advances in the treatment of various cardiovascular diseases, the prevalence of chronic heart failure (CHF) continues to grow.[1,2] This disease is the most common reason for hospitalization among people over 65 years of age. Moreover, about 50% of patients with CHF are re-hospitalized within 6 months, 20%-25% of patients - within 30 days after discharge from the hospital, and 70% of re-hospitalizations are associated with decompensated CHF.[2] Features of the course

of CHF against the background of renal dysfunction (RD) are widely discussed. The type of left ventricle (LV) dysfunction more typical for CHF patients in conditions of impaired renal filtration function has not been determined.[3-6] On the other hand, the influence of the structural and functional reorganization of the heart in CHF on the glomerular-tubular relationship of the kidneys has not been sufficiently studied. Features of arterial wall remodeling in conditions of CHF and chronic kidney disease (CKD) have also not been studied enough and are controversial. The presented controversial issues make it difficult to adequately choose the therapy for patients with CHF and CHD, which also requires the provision of a nephroprotective effect of treatment.[7-9] Further research is needed to determine the patterns of CHF and RD in the conditions of the cardiorenal syndrome and to develop pathogenetically substantiated approaches to their treatment.

The aim of this research was to study the features of changes in the parameters of heart remodeling in patients with coronary heart disease (CHD) and chronic heart failure (CHF), depending on the degree of renal dysfunction (RD).

Materials and Methods

We performed a comprehensive examination of 150 patients with CHD and NYHA functional class (FC) I-IIII of CHF.

The non-inclusion criteria were CHF of non-ischemic origin, stroke, severe or insulin-dependent diabetes mellitus, COPD, high-grade arrhythmias, liver disease, severe kidney disease, and other severe somatic pathologies.

All examined patients were divided into groups according to CHF FC: CHF FCI (n=38), CHF FCII (n=62) and CHF FCIII (n=50) (Table 1). Patients were also subdivided according to the level of GFR: Group A included 81 patients with CHF-FCI-III and eGFR>60 mL/min per 1.73 m^2; Group B included 69 patients with CHF-FCI-III and eGFR ≤60 mL/min per 1.73 m^2.

Table 1.

Clinical characteristics of patients

Variable	CHF FCI (n=38)	CHF FCII (n=62)	CHF FCIII (n=50)
Age, yrs	58.77±0.94	61.3±0.68	62.14±0.79
Women	25 (65.8%)	30 (48.4%)	20 (40.0%)
Men	13 (34.2%)	32 (47.1%)	30 (60.0%)
Arterial hypertension	35 (92.1%)	7 (91.9%)	31 (62.0%)
History of MI	7 (18.2%)	17 (27.4%)	27 (54.0%)

The average age of patients was 58.77±0.94 years, 61.3±0.68 years, and 62.14±0.79 years in Groups 1, 2, and 3, respectively. The duration of the disease was 5.81±0.75 years, 6.6±0.63 years, and 16±0.92 years in Groups 1, 2, and 3, respectively.

Conventional 2D echocardiography was carried out according to the recommendations of the American Society of Echocardiography in M- and B-modes using an MEDISON ACCUVIX V20 device (South Korea) equipped with a 3.25 MHz transducer. The following parameters were measured and calculated: IVST, PWT, LVEDD, LVESD, EF, LVEVD, LVESV, Fs, LA size, Ld, and Ls. LVM was calculated using the formula R. Devereux.[10] LVM was indexed to body surface area (LVMI). Left ventricular hypertrophy (LVH) was defined as LVMI of ≥110 g/m^2 (for women) and ≥134 g/m^2 (for men).

Global LV systolic function was assessed by determining linear and volumetric dimensions, wall thicknesses, ventricular volumes. LVEF was calculated by the Simpson method. LV diastolic function was analyzed by measuring peak early diastolic filling (E) and late diastolic filling (A) velocities, E/A ratio, isovolumic relaxation time (IVRT), and deceleration time (DT).[11]

Assessment of the functional state of the kidneys was carried out on the basis of determining the level of serum creatinine, 24-h urinary albumin excretion, eGFR was calculated using the CKD-EPI equation**.** The eGFR was calculated as described by Levey et al.[12] eGFR was expressed in ml per minute per 1.73 m^2.

Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Baseline characteristics were summarized as frequencies and percentages for categorical variables. For descriptive analysis, results are presented as mean ± standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. 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.

The study protocol was approved by the Ethics Committees of the Republican Specialized Scientific and Practical Medical Center for Therapy and Medical Rehabilitation (Tashkent, Uzbekistan). Written informed consent was obtained from each research participant.

Results and Discussion

The analysis of EchoCG indicators found features in the structural and functional state of the left ventricle in CHD patients with CHF-FCI-III, depending on eGFR. It was found that in patients of Group B, there was a moderate increase in the LA size by 11.3% ($P<0.001$), compared to Group A, which amounted to 3.36±0.04 cm versus 3.74 ±0.05 cm (Table 2).

The progression of RD in patients with CHF was characterized by changes in the LV size. The values of LVESD and LVEDD in Group B were significantly higher than in Group A (4.1±0.05 cm vs. 3.72±0.04 cm, and 5.46±0.05 cm vs. 5.23±0.05 cm, respectively, $P=0.000$). These changes were associated with an increase in LVM in Group B by 11.4%, compared to Group A ($P=0.000$).

Analysis of LV systolic function showed that in Group B, values of LFEF and Fs were significantly lower than in Group A (47.64±0.61% vs. 52.7±0.28%, and 25.40±0.46% vs. 28.23±0.25%, respectively, $P=0.000$) (Table 3). Thus, in Group B, we found CHFrEF, compared to Group A with CHFpEF.

Table 2.

Structural and geometric parameters of the LV in CHD patients with CHF depending on eGFR

Indicator	Group A eGFR>60 mL/min per 1.73m^2	*P*-value	Group B eGFR≤60 mL/min per 1.73m^2
LVPWT, cm	1.085±0.01	0.0691	1.088±0.01
IVST, cm	1.126±0.02	0.2241	1.130±0.02
LVEDD, cm	5.23±0.05	0.000	5.46±0.05
LVESD, cm	3.72±0.04	0.000	4.1±0.05
LA, cm	3.36±0.04	0.000	3.74±0.05
AO, cm	3.13±0.03	0.000	3.36±0.045
RWT	0.45±0,005	0.000	0.42±0.006
Ls, cm	38.6±0.69	0.000	42.2±0.67
Ld, cm	48.8±1.05	0.000	51.9±1.38
LVM, g	249.4±5.55	0.000	277.93±5.71
LVMI, g/cm^2	131.5±3.1	0.000	142.4±3.2

Table 3.

Indicators of LV systolic function in CHD patients with CHF depending on eGFR

Indicator	Group A eGFR>60 mL/min per 1.73m^2	P-value	Group B eGFR≤60 mL/min per 1.73m^2
SV, mL	67.4±1.55	0.244	67.1±1.58
LVEF, %	52.7±0.28	0.000	47.64±0.61
LVEDV, mL	131.22±2.73	0.000	145.9±2.93
LVESV, mL	61.4±1.35	0.000	77.43±1.99
Heart rate, bpm	73.84±1.02	0.000	75.81±1.11
Fs, %	28.23±0.25	0.000	25.40±0.46

Analysis of diastolic function (Table 4) revealed that in Group B, the E/A ratio was statistically higher than in Group A (1.12±0.05 vs. 0.81±0.04, respectively, *P*=0.000). At the same time, in Group B, values of IVRT and DT were significantly lower than in Group A (85.01±0.8ms vs. 91.25±0.99ms, and 177.8±2.1ms vs. 197.5±2.07ms, respectively, *P*=0.000). Thus, the signs of the impaired relaxation (Grade 1 DD) and the pseudonormal filling pattern (Grade 2 DD) were found in Group A and Group B, respectively.

Table 4.

Indicators of LV diastolic function in CHD patients with CHF depending on eGFR

Indicator	Group A GFR>60 mL/min per 1.73m^2 EF≥50%	*P*-value	Group B GFR≤60 mL/min per 1.73m^2 EF<50%
E, m/s	0.58±0.01	0.000	0.76±0.017
A, m/s	0.71±0.017	0.000	0.68±0.02
E/A	0.81±0.04	0.000	1.12±0.05
IVRT, ms	91.25±0.99	0.000	85.01±0.8
DT, ms	197.5±2.07	0.000	177.8±2.1

Thus, the results of these studies have established that RD in patients with CHF is an important factor in worsening the clinical manifestations of the disease, reducing physical performance and quality of life. All this is based on more pronounced damage to the cardiovascular system: the progression of post-infarction heart remodeling with a further deterioration in cardiovascular relationships, as well as a decrease in kidney function with a worsening in cardiorenal relationships. These negative processes develop against the background of complex disorders of autonomic and neurohumoral regulation. All this indicates the need to mitigate and, if possible, eliminate the influence of individual metabolic syndrome components on the body and, first of all, on the cardiovascular system. In this regard, our further research was aimed at studying the effectiveness of standard CHF therapy in patients with and without manifestations of RD.

More pronounced structural changes in the heart, and their further progression, in patients with CHF developing against the background of the cardiorenal syndrome are associated with the activation of the neurohumoral system, which contributes to the activation of a number of pathogenetic mechanisms.[1,2] As a result of activation of the SAS, cardiac output increases, and vasoconstriction of peripheral blood vessels is stimulated. Sympathetic stimulation of the kidneys triggers a powerful mechanism for the development of arterial hypertension - RAAS. Angiotensin II, the main active component of the RAAS, directly and indirectly (indirectly through the activation of the sympathetic nervous system) causes hypertrophy of cardiomyocytes.[2] The combined effect of the components is accompanied by a more powerful activation of the SAS and RAAS. The aggravating effect of RD on the development and prognosis of CHF is due to a number of closely related mechanisms. The impact on the stressed endothelium or stimulation of angiogenesis in patients with CHF can help preserve the function of target organs and slow down the progression of pathology.[11]

Conclusions

1. The analysis of the dimensions of the LV and LA cavities, as well as the LV walls, revealed the peculiarity in structural and functional changes in LF in CHD patients with CHF FCI-III and eGFR >60 mL/min per 1.73 m^2 or GFR<60 mL/min per 1.73 m^2

2. Compared to eGFR>60 mL/min per 1.73m^2, eGFR≤60 mL/min per 1.73 m^2 in CHD patients with CHF FCI-III provides evidence of the role of the severity of RD in reducing systolic function in patients with CHF.

3. Compared to eGFR>60 mL/min per 1.73m^2, eGFR≤60 mL/min per 1.73 m^2 in CHD patients with CHF FCI-III impairs diastolic function of LF.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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sST2 Level at Decompensated Chronic Heart Failure in Patients with Dilated Cardiomyopathy

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Abstract

The purpose of the study was to evaluate the relationship between clinical and functional changes and sST2 levels in patients with dilated cardiomyopathy (DCM) admitted to the hospital due to decompensated chronic heart failure (CHF).

***Methods and Results*:** The study involved 64 DCM patients with clinical signs of decompensated CHF. According to the sST2 level, the patients were divided into two groups. Group 1 included 30 patients with sST2 level <35ng/ml; Group 2 included 34 patients with sST2 level ≥35ng/ml. All patients underwent the following examinations: collection of anamnestic data, physical examination, general clinical and laboratory blood tests, 12-lead ECG, conventional 2D echocardiography in M- and B-modes, the 6MWT, and the assessment of the quality of life according to the Minnesota Living with Heart Failure Questionnaire (MLHFQ). The serum level of sST2 was determined by enzyme immunoassay using the Presage ST2 Assay.

The duration of CHF was significantly longer in Group 2 than in Group 1(48.7±6.5 mth versus 29.6±7 mth $P<0.05$), and the number of hospitalizations per year was more frequent (Table 1). Group 2 patients were characterized by lower blood pressure levels and high heart rate ($P<0.05$). At the same time, the 6MWT value was lower and MLHFQ score was higher in Group 2 than in Group 1 ($P<0.001$ in both cases). In Group 1, LVEF was significantly higher and LVM was significantly lower than in Group 2 ($P<0.001$). All in all, Group 2 patients had more pronounced disorders in LV systolic dysfunction (Table 3). The correlation analysis revealed an inverse correlation between the sST2 level and 6MWT (r=-0.69, $P<0.01$), as well as LVEF (r=-0.26, $P<0.01$). Statistically significant direct correlations were found between the sST2evel and the size and volume of the LV cavities.

Conclusion: sST2 is a clinically relevant biomarker that reflects pathophysiological processes and provides prognostic information in the setting of DCM, especially in patients with HF.(**International Journal of Biomedicine. 2022;12(2):222-226.**)

Key Words: soluble suppression of tumorigenecity 2 • dilated cardiomyopathy • heart failure

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Abbreviations

6MWT, the 6-minute walk test; **CMPs**, cardiomyopathies; **CHF**, chronic heart failure; **DCM**, dilated cardiomyopathy; **DBP**, diastolic blood pressure; **HR**, heart rate; **HF**, heart failure; **LV**, left ventricle; **LVEF**, left ventricular ejection fraction; **LVM**, left ventricular mass; **LVEDD**, left ventricular end-diastolic diameter; **LVEDV**, left ventricular end-diastolic volume; **LVESD**, left ventricular end-systolic diameter; **LVESV**, left ventricular end-systolic volume; **LVPWT**, left ventricular posterior wall thickness; **MLHFQ**, Minnesota Living with Heart Failure Questionnaire; **mPAP**, mean pulmonary artery pressure; **NP**, natriuretic peptide; **sST2**, soluble suppression of tumorigenecity 2. **SBP,** systolic blood pressure.

Introduction

In recent decades, interest in the study of cardiomyopathies (CMPs) has grown. CMPs are a group of often inherited diseases characterized by structural and functional cardiac abnormalities, an unclear etiology, chronic progressive course and, ultimately, cardiomegaly, progressive heart failure (HF), arrhythmic, and thromboembolic syndrome, often resulting in sudden cardiac death.

Dilated cardiomyopathy (DCM), characterized by heterogeneity of clinical manifestations, is inevitably aggravated by the development of severe CHF, which is

associated with a decrease in the quality of life, frequent hospitalizations, and a high mortality rate, reaching 50% per year. Though this condition has been studied for more than half a century, these studies did not contribute to solving a number of issues related to the etiology, pathogenesis, and effective drug therapy. Based on current research data, it can be stated that the vast majority of cases of DCM are genetically determined, although the presence of the disease in close relatives cannot always be detected.[1,2] It is not yet possible to conduct extensive studies to identify genetic mutations before the onset of clinical symptoms or before the incidental detection of myocardial pathology. Moreover, the presence of an established genetic defect in the family is not always accompanied by clinical and/or morphological manifestations.

The biomarker strategy in diagnosis, risk assessment, and treatment seems to be the most optimal choice due to its high specificity to the main etiopathogenetic mechanisms of disease development and progression, such as inflammation, stress, myocyte injury, extracellular matrix remodeling, oxidative stress, and neurohormonal disorders. Clinical biomarkers such as cardiac troponin and N-terminal pro-BNP (NT-proBNP) are widely used in the diagnosis of heart failure (HF).[3-5] NPs recommended by international communities to diagnose heart failure have firmly entered the toolkit of routine use throughout the world. Meanwhile, in addition to NPs, the potential effectiveness of new biomarkers has been identified, in particular, ST2. For the first time, it has become known as a participant in inflammatory and autoimmune reactions.[3,6-8] ST2 is defined as the IL-33 receptor, as it binds to IL-33.[7,9] ST2 has two main isoforms: transmembrane or cellular (ST2L) and soluble or circulating (sST2) forms.[6,10] The blood sST2 level is increased in inflammatory diseases and in various heart diseases.[11] sST2 is released when cardiomyocytes stretch, neutralizing its ligand IL-33.[4,12] It is also associated with inflammation during myocardial infarction and HF.[13] Recently, sST2 is frequently reported to be associated with HF.[14-16] The 2013 American College of Cardiology and American Heart Association guidelines recommend measurement of ST2 for additive risk stratification in patients with acute or chronic ambulatory HF.[17]

A high risk of rehospitalization and death from HF is observed in the group of patients with decompensated CHF. In this group, mortality rate during the year, according to different authors, ranges from 17.4% to 23.7%, and taking into account hospital mortality rate, it reaches 29%. At the same time, the frequency of repeated hospitalizations during the first 30 days after discharge from the hospital is 20%-25%.

The purpose of the study was to evaluate the relationship between clinical and functional changes and sST2 levels in patients with the DCM admitted to the hospital due to decompensated CHF.

Materials and Methods

The study involved 64 DCM patients with clinical signs of decompensated CHF. The non-inclusion criteria were the presence of bronchopulmonary diseases(including asthma, COPD), chronic kidney disease(3b stages and higher), diabetes mellitus or taking hypoglycemic drugs, permanent atrial fibrillation, anemia, diseases of musculoskeletal system (coxarthrosis, gonarthrosis, etc., reducing motor activity), obesity (2-3 classes) and other severe somatic pathologies.

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All patients gave informed consent to participate in the study. The diagnosis of CHF was established according to 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure.[18]

According to the sST2 level, the patients were divided into two groups. Group 1 included 30 patients with sST2 level <35ng/ml (mean value of 20.6±5.7 ng/ml); Group 2 included 34 patients with sST2 level ≥35ng/ml (mean value of 77.3±8.8 ng/ml).

All patients underwent the following examinations: collection of anamnestic data, physical examination, general clinical and laboratory blood tests, 12-lead ECG, conventional 2D echocardiography according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging (2015) in M- and B-modes, the 6MWT, and the assessment of the quality of life according to the Minnesota Living with Heart Failure Questionnaire (MLHFQ).

The serum level of sST2 was determined by enzyme immunoassay using the Presage ST2 Assay (Critical Diagnostics, San Diego, CA, USA).

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation (SD) for continuous variables. Group comparisons with respect to categorical variables 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

Our results showed that Group 1 patients were slightly younger than Group 2 patients, and males predominated in both groups (67% and 70%, respectively). The duration of CHF was significantly longer in Group 2 than in Group 1 (48.7±6.5 mth vs. 29.6±7 mth $P<0.05$), and the number of hospitalizations per year was more frequent (Table 1). Group 2 patients were characterized by lower blood pressure levels and high heart rate ($P<0.001$). At the same time, the 6MWT value was lower and MLHFQ score was higher in Group 2 than in Group 1 ($P<0.001$ in both cases) (Table 2). In Group 1, LVEF was significantly higher and LVM was significantly lower than in Group 2 ($P<0.001$). All in all, Group 2 patients had more pronounced disorders in LV systolic dysfunction (Table 3). The correlation analysis revealed an inverse correlation between the sST2 level and 6MWT($r=-0.69$, $P<0.01$), as well as LVEF($r=-0.26$, $P<0.01$). Statistically significant direct correlations were found between the sST2evel and the size and volume of the LV cavities.

Table 1.

Characteristics of patients in both groups

Variable	Group 1, n=30 sST2<35ng/ml	Group 2, n=34 ST2≥35ng/ml	*P*-value
Average age, yrs	36.95±5.38	40.53±5.14	0.0084
Male, n (%)	20 (67)	24 (70)	>0.05
Duration of CHF, months	29.6±7	48.7±6,5	<0.001
Number of hospitalizations per year, n (%): -more than 4 times a year -2-4 times a year -less than 2 times a year	 4 (13.3) 8 (26.7) 18 (60.0)	 14 (41.2) 14(41.2) 6(17.6)	 0.024 0.05 0.002

Table 2.

Clinical and systemic hemodynamic parameters of patients in both groups

Variable	Group 1, n=30 sST2 <35 ng/ml	Group 2, n=34 ST2 ≥35 ng/ml	*P*-value
SBP, mmHg	118.3±5.4	101.3±5.5	<0.0001
DBP, mmHg	75.3±3.5	60.3±4.5	<0.0001
HR, bpm	75.3±12.5	85.3±17.5	0.0117
6MWT, m	232.3±29.8	113.3±32.8	<0.0001
MLHFQ, total score	46.4±12.4	58.4±12.4	0.0003

Table 3.

Indicators of intracardiac hemodynamics in patients of both groups

Indicators	Group 1, n=30 ST2<35ng/ml	Group 2, n=34 ST2≥35ng/ml	P-value
LVEDD, mm	61.6±4.5	73.8±3.1	<0.0001
LVESD, mm	52.7±4.8	65.7±4	<0.0001
LBEDV, ml	210.7±20.6	270±21.4	<0.0001
LVESV, мл	131.6±26.2	189±11.5	<0.0001
LVEF, %	33.1±2.1	24.9±3.3	<0.0001
LA, mm	41.5±3.3	46.6±2.2	<0.0001
RV, mm	40±9.2	41.2±6.1	>0.05
LVM, g	273.3±23	345.4±24.5	<0.0001
PWLV, mm	8.15±0.69	9.54±0.75	<0.0001
mPAP, mmHg	45.1±6.2	62±5.7	<0.0001

Discussion

The success of using NT-proBNP as "gold standard" [19] both in practical medicine (as a reference for the diagnosis of CHF) and in scientific research is limited by many factors (impaired renal function, anemia, COPD, obesity) that can affect its level. sST2 has emerged as a new biomarker that may be used to improve management of heart failure patients beyond the diagnostic value of NPs.[10] Recently, sST2 was found to independently predict all-cause mortality and heart failure hospitalization in patients with CHF.[15] Elevated blood sST2 values have also been significantly correlated with LVEF and NYHA class.[20] Ky et al.[21] demonstrated that patients with sST2 higher than 36.6ng/mL have a three times higher risk of death or cardiac transplantation than those with lower values.

Under local inflammation and/or mechanical or biochemical stress, cardiac tissues produce protective cytokines such as IL-33 and growth differentiation factor-15.[22] In experimental models, the interaction between IL-33 and ST2L provided the cardioprotective effects, reducing myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis, as well as improving myocardial function. sST2 avidly binds to IL-33, competing with ST2L.[10] The interaction of sSH2 with IL-33 blocks the IL-33/ST2L system and, as a result, eliminates the IL-33 cardioprotective effects.[10] sST2 secreted by damaged cardiac tissue acts as a decoy receptor for IL-33, and can completely attenuate the protective effects of IL-33.[7]

The results of our study agrees with several studies that have established a direct correlation between the sST2 levels and the severity of the CHF symptoms, impaired systolic myocardial function,[23,24] as well as the severity of HF.[24-27] In a study performed by You et al.,[28] 94 patients with pediatric DCM were enrolled after admission from two centers in China and followed up for adverse events. Patients in the highest tertile of sST2 levels had increased risk of short-term (<6 months) and long-term adverse events (2 years) than those in lower tertiles. A study by Binas et al.[20] revealed that sST2 predicts all-cause mortality and cardiac mortality in 262 DCM patients with CHF. P Jirak [30] also observed a significant increase in and correlation with disease severity of sST2 in chronic HFrEF patients of both ischemic and non-ischemic origin.

Lupon et al.[31] developed the ST_2-R2 score to predict reverse remodeling in HF with systolic dysfunction; patients with sST_2 values above 48 ng/mL will unlikely experience LV reverse remodeling. In a study performed by Wojciechowska et al.,[32] 107 DCM patients of 39-56 years were followed up for mean 4.8 years. The ROC curve indicated a cut-off value of ST2-17.53 ng/ml, AUC-0.65(0.53-0.76) for prediction of death. In multivariate analysis, ST2 was a predictor of death (HR per unit increase in log ST2 2.705, 95% CI 1.324-5.528, *P*=0.006) and combined endpoint (HR per unit increase in log ST2 2.753, 95% CI 1.542-4.914, *P*<0.001). The authors concluded that sST2 may be useful for predicting adverse outcomes in stable DCM patients.

Therefore, sST2 is a clinically relevant biomarker that reflects pathophysiological processes and provides prognostic information in the setting of DCM, especially in patients with HF.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Synchronization of Wave Flows of Arterial and Venous Blood with Phases of the Cardiac Cycle in Norm: Part 5

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Abstract

The muscular-fibrous frame of the heart (MFFH) synchronizes and compensates for the pressor effect of the myocardium between the high/low-energy regions (left/right) of the heart. The anatomical structures of MFFH (plastic muscular-fibrous formation with a phase change in contours, valves, valve rings) form the "cardiac mean integral pressure" (CMIP). MFFH, a variable spatial structure, forms the starting pressor levels of "opening/closing" of valves and hemodynamic vectors of the heart chambers, systemic and pulmonary circulation, and also compensates for excess pressor pressure (having a variable gradient) at the boundaries of the heart chambers. Throughout the cardiac cycle (CC), on the path "venous block-lung-arterial block-aorta," variable pressure values, compensated by the structures of MFFH, are formed between the blood flows of the right and left parts of the heart. A mutual adaptation of stroke volume (SVs) of the ventricles is formed by phase-by-phase compensatory plastics of MFFH. CMIP of MFFH is an integral indicator, where each point reflects: 1) CC phase (time and place); 2) the average value of the range of values in which the equilibrium point of pressor compensation between the high/low-energy processes of this CC phase is located. CMIP is a vector of the MFFH phase dynamics, which compensates for the excess pressor effect with a changing gradient through CC. (**International Journal of Biomedicine. 2022;12(2):227-231.**)

Key Words: cardiac cycle • muscular-fibrous frame of the heart • cardiac mean integral pressure

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Abbreviations

Ao, aorta; **BB**, "bolus" of the blood; **CC**, cardiac cycle; **CMIP**, cardiac mean integral pressure; **CS**, coronary sinus; **HDVM**, hydrodynamic volumetric module; **IC**, integral curve; **IVC,** inferior vena cava; **LV**, left ventricle; **MV**, mitral valve; **MFF**, muscular fibrous frame; **MFFH**, MFF the heart; **PC**, pulmonary circulation; **PV**, pulmonary valve; **PT**, pulmonary trunk; **PW**, pulse wave; **PVs**, pulmonary veins: **RV**, right ventricle; **RA**, right atrium; **SC**, systemic circulation; **SB**, stroke volume; **PW**, pulse wave; **MC**, magnetograph curve; **SVC,** superior vena cava; **TV**, tricuspid valve; **TP**, trigger point; **ZTEP**, zone of temporal equalization of pressure.

Basic Part

The heart, the central structural element of the cardiovascular system, we consider as a generator of wave, hemodynamic,[1-5] electrical, and magnetic information impulses (packets). Life activity, in general, along with metabolism and energy, is carried out by the transfer, storage, and implementation of information. Being neither matter nor energy, information needs fixation and a carrier.[6] Along with metabolites, hormones, hemodynamic, nervous, and electrical impulses, the information carrier is also a pulsed magnetic field and a change in the architectonics of the MFFH. A synchronization of integral parameters of hemodynamics, pulse wave, and electrical and magnetic parameters of CC

reduces the complexity of the system, on the whole, leading to the appearance of predictable structures in the CC period.

In Figure 1, integral curves (ICs) of the high/low-energy processes of CC, CMIP, ECG, and heart magnetograms are synchronized.

We emphasize that the magnetogram allows us:

a) to measure quasi-constant signals, which are masked on the ECG by the electrical conductivity of tissues;

b) to decipher "weak signals";

c) to reflect in full the electrical activity of the heart, not distorted by membranes, bones, skin. The inclusion of the magnetogram in the general system of synchronization of CC is the subject of our next publication.

We believe, understanding the depth of simplification, that the heart conditionally can be divided into two blocks:

1) The outer muscular frame ("outer heart"), which creates phase-changing pressor-depressor effects throughout CC, participates in the creation of arterial and venous BBs in the chambers of the heart with subsequent transit to the "exchange zones," and creates pressure gradients in the chambers of the heart, SC, and PC;

2) The internal muscular-fibrous frame ("inner heart"), which forms the chambers of the heart, determines the dynamics of the interaction of high/low-energy parts of the heart and the sequence and vectors of wave and hemodynamic structures of the heart.

The "inner heart" MFF[7] consists of fibrous rings of the valves (mitral, tricuspid, aortic, pulmonary artery, connected by the membranous part of the interventricular septum), central fibrous body, aortopulmonary fibrous junction, and left and right fibrous triangles. We believe that the entire muscular array of the interventricular and interatrial septa, including the foramen ovale membrane, is also a constructive part of this frame. It is this anatomical formation (MFF of the "inner heart") that synchronizes and compensates for the pressor effect of the myocardium between the high/low-energy regions (left-right) of the heart. The phase-changing architectonics of MFFH provides all the vectors of the hemodynamic processes of the heart. MFFH anatomical structures (a plastic muscular-fibrous formation with a phase change of contours, valves, and valve rings) are the zone of formation and functioning of the CMIP.[4,5]

We emphasize that the fibrous fibers, penetrating all layers of the myocardium and endocardium, and the papillary muscles, pass into the connective tissue apparatus of the vessels of the SC and PS. From the "fibrous center,"[7] the fibrous rings of all valves, bundles of collagen fibers depart, along with valves, membranes, chords, ligaments, and tendons, which make up the supporting frame of the heart. We believe that fibrous fibers with limited extensibility can be considered not only as structural formations affecting the amplitude of working cardiomyocytes, but also as tensor sensors.

MFFH is a variable spatial construct that forms hemodynamic vectors and compensates for the pressor effect between the heart chambers' high/low-energy processes. MFFH, vertically separating the right and left parts of the heart horizontally (valve moderation), participates in forming three pairs of phase-changing volumetric modules of variable capacitance.

1) The first pair is formed in proper ventricular diastole when the ventricular block unites with the atrial block and is disconnected from the aortopulmonary block (open TV and MV). The variable capacitances of HDVM-1 (SVC, IVC, RA, RV, and CS) and HDVM-2 (PVs, LA, LV) are separated from arterial volume modules of the variable capacitance by two valve membranes at the heart output (Ao, PT).

2) The second pair of the variable capacitance is formed in the phase of isometric contraction of the ventricles, with closed heart valves, partial connection with the atrial block (CS), and disconnection from the aortopulmonary block. At the same time, the variable capacitances of HDVM-1 (SVC, IVC, RA) and HDVM-2 (PVs, LA) are separated from arterial volume modules of the variable capacitance of SC (Ao) and PC (PT) by two pairs of membranes at the input and output of both ventricles: TV-PT, MV-Ao

3) The third pair of modules of the variable capacitance is formed in systole when the ventricular block is combined with the aortopulmonary block and partially connected to the atrial block (through the spongy venous chamber of the myocardium). The variable capacitances of HDVM-1 (SVC, IVC, RA, and CS) and HDVM-2 (PVs, LA) are separated from arterial volumetric modules of the variable capacitance of SC (LV) and PC (RV) by two membranes at the entrance to the block of ventricles (MV, TV).

Synchronization of the phase dynamics of arterial and venous volumetric modules of the variable capacitance under the influence of the pressor-depressor effect of the myocardium is carried out through the valvular apparatus of the heart, a constructive component of the MFFH.

The phase-changing architectonics of the MFFH creates the hemodynamic vectors of the heart; its anatomical contours are the zone of formation and functioning of CMIP. In other words, CMIP is an integral characteristic of the "inner heart," which is formed by the plastic structures of the MFFH at the boundaries of the heart's chambers by the dynamics of excess pressure with a variable gradient. MFFH provides a) compensation of the pressor interaction between the myocardium ("external heart") and the contents of the heart's chambers; b) the starting pressor level of valve modulation and vectors of intra- and extracardiac hemodynamics.

CMIP of MFFH is simultaneous: 1) an integral derivative of the total pressor interaction of all hemodynamic tracts with structural elements of the heart; 2) a derivative of the myocardial pressor effect, which regulates the starting pressure of opening/closing valves, interaction, synchronization, and vectors of the tracts of BBs of the right and left sections of the heart.

The primary link of intracardiac transformations is the pressor effect of the functional syncytium, which includes cascade dynamics of synchronous interaction between different zones and layers of the myocardium, valvular, fibrous, and ligamentous apparatus of the heart. With a trigger type of response, syncytium has synchronized differences in the power and sequence of contraction of various myocardial zones.

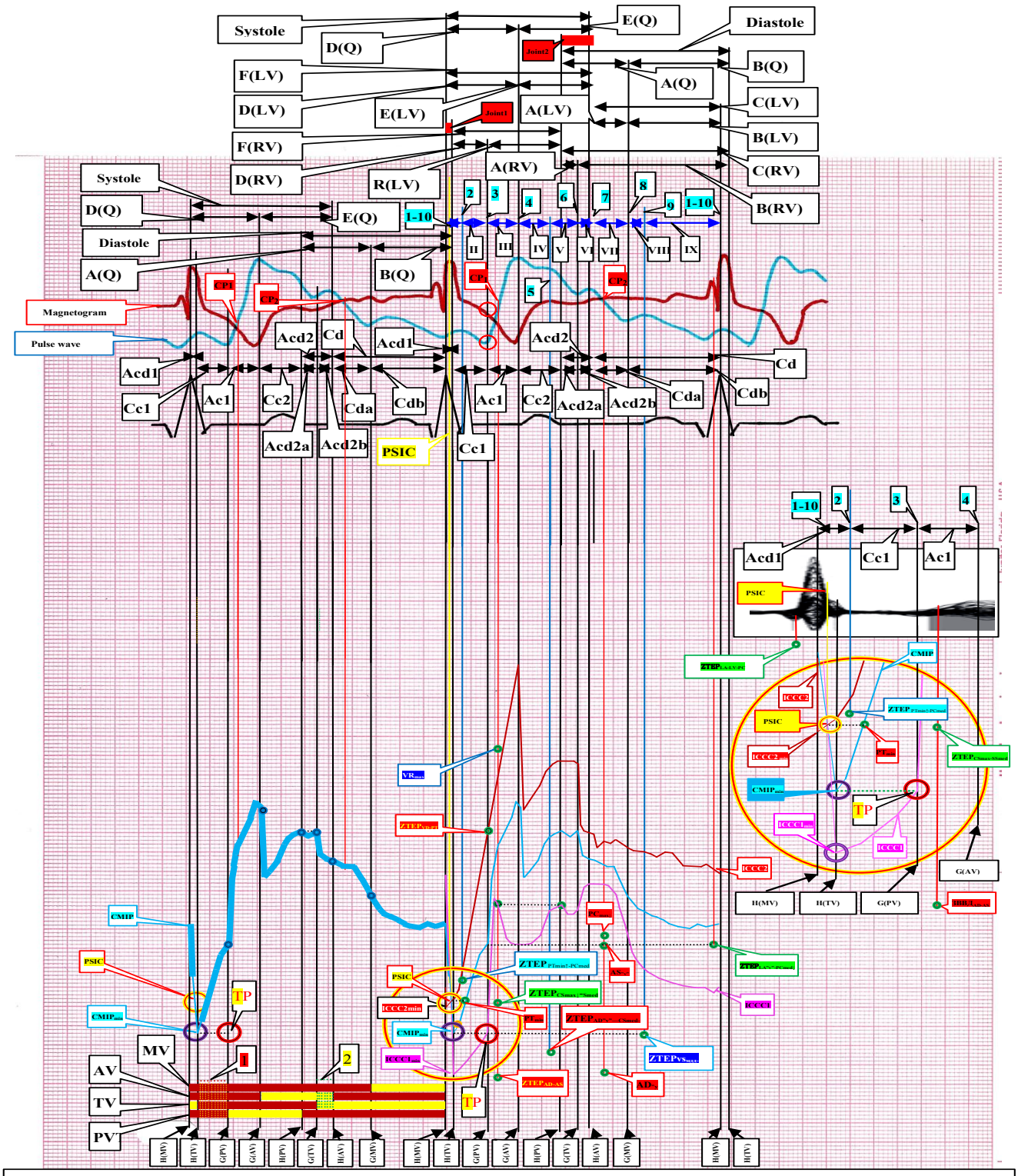

Figure 1.

1-10 – asynchronous phase of the contraction (AC)
2 – phase of the isometric contraction (IC)
3 – rapid ejection phase (Em)
4 – slow ejection phase (Er)
5 – protodiastole (P)
6 – isometric relaxation phase (IR)
7 – rapid filling phase (Fr)
8 – slow filling phase (Dy)
9 – atrial systole
A(Q) – isometric ventricular relaxation
B(Q) – actual ventricular diastole
C(LV) – LV diastole
A(LV) – isometric LV relaxation
B(LV) – actual LV diastole
C(RV) – RV diastole
A(RV) – isometric RV relaxation
B(RV) – actual RV diastole
D(Q) – isometric ventricular contraction
E(Q) – actual ventricular systole
F(LV) – LV systole
D(LV) – isometric LV contraction
E(LV) – actual LV systole

F(RV) – RV systole
D(RV) – isometric RV contraction
E(RV) – actual RV systole
G(AV) – opening of AV
H(PV) – closing of PV
G(TV) – opening of TV
H(AV) – closing of AV
G(MV) – opening of MV
H(TV) – closing of TV
H(MV) – closing of MV
G(PV) – opening of PV
H(PV) – closing of PV
Acd1 – asynchronous period of ventricular systole-diastole -1
Cc1 – synchronization period of isometric ventricular contraction -1
Ac1 – asynchronous period of ventricular systole -1
Cc2 – synchronization of the actual ventricular systole -2
Acd2 – asynchronous period of ventricular systole-diastole -2
Acd2a – from closing of PV to opening of TV
Acd2b – from opening of TV to closing of AV
Cd – period of synchronization of ventricular relaxation
Cda– isometric relaxation of LV
Cdb– actual LV diastole

– phases of PW (V.V. Boronoev)
– cross point of the ZTEPs
– trigger point (TP), including ZTEP: SS-VH-SVC-CS-RV-LA
– IC of the low-energy phase of CC - ICCC1
– IC of the high-energy phase of CC - ICCC2
– CMIP
– "stabilization" point for ICC1 и ICC2 - PSIC
– "cross" point between PW and MC - CP
–"destruction" point of the CMIP gradient at the borders of the heart chambers
.... points of ZTEP coincidence
MV – heart valve closed AV – heart valve open
1 – "isolation" of the ventricular block from atrial block and SC and PC
2 – "isolation" of the ventricular block, **RA** and SC from **LA** and PC
CP1 – "cross" of magnetogram with PW
Joint – asynchronous period of ventricular systole-diastole
$ZTEP_{AD-AS}$ – min. excessive pressor effect of the myocardium AD-AS
$ZTEP_{VD-LV}$ – min. excessive pressor effect of the myocardium VD-LV

AV– aortic valve; **CC** – cardiac cycle; **CS** – coronary sinus; **IVC** – inferior vena cava; **SVC** – superior vena cava; **VH** – vein hepatic ; **LA** – left atrium; **LV** – left ventricle; **MV** – mitral valve; **PV** – pulmonary valve; **PT** – pulmonary trunk; **RA** – right atrium; **RV** – right ventricle; **TV** – tricuspid valve; **SS** – sigmoid sinus; **SC** – systemic circulation; **PC** – pulmonary circulation; **ZTEP** – zone of temporary equalization of pressure.

The result is differences in the pressor parameters of the formation and transit of BBs in the right and left parts of the heart. BBs differ in the magnitude of the forming pressure, density, viscosity, gas composition, electrical, magnetic, and other parameters. Synchronized pressor action of the myocardium initiates the interaction of BBs through anatomical septa with different plasticity properties: RA and LA through the foramen ovale and interatrial septum; RA and LV through the membranous part of the interventricular septum; RV and LV through the muscular part of the interventricular septum, etc. In other words, throughout CC in the "inner heart" on the tract "venous block-lung-arterial block-aorta," there is the formation of the averaged variable pressure values (compensated by the plastic septa and BB volumes) between synchronous, spatially separated blood flows of the right and left parts of the heart.

Thus, there is a mutual adaptation of the SVs of the heart's ventricles, formed by 1) the pressor amplitude of the syncytium; 2) incompressible volume of BBs; 3) MFFH plasticity. The combination of these values creates a dynamic intracardiac pressor balance at the border of low/high energy processes of the heart, which we designated as CMIP.

We believe that ventricular ejections are synchronized and ventricular SVs are mutually adapted precisely by the interaction of the myocardium, BBs and MFFH. Hemodynamic factors[1-4] phase-by-phase form the compensatory plasticity of MFFH during CC. In other words, compensation for the difference in pressor influences between the high/low-energy parts of the heart occurs through changes in the architectonics and utilization of excess pressure by the MFFH structures.

IC1 and IC2 [3,4] reflect the synchronous dynamics of the bilateral pressor effect of the myocardium on MFFH, being indicators of the dynamics of the average pressure between the polar pressor values of the CC processes located in the same phase segment of CC.

CMIP is an integral indicator, where each point reflects:

1) CC phase (time and place)

2) The average value of the range of values in which the equilibrium point of pressor compensation between the high/low-energy processes of this CC phase is located. In other words, CMIP is a vector of the "inner heart" phase dynamics, which compensates for the excess pressor effect with a changing gradient in the system of high/low-energy CC processes.

In this work, the asynchronous period of ventricular systole (Acd1) is of greatest interest to us, during which low-energy CC processes, formed by the right heart sections, are "launched." In this period, the hemodynamic "point of stabilization" (PSIC) is formed, which determines the pressor dynamics of the sequential triggering of the phases of isometric contraction (left, then right) of the ventricles.[3,4] At the end of the period Acd1, CMIPmin forms the pressor level of the "trigger point," determining the pressor level of opening of the PT valves and RVSV.[4,5] CMIPmax determines the pressor level of AV opening and LVSV.[5] The phase sequence of this period ensures the synchronization of hemodynamics in the aortopulmonary, ventricular and atrial blocks.

Next period: asynchronous period of ventricular systole and diastole (Acd2), when, with incomplete LV systole, RV diastole begins. The period of minima of CMIP and IC1-IC2 with the intersection of their graphs at the PSIC point includes: asynchronous period of ventricular systole-diastole (Acd1) and the initial phase of isometric LV contraction, against the background of the completion of the RV diastole and RA systole.

We believe that the pressor interaction of the myocardium, BBs and MFAP in the Acd1 phase forms pressure minima at the «input» and «output» of the right heart, determining the level of «starting» pressure - the «trigger» point.[3,4]

CMIP is an indicator of intracardiac pressor balance, which is a systemic hemodynamic regulator[5] that has characteristic components: topographic (variable architectonics of MFFH), pressor, electrical, magnetic, rheological, etc. CMIP is considered by us as a vector of the system regulator of the first hierarchical level,[4,5] which influences the third order regulators (organ) through the second order regulators (SVs of LV and RV, PW).

In other words, the interaction of the myocardium, BBs and MFFH during CC forms a closed cycle of direct and feedback links of the first, second, and third hierarchical levels of the regulatory central (cardiac) and peripheral (organ) control of homeostasis by wave patterns. We believe that it is this variable structure that is the "central rhythmic process" that determines the heart rhythm and the synchronization of electrophysiological and behavioral equivalents, the assumption of which was made.[8]

Conclusion

The pressor dynamics of the myocardium phase-by-phase forms compensatory plastics of MFFH. Changes in the architectonics of MFFH determine the starting pressor levels of opening/closing of the heart valves and hemodynamic flow vectors, adapt and synchronize the ventricle SVs, utilize excess pressure, and form a pressor balance between the high/low-energy parts of the heart.

CMIP is an integral indicator, where each point reflects:

1) CC phase (time and place)

2) The average value of the range of values in which the equilibrium point of pressor compensation between the high/low-energy processes of this CC phase is located.

CMIP is a vector of the MFFH phase dynamics, which compensates for the excess pressor effect with a changing gradient through CC.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Diabetes Mellitus

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Lipids Peroxidation Products in Young Men with Type 1 Diabetes Mellitus

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Abstract

The aim of this research was to assess the level of lipid oxidation products level and the antioxidant defense state in young male patients with type 1 diabetes mellitus (T1DM) depending on the disease duration.

Methods and Results: A total of 57 men of young reproductive age (average age of 30.25±8.51 years) with T1DM and an unsatisfactory glycemic profile, depending on the disease duration, were divided into 2 groups. Group 1 included 29 men (average age of 27.69±6.92 years) with a T1DM duration <5 years (2.72±1.61 years) and HbA1c level of 11.37±2.74%. Group 2 included 28 men (average age of 32.89±9.28 years) with a T1DM duration ≥5 years (12.93±5.69) and HbA1c level of 10.19±2.18%). The control group consisted of 28 men of the same age (29.71±4.59 years). Spectrophotometric/spectrofluorometric methods and enzyme immunoassay were used. We found a significant increase in the values of CDs (by 2.04 times, $P<0.0001$), KD and CT (by 2.38 times, $P<0.0001$), TBARs (by 1.17 times, $P=0.001$), SB (by 2.6 times, $P<0.0001$), and retinol (by 1.44 times, $P<0.0001$) in Group 1 compared to the control group. In Group 2, there was a statistically significant increase in the levels of CDs (by 2.59 times, $P<0.0001$), KD and CT (by 2.94 times, $P<0.0001$), TBARs (by 1.49 times, $P=0.001$), SB (by 3.27 times, $P<0.0001$), and retinol (by 1.4 times, $P=0.001$) compared to the control group. The differences between the two groups with different duration of T1DM were characterized only by the CDs level, which was increased in Group 2 patients with a T1DM duration of ≥5 years (by 1.27 times, $P=0.048$) compared to Group 1 patients with a T1DM duration of <5 years.

Conclusion: LPO parameters can serve as additional laboratory markers that characterize the course of T1DM and can be used to develop potential prevention and therapy strategies.(**International Journal of Biomedicine. 2022;12(2):232-236.**)

Key Words: type 1 diabetes mellitus • men • diabetes duration • lipid peroxidation • antioxidant defense

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Abbreviations

AOD, antioxidant defense; **CDs**, conjugated dienes; **DM**, diabetes mellitus; **KD-CT**, ketodienes and conjugated trienes; **LPO**, lipid peroxidation; **MDA**, malondialdehyde; **OS**, oxidative stress; **RBC**, red blood cells; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TAA**, total antioxidant activity; **T1DM**, type 1 diabetes mellitus; **TBARs**, thiobarbituric acid reactants; **SB**, Schiff bases; **T2DM**, type 2 diabetes mellitus.

Introduction

Diabetes mellitus (DM) is one of the most critical problems of our time due to its high prevalence, early disability, and a decrease in the patients` life expectancy.[1] According to WHO (2021), between 2000 and 2016, there was a 5% increase in premature mortality from diabetes. More than 10 million patients with DM are officially registered in the Russian Federation, of which more than 300 thousand are patients with type 1 diabetes mellitus (T1DM).[2]

Reactions of free radical oxidation, including lipid peroxidation (LPO), play an essential role in the pathogenesis of T1DM and its complications.[3] LPO is a universal metabolic process represented in all organs and tissues. LPO reactions,

having the ability to modify the structure and functions of cell membranes, can determine the nature of intercellular and inter-organ relationships within a certain functional system, as evidenced by the data on the direct participation of LPO in the xenobiotic metabolism, in the regulation of the immune response, cell proliferation, vascular permeability, receptor sensitivity, etc.[4,5] Under physiological conditions, the LPO reaction state can inform us about the nature of adaptive reactions.[6] At the same time, the LPO intensification and the insufficiency of antioxidant response define the pathogenesis of many diseases, including T1DM.[7,8]

The main molecular mechanisms associated with oxidative damage reactions in T1DM, associated with glucose and lipid metabolism, have been identified.[9] They include the glycolytic pathway, enhanced formation of advanced glycation end products, the hexosamine pathway, protein kinase C activation, polyol pathway, and insulin signaling pathway deactivation.[7,9] Oxidative stress (OS) can play a dual role concerning T1DM, contributing not only to its manifestation, but also to the exacerbation of the disease and related complications.[10] Despite numerous studies of these reactions in T1DM, the age and gender of the subjects usually were not taken into account.

In this regard, the aim of our work was to assess the lipid oxidative damage products level and the antioxidant defense state in young men with T1DM, depending on the disease duration.

Material and Methods

Design of study

A total of 57 men of young reproductive age (average age of 30.25±8.51 years) with T1DM and an unsatisfactory glycemic profile, depending on the disease duration, were divided into 2 groups. Group 1 included 29 men (average age of 27.69±6.92 years) with a T1DM duration of <5 years (2.72±1.61 years) and HbA1c level of 11.37±2.74%. Group 2 included 28 men (average age of 32.89±9.28 years) with a T1DM duration of ≥5 years (12.93±5.69) and HbA1c level of 10.19±2.18%). The patients were treated in the endocrinology department of the Irkutsk Regional Clinical Hospital. The control group consisted of 28 men of the same age (29.71±4.59 years). Inclusion criteria for Groups 1 and 2 were confirmed diagnosis of T1DM, men aged 18-40 years, and residence in the specified territory. Exclusion criteria for Groups 1 and 2 were T2DM or other types of diabetes, severe DM complications (proteinuria, renal failure, and macrovascular complications), and other endocrine diseases, pronounced concomitant somatic pathology. Inclusion criteria for the control group were the absence of acute or exacerbation of chronic diseases at the time of the examination, normal indicators of glucose tolerance, absence of a hereditary predisposition to DM.

The study complied with the ethical principles of World Medical Association Declaration of Helsinki (1964, ed. 2013) and it was approved by the Biomedical Ethic Committee at the Scientific Centre for Family Health and Human Reproduction Problems (No. 8.2 dated November 2, 2018). Written informed consent was obtained from all participants.

Biochemical measurements

Plasma, serum and erythrocyte hemolysate were used as the material for the study. Plasma concentrations of primary/secondary/final products of LPO (CDs/KD-CT, TBARs/SB) were estimated.[11] TBARs content was detected by fluorometry.[12]

The state of the AOD system was determined by TAA in blood serum (using a commercial kit from Randox (UK)), the SOD activity in hemolysate (using a commercial kit from Randox (UK)), and the content of α-tocopherol and retinol in the blood plasma.[13] The measurements were carried out using a Shimadzu RF-1501 spectrofluorophotometer (Japan) and Shimadzu RF-1650 spectrofluorophotometer (Japan). Enzyme immunoassay was performed usionga MultiSkan ELX808 microplate reader (Biotek, USA).

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 mean±standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. The Spearman correlation coefficient r_s, was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

We found a significant increase in the values of CDs (by 2.04 times, $P<0.0001$), KD and CT (by 2.38 times, $P<0.0001$), TBARs (by 1.17 times, $P=0.001$), and SB (by 2.6 times, $P<0.0001$) in Group 1 compared to the control group (Fig. 1). Among AOD parameters, in Group 1, only the retinol level was significantly greater (by 1.44 times, $P<0.0001$) than in the control group.

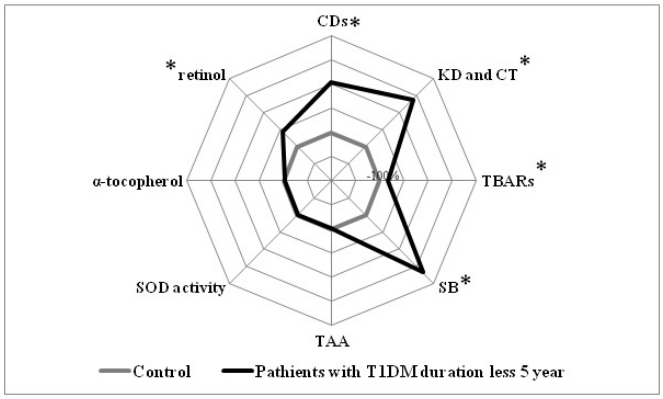

Fig. 1. *State of the LPO-AOD system in patients with T1DM duration of <5 years (*- statistically significant differences compared to the control (values are taken as 100%)).*

Group 2 was characterized by similar changes in the LPO-AOD system. Thus, there was a statistically significant

increase in the levels of CDs (by 2.59 times, $P<0.0001$), KD and CT (by 2.94 times, $P<0.0001$), TBARs (by 1.49 times, $P=0.001$), SB (by 3.27 times, $P<0.0001$), and retinol (by 1.4 times, $P=0.001$) in Group 2 compared to the control group (Fig. 2).

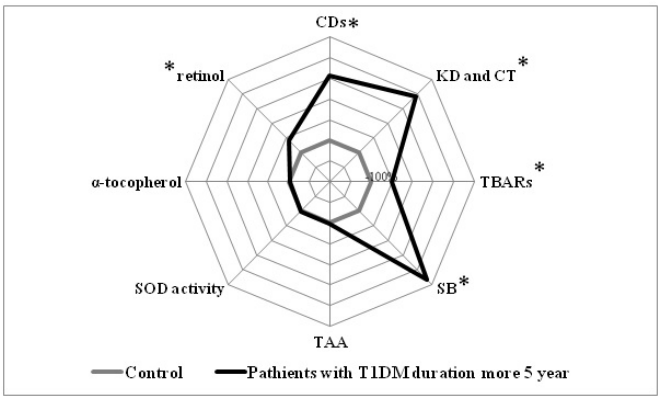

Fig. 2. *State of the LPO-AOD system in patients with T1DM duration of ≥5 years (*- statistically significant differences compared to the control (values are taken as 100%)).*

The differences between two groups with different duration of T1DM have characterized only the CDs level, which was increased in Group 2 patients with a T1DM duration of ≥5 years (by 1.27 times, $P=0.048$) compared to Group 1 patients with a T1DM duration of <5 years.

Data analysis found that in patients with T1DM, regardless of the disease duration, a significant increase in the content of primary LPO products occurs at all stages of the LPO process. However, when the duration of T1DM was more than 5 years, the changes were more pronounced.

According to the literature data, the activation of LPO processes in erythrocyte membranes occurs at the T1DM debut. The presence of atherogenic dyslipidemia and LPO activation manifested by an increased content of primary and secondary LPO products in patients with newly diagnosed T1DM has been shown in numerous studies.[14] At the same time, the assumption that the LPO-AOD system imbalance is closely related to the degree of compensation and severity of T1DM turned out to be significant.[15,16] The state of metabolic decompensation in T1DM, defined as "metabolic stress," also includes lipid metabolism disturbances.[8,17,18] Many studies established a close positive relationship between the accumulation of MDA with the duration of diabetes and HbA1c and a negative association with the ferric reducing ability of plasma.[19,20] Perhaps this is due to insulin insufficiency, which is involved in lipoperoxide utilization.[21] It was also shown that the primary LPO product (CDs) accumulation might depend on the content of free fatty acids.[3,14,22] With the enhanced LPO activity, the interactions between insulin and its receptors can be changed due to the MDA's ability covalently binding to cell membranes of lipids and proteins with the crosslinking formation.[5] All of the above leads to the alteration of insulin receptor internalization—the number of insulin-binding sites decreases, which can serve as one of the causes of insulin resistance.[23,24] It was noted that under conditions of poorly controlled T1DM, there is a direct dependence of the malondialdehyde level and SOD activity from the ketone bodies content due to their excess formation in T1DM.[25] The products of toxic MDA reactions with proteins, phospholipids, and nucleic acids are not destroyed due to strong bonds. They are accumulated in the body, leading to the violations of biopolymers properties, which can worsen a patient's condition in conditions of AOD system poor functioning.[3]

The disease duration can also aggravate the LPO-AOD system disturbances in T1DM patients. Thus, a pronounced LPO process activation can increase in patients with diabetes duration of more than 10 years.[3,20] Many studies have reported that in patients with T1DM complications, the content of LPO products was more pronounced, suggesting their participation in the vascular damage initiation.[26-28] Toxic products of LPO can cause microvascular complications through various mechanisms. In DM, the increased oxidative stress, the alteration of lipogenesis, the reduction of nitric oxide, and the alteration of endothelial progenitor cell function create damage to the vessel wall leading to the pathogenesis of arterial thrombus.[29] The pathogenesis of endothelial dysfunction in T1DM is complex and involves metabolic and hormonal changes. In particular, insulin deficiency leads to decreased number of endothelial progenitor cells, decreased nitric oxide production, increased oxidative stress in the vascular milieu, and a consequent decrease in the ability to promote vessel dilation.[30] It was found that the cell membranes of T1DM patients undergo significant structural changes. Lee et al.[31] demonstrated that hyperglycemia in T1DM patients severely impairs RBC deformability by remodeling the mechanical properties of the cell membrane. Currently, several impaired biochemical pathways such as glycolytic, hexosamine, protein kinase C, polyol, and advanced glycation end-product (AGE) pathways have been identified as pro-oxidative processes in the diabetics.[32-38] Inhibition of glyceraldehyde-3-P dehydrogenase by poly-ADP-ribose polymerase 1 and subsequent accumulation of the enzyme substrate (glyceraldehyde-3-P) appears to be central to diabetes-associated oxidative stress.[9]

Any impact that causes an increase in the peroxidation process exerts a different effect depending on the activity of the antioxidant system. We found no significant differences in most of the studied parameters, with the exception of retinol, the values of which were elevated in both two groups with T1DM. Insulin insufficiency may be the main cause of the antioxidant status changes in T1DM.[39]) It was noted that the vascular pathology progression in T1DM is associated with increasing AOD insufficiency, manifested in the form of the main antioxidants concentration decrease.[40-43]

Thus, in patients with T1DM and diabetic nephropathy (decompensated form), against the background of metabolic disorders, the LPO activation and the antioxidant system inhibition are noted.[10] Very contradictory information was obtained while studying the activity of antioxidant enzymes in the RBC of patients with T1DM. Analysis of serum TAA using biochemiluminescence revealed a significant decrease in this indicator in patients with T1DM decompensation stage compared with healthy people.[7] In our study, we observed an increase in retinol values regardless of the disease duration. It can be assumed that retinol plays the role of both an

independent antioxidant that ensures the preservation of the cell membrane functional stability, blocking the LPO processes in the cell membrane, and serve as a synergist of α-tocopherol–the main fat-soluble antioxidant.

Thus, an increase in retinol content can be regarded as a compensatory phenomenon. However, despite the increase in its values, patients with T1DM have a significant LPO activity at all disease stages, which can be characterized as a shift in the redox balance toward pro-oxidant factors.

Conclusion

We identified the significant increase in the LPO product content and the retinol level with longer disease duration in young men with T1DM. LPO parameters can serve as additional laboratory markers that characterize the course of T1DM and can be used to develop potential prevention and therapy strategies.

This work was performed with the use of equipment of the collective research center "Centre for the development of progressive personalized health technologies" SC FHHRP, Irkutsk.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

COVID-19

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The Association of C-Reactive Protein and Ferritin Levels with the Severity of COVID-19 in Ajman, UAE

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Abstract

Background: SARS-Cov-2, a new strain of coronavirus first identified in Wuhan city, China, has spread worldwide, causing severe illnesses and a high mortality rate. Many studies have shown the association of elevated levels of pro-inflammatory markers, such as ferritin and C-reactive protein (CRP), with the severe course of coronavirus disease The aim of this research was to investigate the association between CRP and ferritin levels, and the severity of COVID-19.

Methods and Results: This cross-sectional study was performed in Thumbay Hospital, Ajman, United Arab Emirates, from January 2021 to October 2021. A total of 100 COVID-19 positive patients were included in this study. Serum CRP and ferritin were measured by immunoturbidimetric assay. We found statistically significant differences between ferritin levels and disease severity (P=0.005), age category (P=0.030), and the clinical wards (P=0.016). Statistically significant differences were found between the ferritin levels in mild to moderate cases (P=0.023) and mild to severe cases (P=0.007). There were significant differences in CRP in mild to moderate cases (P=0.012), and in mild to severe cases (P=0.000). Thus, the results obtained showed that CRP and ferritin levels are considerably greater in severe cases than in mild and moderate cases of COVID-19. The findings of the current study indicate that CRP and serum ferritin levels might be considered as an essential indication of the progression and severity of COVID-19.(**International Journal of Biomedicine. 2022;12(2):237-241.**)

Key Words: SARS-Cov-2 • C-reactive protein • ferritin

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Introduction

SARS-Cov-2, a new strain of coronavirus first identified in Wuhan city, China, has spread worldwide, causing severe illnesses and a high mortality rate.[1-3] Patients with SARS-CoV-2 infection can experience a range of clinical manifestations, from no symptoms to critical conditions.[4-6] Many studies have shown the association of elevated levels of pro-inflammatory markers, such as ferritin and C-reactive protein (CRP), with the severe course of coronavirus disease.[7,8] Ferritin is a protein that binds iron molecules and stores iron. It comprises two subunits: light chain and heavy chain. Typically, in response to inflammation, ferritin levels increase in blood, whereas hyperferritinemia is associated with a significantly increased mortality in infected patients. Iron availability in the blood is the main modulator of ferritin levels.[9,10] CRP is the first inflammatory marker that increases significantly at the early stage of the disease. It plays an important role in cases of infections and inflammations.[11] Many studies have shown a positive correlation between increased CRP and ferritin levels and COVID-19 severity.[12,13]

The aim of this research was to investigate the association between CRP and ferritin levels, and the severity of COVID-19.

Materials and Methods

This cross-sectional study was conducted in Thumbay Hospital, Ajman, United Arab Emirates, from January 2021 to October 2021. A total of 100 COVID-19 positive patients were included in this study. Written informed consent was obtained from all participants.

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Serum CRP and ferritin were measured by immunoturbidimetric assay using Beckman Coulter AU Chemistry Analyzers. The validation procedure was done according to CAP and CLIA for precision, accuracy, and linearity. The severity and diagnosis of pneumonia and the severity of the illness (mild, moderate, or severe) were assessed according to WHO recommendations. Participants without symptoms or with mild symptoms did not require hospitalization, but they were included in the study as they tested positive for SARS-CoV-2.

Clinical symptoms in the mild group included nausea, headaches, stomach pain, and vomiting. The moderate group was hospitalized due to symptoms such as fever, cough, and pneumonia. The severe group of patients who presented with high temperatures, coughing, pneumonia, and shortness of breath required intensive care.

Statistical analysis was performed using the statistical software package SPSS version 16.0 (SPSS Inc, Chicago, IL). Variables were presented as the mean (M) and standard deviation (SD). A 95% confidence interval (CI) was calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Multiple comparisons were performed with one-way ANOVA. 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.

Results

Among 100 COVID-19 positive patients, men accounted for 77.0%. Age subgroups were as follows: 26-35 yrs – 19(19.0%), 36-45 yrs – 40(40%), and >45 yrs – 41(41.0%). A total of 45(45%) patients had no chronic diseases. Sixty-one (61.0%) patients were admitted to the Internal Medicine Ward (IMW), 24(24.0%) to the Pulmonary Ward (PW), and 15(15.0%) patients were admitted to the Intensive Care Unit (ICU). The mild, moderate, and severe courses of the disease were found in 29(29%), 25(25.0%), and 46(46.0%) cases, respectively (Table 1). Summary of patients' symptoms: 58(58.0%) patients had shortness of breath, 88(88.0%) - fever, 58(58.0%) - pneumonia, 76(76.0%) - cough, 58(58.0%) - fatigue, 77(77.0%) - headache. A few patients experienced nausea and vomiting, diarrhea, loss of taste & smell, sore throat, and abdominal pain (Table 2). We observed a statistically significant difference between clinical course groups and CRP (Table 3).

We also found significant differences in CRP in patients of IMW to ICU (P=0.001). At the same time, there were no statistically significant differences between the PW and IMW patients (P=0.950) (Table 4). There were significant differences in CRP in mild to moderate cases (P=0.012), and in mild to severe cases (P=0.000); but there were no statistically significant differences between the moderate and severe groups of patients cases (P=0.283) (Table 5). We found statistically significant differences between ferritin levels and disease severity (P=0.005), age category (P=0.030), and the clinical wards (P=0.016). There were no associations between comorbidities and ferritin levels (Table 6). There were significant differences in ferritin levels in patients of PW and ICU (P=0.012) but no statistically significant differences between IMW and ICU (P=0.165) patients (Table 7). Statistically significant differences were found between the ferritin levels in mild to moderate cases (P=0.023) and mild to severe cases (P=0.007). However, there were no statistically significant differences between the moderate and severe cases (P=1.000) (Table 8). There were associations between ferritin levels and age subgroups (Table 9). We found weak positive correlations between ferritin and CRP levels (r=0.273, P=0.006), ferritin levels and age (r=0.239, P=0.017), and CRP levels and age (r=0.246, P=0.014) (Table 10). The ferritin and CRP levels did not differ between men and women (Tables 11 and 12).

Table 1.
Baseline characteristics of the patients included in the study

Variables	Group	Frequency	Percentage
Gender	Males	77	77.0 %
	Females	23	23.0 %
Age Category	26 – 35	19	19.0 %
	36 – 45	40	40.0 %
	> 45	41	41.0 %
Comorbidities	DM	20	20.0 %
	HTN	8	8.0 %
	DM & HTN	12	12.0 %
	Multiple comorbidity	3	3.0 %
	Other	14	14.0 %
	No Chronic Diseases	43	43.0 %
Ward	IMW	61	61.0 %
	PW	24	24.0 %
	ICU	15	15.0 %
Disease Severity	Mild	29	29.0 %
	Moderate	25	25.0 %
	Severe	46	46.0 %
Contentious Variables			
Variable	Mean±SD	Minimum	Maximum
Age	44.39±11.892	26	84
Length of Stay,day	10.310±8.3566	2.0	70.0
CRP, mg/L	82.343±76.5985	5.0	518.8
Ferritin, ng/mL	561.096±516.8643	8.8	2616.0

Table 2.
Summary of Patients' Symptoms

Variables	Group	Frequency	Percentage
Abdominal Pain	No	98	98.0 %
	Yes	2	2.0 %
Sore throat	No	97	97.0 %
	Yes	3	3.0 %
Loss of taste & Smell	No	91	91.0 %
	Yes	9	9.0 %
Nausea	No	94	94.0 %
	Yes	6	6.0 %
Vomiting	No	97	97.0 %
	Yes	3	3.0 %
Diarrhea	No	94	94.0 %
	Yes	6	6.0 %
Headache	No	77	77.0 %
	Yes	23	23.0 %
Fatigue	No	42	42.0 %
	Yes	58	58.0 %
Cough	No	24	24.0 %
	Yes	76	76.0 %
Pneumonia	No	42	42.0 %
	Yes	58	58.0 %
Fever	No	12	12.0 %
	Yes	88	88.0 %
SOB	No	42	42.0 %
	Yes	58	58.0 %

Table 3.

Analysis of variance (ANOVA) of CRP with Severity, Age Category, Comorbidities, and Wards

Variables		Sum of Squares	Df	Mean Square	F	Sig.
Severity	Between Groups	118260.683	2	59130.342	12.399	0.000
	Within Groups	462604.442	97	4769.118		
	Total	580865.125	99			
Age Category	Between Groups	30898.900	2	15449.450	2.725	0.071
	Within Groups	549966.225	97	5669.755		
	Total	580865.125	99			
Co-morbidities	Between Groups	56303.745	5	11260.749	2.018	0.083
	Within Groups	524561.380	94	5580.440		
	Total	580865.125	99			
Ward	Between Groups	79802.979	2	39901.490	7.724	0.001
	Within Groups	501062.146	97	5165.589		

Table 4.

Multiple comparisons (Post-Hoc) of CRP with Wards

Dependent Variable: CRP						
Tukey HSD						
(I) Ward	(J) Ward	Mean Difference (I-J)	Std. Error	Sig	95% CI	
					Lower Bound	Upper Bound
IMW	PW	-5.2903	17.3180	0.950	-46.511	35.930
	ICU	-80.3686	20.7136	0.001	-129.672	-31.066
PW	IMW	5.2903	17.3180	0.950	-35.930	46.511
	ICU	-75.0783	23.6560	0.006	-131.385	-18.772
ICU	IMW	80.3686	20.7136	0.001	31.066	129.672
	PW	75.0783	23.6560	0.006	18.772	131.385

Table 5.

Multiple comparisons (Post-Hoc) of CRP with Disease Severity

Dependent Variable: CRP						
Tukey HSD						
(I) Severity	(J) Severity	Mean Difference (I-J)	Std. Error	Sig	95% CI	
					Lower Bound	Upper Bound
Mild	Moderate	-55.1719	18.8472	0.012	-100.032	-10.311
	Severe	-81.3606	16.3746	0.000	-120.336	-42.385
Moderate	Mild	55.1719	18.8472	0.012	10.311	100.032
	Severe	-26.1888	17.1593	0.283	-67.032	14.654
Severe	Mild	81.3606	16.3746	0.000	42.385	120.336
	Moderate	26.1888	17.1593	0.283	-14.654	67.032

Table 6.

Analysis of variance (ANOVA) of Ferritin with Severity, Age Category, Comorbidities, and Wards

Variables		Sum of Squares	df	Mean Square	F	Sig.
Severity	Between Groups	2702780.636	2	1351390.318	5.521	0.005
	Within Groups	23744937.223	97	244793.167		
	Total	26447717.858	99			
Age Category	Between Groups	1838445.278	2	919222.639	3.623	0.030
	Within Groups	24609272.581	97	253703.841		
	Total	26447717.858	99			
Co-morbidities	Between Groups	2419822.403	5	483964.481	1.893	0.103
	Within Groups	24027895.455	94	255615.909		
	Total	26447717.858	99			
Ward	Between Groups	2152206.232	2	1076103.116	4.296	0.016
	Within Groups	24295511.626	97	250469.192		
	Total	26447717.858	99			

Table 7.

Multiple comparisons (Post-Hoc) of Ferritin with Wards

Dependent Variable: Ferritin						
Tukey HSD						
(I) Ward	(J) Ward	Mean Difference (I-J)	Std. Error	Sig.	95% CI	
					Lower Bound	Upper Bound
IMW	PW	216.1854	120.5913	0.177	-70.849	503.219
	ICU	-264.0230	144.2359	0.165	-607.336	79.290
PW	IMW	-216.1854	120.5913	0.177	-503.219	70.849
	ICU	-480.2083	164.7245	0.012	-872.289	-88.128
ICU	IMW	264.0230	144.2359	0.165	-79.290	607.336
	PW	480.2083	164.7245	0.012	88.128	872.289

Table 8.

Multiple comparisons (Post-Hoc) of Ferritin with disease severity

Dependent Variable: Ferritin						
Tukey HSD						
(I) Severity	(J) Severity	Mean Difference (I-J)	Std. Error	Sig.	95% CI	
					Lower Bound	Upper Bound
Mild	Moderate	-363.0014	135.0291	0.023	-684.401	-41.602
	Severe	-361.9283	117.3147	0.007	-641.163	-82.693
Moderate	Mild	363.0014	135.0291	0.023	41.602	684.401
	Severe	1.0730	122.9362	1.000	-291.542	293.688
Severe	Mild	361.9283	117.3147	0.007	82.693	641.163
	Moderate	-1.0730	122.9362	1.000	-293.688	291.542

Table 9.

Multiple comparisons (Post-Hoc) of Ferritin levels with age category

Dependent Variable: Ferritin						
Tukey HSD						
(I) Age	(J) Age	Mean Difference (I-J)	Std. Error	Sig.	95% CI	
					Lower Bound	Upper Bound
26 - 35	36 - 45	-232.7158	140.3404	0.227	-566.757	101.325
	> 45	-374.9402	139.7882	0.023	-707.667	-42.213
36 - 45	26 - 35	232.7158	140.3404	0.227	-101.325	566.757
	> 45	-142.2244	111.9397	0.415	-408.666	124.217
> 45	26 - 35	374.9402	139.7882	0.023	42.213	707.667
	36 - 45	142.2244	111.9397	0.415	-124.217	408.666

Table 10.
Relationship between CRP levels, Ferritin levels, and age

		Age in years	CRP	Ferritin
Age in years	Pearson Correlation		0.246	0.239
	Sig. (2-tailed)		0.014	0.017
	N		100	100
CRP	Pearson Correlation	0.246		0.273
	Sig. (2-tailed)	0.014		0.006
	N	100	100	100
Ferritin	Pearson Correlation	0.239	0.273	
	Sig. (2-tailed)	0.017	0.006	
	N	100	100	

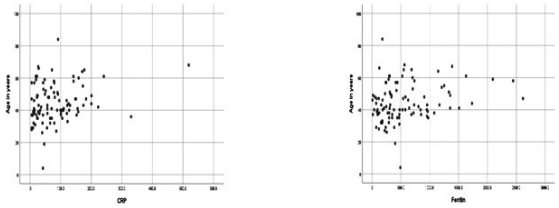

Table 11.
Group Statistics of Gender with CRP and Ferritin

Group Statistics					
	Gender	N	Mean	Std. Deviation	Std. Error
Ferritin	Male	77	622.565	537.1310	61.2118
	Female	23	355.309	384.7094	80.2175
CRP	Male	77	84.634	64.5451	7.3556
	Female	23	74.674	109.2301	22.7761

Table 12.
Comparison between Gender with CRP and Ferritin

Variable	Group	N	Mean±SD	Mean Difference	95% CI		Sig.
					Lower	Upper	
Ferritin	Male	100	622.565 ± 537.1310	267.2562	28.2147	506.2978	0.226
	Female	100	355.309 ± 384.7094				
CRP	Male	100	84.634 ± 64.5451	9.9599	18.2666	-26.2896	0.224
	Female	100	74.674 ± 109.2301				

Discussion

The results obtained showed that CRP and ferritin levels are considerably greater in severe cases than in mild and moderate cases of COVID-19. A high ferritin level was associated with admission to ICU. The current findings are consistent with data obtained by Cheng et al.[5] The close positive association between CRP values and severity of tissue damage in many different pathologies, notably including COVID-19, was illustrated by Smilowitz et al.[11] CRP bound to tissues damaged by the virus and/or host response activates a complement locally, thereby exacerbating damage and promoting systemic complement activation. A novel small-molecule drug that inhibits CRP binding in vivo is currently being developed to test whether this CRP-complement mechanism significantly contributes to the severity of COVID-19 and other diseases.[12] The findings of the current study indicate that serum ferritin levels might be considered as an essential indication of the progression and severity of COVID-19.[13] Henry et al.[14] found that ferritin levels were high at admission to the hospital and during the hospital stay in patients who died from COVID-19. In a study by Liu et al.,[9] an analysis of the peripheral blood of 69 patients with severe COVID-19 revealed elevated ferritin levels, compared with patients with a non-severe course of the disease. In addition, a positive correlation between ferritin and IL-6 was noted. A retrospective study of over 900 patients admitted with COVID-19 showed that higher ferritin levels were associated with all-cause mortality.[8] Thus, multiple publications are showing that higher ferritin levels, along with other pro-inflammatory markers, including CRP and IL-6, are correlated with worse outcomes and may even help predict these outcomes of COVID-19.[15-21] Zhou et al.[7] found that a combination test of hepcidin and serum ferritin provided the best specificity and sensitivity in the prognosis of COVID-19 severity. Hepcidin and serum ferritin tandem testing predicted COVID-19 severity with 94.6% specificity. It is quite obvious that iron homeostasis had a robust association with the occurrence of severe COVID-19.

Conclusion

The findings of the current study indicate that CRP and serum ferritin levels might be considered as an essential indication of the progression and severity of COVID-19.

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Competing Interests

The authors declare that they have no competing interests.

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COVID-19

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Oxidative Stress Intensity in Children and Adolescents with a New Coronavirus Infection

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Abstract

The aim of our research was to assess the intensity of oxidative stress (OS) in children and adolescents with COVID-19 using the oxidative stress index (OSI).

***Methods and Results*:** The study was conducted between May 2020 and March 2021. The main group included 17 children and adolescents [8(47.1%) boys and 9(52.9%) girls; mean age of 12.35±4.01 years] with diagnosed COVID-19 infection (mild to moderate course) selected as a result of the primary diagnostic examination from among those admitted to hospitalization at the Irkutsk Regional Infectious Diseases Clinical Hospital. The control group included 17 healthy children and adolescents (average age of 12.35±4.01 years) matched by copy-pair type. The obtained data indicated statistically significant differences in a number of parameters between children and adolescents with COVID-19 and the control groups. We found statistically significant higher levels of lipid peroxidation (LPO) products (CDs, $P<0.0001$; KD and CT, $P=0.006$; and TBARs, $P=0.013$) in the study group than in the control group. Among antioxidant defense (AOD) system parameters, the levels of retinol ($P=0.015$) and reduced glutathione ($P=0.048$) and SOD activity ($P<0.0001$) were statistically lower in the study group than in the control group. The OSI level was significantly greater (by 8.5 times, $P=0.028$) in the study group than in the control group, which confirms the development of antioxidant deficiency in COVID-19.

Conclusion: The results of the assessment of OSI in children and adolescents with COVID-19 indicate insufficient activity of some critical components of AOD and a shift of the redox balance toward pro-oxidant factors, which can have extremely negative consequences in the development of the disease. In this regard, we recommend carrying out corrective measures to stabilize LPO/AOD parameters by including drugs with antioxidant properties in the treatment complex.(**International Journal of Biomedicine. 2022;12(2):242-246.**)

Key Words: COVID-19 • oxidative stress index • children • adolescents • polymerase chain reaction

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Abbreviations

AOD, antioxidant defense; **ACE2**, angiotensin-converting enzyme 2; **CDs,** conjugated dienes; **COVID-19**, coronavirus disease 2019; **GSH**, reduced glutathione; **GSSG**, oxidized glutathione; **KD and CT,** ketodienes and conjugated trienes; **LPO,** lipid peroxidation; **OS,** oxidative stress; **OSI,** oxidative stress index; **ROS**, reactive oxygen species; **SARS-CoV-2**, severe acute respiratory syndrome-related coronavirus 2; **SOD,** superoxide dismutase; **TAA**, total antioxidant activity; **TBARs**, thiobarbituric acid reactants.

Introduction

The pandemic of coronavirus infection (COVID-19) is the most devastating disease since the turn of the 21th century, but unfortunately, many of its clinical manifestations, pathogenetic characteristics, and treatments are still unclear. The number of cases of infection worldwide has exceeded 500 million to date, and the rates are still increasing.[1] An important distinguishing

feature of COVID-19 is the different severity of the course depending on the age of the patient. Thus, if the disease is mild or moderate for children and adolescents, for the elderly the course will be more severe, with a high mortality rate, especially in the presence of chronic comorbidities such as diabetes mellitus, arterial hypertension, and cardiovascular diseases.[2]

According to worldwide studies, children and adolescents are significantly less likely to have a new coronavirus infection, usually within 1%-5% of patients with diagnosed clinical cases of COVID-19 and up to 11% of those infected with SARS-CoV-2.[3,4] Moreover, there is a direct correlation between emergency department visits and the vaccination intensity of the population in the region where the child lives. In regions with high vaccination coverage, the data tend to be lower.[5] Schoolchildren and preschool-age children, make up the bulk of infected children.[3] Children are characterized by mild clinical symptoms, with the main symptoms in the form of fever, cough, signs of intoxication, etc.[6] The majority of cases are asymptomatic, while about 10% require hospitalization.[7] There are some cases with a severe course (about 1% of children), especially in children with a burdened pre-morbid and comorbid background and severe comorbid disorders.[4] Overall, no particular changes were found in the structure of morbidity in children in different "waves" of COVID-19. However, some differences concern the so-called "third wave" (May-June 2021): an increase in the number of hospitalized children, early hospital admission from the beginning of the disease, prolongation of the virus release period, reduction of cases with development of multisystem inflammatory syndrome, which characterizes modern features of the course of COVID-19 infection in children.[3] In addition, the pediatric population should receive no less attention than the adult population because of the active participation of children in the spread of the new infection.

Many respiratory infections are accompanied by excessive generation of ROS and insufficient activity of the antioxidant defense (AOD) system.[8] The disproportion between the presence of antioxidants and free radicals or pro-oxidants in the biological system is defined as oxidative stress (OS). Studies show that OS plays an important role in the genesis of coronavirus infections, including COVID-19.[9-12] The integral coefficient evaluating the intensity of OS reactions can characterize the stage of pathological process formation in the body, including in the presence of infectious diseases.[13-16] There is virtually no information on the state of OS reactions in children with coronavirus infection. At the same time, such studies are highly relevant and can improve the effectiveness of preventive and therapeutic measures.

The aim of our research was to assess the OS intensity in children and adolescents with COVID-19 using the oxidative stress index (OSI).

Material and Methods

Design of study

The study was conducted between May 2020 and March 2021. The main group included 17 children and adolescents [8(47.1%) boys and 9(52.9%) girls; mean age of 12.35±4.01 years] with diagnosed COVID-19 infection (mild to moderate course) selected as a result of the primary diagnostic examination from among those admitted to hospitalization at the Irkutsk Regional Infectious Diseases Clinical Hospital. The control group included 17 healthy children and adolescents (average age of 12.35±4.01 years) matched by copy-pair type. Inclusion criteria were the age of subjects, informed consent to participate in the study, and presence of laboratory-confirmed SARS-CoV-2. Exclusion criteria were a severe disease, refusal to participate in the study, failure to meet inclusion criteria, and other reasons. On admission to the hospital, we performed a general clinical examination of patients, analysis of medical records, and questionnaire survey. The questionnaire included information on the presence of a positive PCR test for SARS-CoV-2 RNA (laboratory confirmation of infection), dynamic monitoring of enzyme immunoassay and PCR, the course of the disease, and epidemiological history. We collected data on complications of the underlying disease, need for respiratory support, renal replacement therapy, presence of viral or fungal co-infection, and laboratory findings. The main group of children and adolescents was homogeneous in terms of the nature and duration of the infection and the therapeutic effect.

Biochemical measurements

The OSI [the ratio of the LPO-AOD system indicators in the study group to average indicators in the control group], as integral indicator the OS intensity, was calculated using the formula that was developed and modified in our previous study.[13-16] The formula takes into account not only the accumulation of LPO products at various stages (from primary to final), but also the activity of various parts of the AOD system (enzymatic and non-enzymatic).

Plasma concentrations of primary/secondary/final products of LPO (CDs/KD-CT/ TBARs) were estimated.[17,18] The state of the AOD system was assessed by the content of α-tocopherol and retinol,[19] GSH and GSSG,[20] and the SOD activity.[21]

The measurements were carried out using a Shimadzu RF-1501 spectrofluorophotometer (Japan) and Shimadzu RF-1650 spectrofluorophotometer (Japan).

Statistical analysis was performed using STATISTICA 8.0 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 mean±standard deviation (SD), 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 $P<0.05$ was considered statistically significant.

Ethics approval of research

All participants or their parents signed an informed consent to participate in the study in accordance with the World Medical Association Declaration of Helsinki (1964, 2013 ed.). The study was approved the Biomedical Ethics Committee at the Scientific Centre for Family Health and Human Reproduction Problems, Russia (No. 6/1 dated June 19, 2020).

Results and Discussion

The obtained data indicated statistically significant differences in a number of parameters between children and adolescents with COVID-19 and the control groups. Thus, we found statistically significant higher levels of LPO products (CDs, $P<0.0001$; KD and CT, $P=0.006$; and TBARs, $P=0.013$) in the study group than in the control group. Among AOD system parameters, the levels of retinol ($P=0.015$) and reduced glutathione ($P=0.048$) and SOD activity ($P<0.0001$) were statistically lower in the study group than in the control group (Table 1).

Table 1.

Content of LPO products and AOD components in children and adolescents with COVID-19 (Me, 25%-75%).

Parameters	Control group	Main group
CDs, μmol/L	1.18 (0.84-1.82)	7.03 (5.51-7.58) *
KD and CT, units	0.22 (0.14-0.32)	0.9 (0.43-1.2) *
TBARs, μmol/L	0.86 (0.67-1.36)	1.33 (1.14-1.91) *
SOD activity, units	1.66 (1.6-1.74)	1.01 (0.94-1.14) *
α- tocopherol, μmol/L	7.71 (5.95-11.7)	7.85 (5.17-8.79)
retinol, μmol/L	1.25 (0.59-2.21)	0.85 (0.63-0.99) *
GSH, mmol/L	2.32 (2.08-2.85)	1.79 (1.67-2.45) *

* - *statistically significant differences.*

According to our data, the OSI level was significantly greater (by 8.5 times, $P=0.028$) in the study group than in the control group, which confirms the development of antioxidant deficiency in COVID-19 (Figure 1).

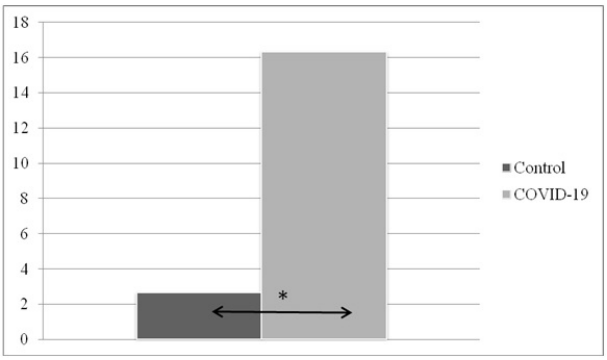

Fig. 1. *The level of OSI in children and adolescents with COVID-19 (* - statistically significant differences between the two groups).*

Currently, there are very few works concerning metabolic responses in the pediatric and adolescent population with a new coronavirus infection. Most of them are theoretical in explaining the reasons for a milder course of infection in this cohort of patients. Possible causes include a number of factors, such as differences in the ACE2 expression, immune response reactivity, the nature of the inflammatory response, melatonin secretion levels, and other metabolic parameters.[22,23] Differences in the distribution, maturation, and function of viral receptors, as well as the likely protective effect of low levels of ACE2 in children, have also been noted.[24] On the other hand, it is well known that some infectious diseases, such as paralytic polio and rubella, are milder in children than in adults.[25] There are suggestions that the more favorable course of infection in children may be due to the high intensity of metabolic reactions.[23,25]

Our analysis of the OSI values indicates the presence of a higher intensity of OS reactions in children and adolescents with COVID-19 relative to healthy children.

Several studies have shown that OS plays an important role in viral infections such as SARS-CoV and SARS-CoV-2.[26,27] Coronavirus contains an S-protein that enables virus access to target cells via the ACE2 receptor. The latter is expressed on the surface of epitheliocytes of the respiratory and digestive tracts, the upper parts of which are the entry gates of infection. Accumulation of intermediate products of viral metabolism is accompanied by the generation of ROS and mitochondrial damage.[12] The infection progresses and descends to the lower parts of the respiratory tract, affecting alveolar type I and type II cells and endotheliocytes, where the expression and subsequent secretion of pro-inflammatory cytokines occurs.[9] When this occurs, alveolar macrophages and infiltrated immune cells are activated, which increases oxygen consumption and aggravates the process of hypoxia.[28] Activated alveolar macrophages release pro-inflammatory cytokines in the alveoli, which then enter the great circle of the circulation. Intense inflammation leads to excessive production of ROS, activation by hypoxia-induced factors (HIF-1α, NF-κB), etc.[29] Experimental studies of coronavirus infections of past decades showed that the pro-inflammatory response was secondary to an impaired AOD system.[25] This conclusion was supported by similar clinical data. A number of papers have also identified low AOD activity as a key component determining the severity of viral infections.[10,12,27] Viral respiratory infections, including respiratory syncytial virus, human metapneumovirus, and influenza virus infections, were shown to suppress the expression and activity of antioxidant enzymes, resulting in reduced antioxidant capacity.[8] Although the exact role of OS in viral virulence is not clear, it may be secondary to the modulation of the immune system caused by oxidative damage.[28,29] Strong evidence for the role of OS comes from studies of ACE2 involvement. ACE2 plays an important role in determining the severity level of SARS-CoV-2 infections because it is not only a condition for SARS-CoV-2 entry into the cell but also acts as a modulator of OS and inflammation.[30]

Viral infection causes increased synthesis of ROS as a result of the lack of appropriate activity of the AOD system components to neutralize toxic metabolites. It is likely that in our study, the increased OSI values in children and adolescents with COVID-19 were associated with the insufficiency of a number of antioxidants. Thus, in particular, the insufficient activity of SOD, GSH, and retinol was noted. SOD is a key enzyme of the first line of defense against ROS, responsible for the innate antioxidant response in aerobic organisms.[31,32] It was noted

that even a slight decrease in the activity of SOD causes a shift of metabolic reactions toward the prevalence of pro-oxidant processes.[33] Glutathione (γ-1-glutamyl-1-cysteinylglycine) is a tripeptide that plays an important role in cellular detoxification through glutathione S-transferase activity, and participates in antioxidant defense, regulates the synthesis and recovery of fat- and water-soluble vitamins, supports "thiol status," and modulates cell proliferation.[26] Lower levels of glutathione are associated with immune dysfunction, which leads to higher susceptibility to viral infections, particularly SARS-CoV-2 infection.[27] Vitamin A and related retinols are involved in modifying the immune system by expressing the key antiviral antibodies.[34] Consequently, retinol deficiency in the blood of children with COVID-19 may adversely affect the course of the disease. Considering the vital role of vitamins as regulators of growth and tissue morphological differentiation, changes in this metabolic link in children and adolescents seem to be highly significant.[35-37]

Conclusion

Thus, the results of the assessment of OSI, the integral index of OS, in children and adolescents with COVID-19 indicate insufficient activity of some critical components of AOD and a shift of the redox balance toward pro-oxidant factors, which can have extremely negative consequences in the development of the disease. In this regard, we recommend carrying out corrective measures to stabilize LPO/AOD parameters by including drugs with antioxidant properties in the treatment complex.

This work was performed with the use of equipment of the collective research center "Centre for the development of progressive personalized health technologies" SC FHHRP, Irkutsk.

Competing Interests

The authors declare that they have no competing interests.

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Reproductive Health

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Reproductive Attitudes and Sexual Behavior of Women and Men Living with HIV

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Abstract

An anonymous survey was conducted, covering the issues of reproductive attitudes and contraceptive behavior in 50 women (mean age of 30.9±4.5 years) and 35 men (mean age of 31.1±3.2 years) with HIV infection of stages 4B and 4C. The study was carried out using a structured questionnaire that included questions about age, marital status, methods of contraception, reproductive plans, and reproductive and medical history. An anonymous interview with 85 HIV-infected respondents revealed that most of the women (82.0%) had contracted HIV through heterosexual contact, while in the men, the parenteral route of HIV infection predominated (57.1%) (P=0.0126). Statistically significant differences were found between the rate of single women and men: 21(42%) women versus 2(5.7%) men (P<0.001). An analysis of contraceptive behavior revealed statistically significant differences: 26% of the women and 8.6% of men (P<0.05) did not use any methods of contraception with regular or irregular sexual life. The majority of respondents used a barrier method of contraception and coitus interruptus. The vast majority of women (80%) had a desire to have children in an indefinite future, as opposed to 37.1% of the men (P<0.001). About 11.4% of men and 10% of women (P>0.05) definitely did not plan pregnancy in the future, mainly because of their unsatisfactory financial situation and the presence of a current HIV infection. Forty-two percent of the women did not undergo periodic medical examinations by a gynecologist or other specialists, and 44% of them indicated the reason for the lack of visits to the doctor as an unsatisfactory attitude of medical personnel towards them. The results obtained are important for the development of optimal medical care that alleviates the burden of HIV infection. In addition to medical care, health care providers must consider social and psychological needs to help HIV patients improve their health, including their sexual and reproductive health.(**International Journal of Biomedicine. 2022;12(2):247-250.**)

Key Words: hyperpigmentation • melasma • diagnosis • dermoscopy • treatment

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Introduction

The HIV/AIDS epidemic is the leading cause of death among women of reproductive age worldwide, from the discovery of HIV in the early 1980s to the end of 2021. HIV infection is considered to be a chronic, rather than a fatal, disease in terms of life expectancy as a result of increased access to effective antiretroviral treatment.[1] In addition to medical care, health care providers must consider social and psychological needs to help HIV patients improve their health, including sexual and reproductive health.[2,3] Studies show that the majority of HIV-infected women are sexually active after diagnosis.[4,5]

For HIV-serodiscordant couples who desire a child, there are several safe ways to achieve pregnancy safely: the antiretroviral therapy (ART) for a woman living with HIV to suppress viral load; pre-exposure prophylaxis (PrEP) for HIV-negative persons with partners living with HIV: identification and treatment of sexually transmitted infections (STIs) in both partners; behavioral strategies (timed vaginal insemination, timed intercourse without a condom around the most fertile period of the woman's menstrual cycle).[2,6-9]

All couples with childbearing problems need to be screened for infertility and treated appropriately.[3,10-12]

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Reproductive behavior, as an integral part of reproductive health, includes a system of actions and relationships that mediate the birth of, or refusal to have, a child in or out of wedlock. Thus, contraceptive behavior is a system of personality actions aimed at preventing the birth of a child, implemented through the use of contraceptive methods, artificial abortion, or abstinence.[13] Sexual behavior is a form of interaction between individuals motivated by sexual need, as well as social behavior, in which a person pursues certain goals, and seeks to realize certain interests, based on the use of biological mechanisms. Thus, sexual behavior is determined by the reproductive, hedonic, and communicative functions of the human individual.[14] The reproductive attitude is considered by researchers as a regulator of reproductive behavior and the psychological readiness of an individual to have a particular number of children under specific conditions and within certain periods of life. The reproductive attitude is often considered from the standpoint of three components: cognitive (cognitive), affective (emotional), and behavioral (motivational).[13] Accordingly, sexual and reproductive health issues need to be considered in high-quality HIV services that can improve quality of life and improve HIV prevention. [2,15]

Materials and Methods

This work was carried out at the Scientific Center for Family Health and Human Reproduction Problems as part of a clinical examination of women and men with HIV infection of stages 4B and 4C at the Irkutsk Regional Infectious Clinical Hospital. . The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013). The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each participant.

Inclusion criteria were reproductive age (18–45 years), HIV infection stages 4B-4C, diagnosed on the basis of epidemiological, clinical data and confirmed by detection of specific antibodies by ELISA and immune blotting to HIV type 1 proteins.

An anonymous survey was conducted, covering the issues of reproductive attitudes and contraceptive behavior in 50 women (mean age of 30.9±4.5 years) and 35 men (mean age of 31.1±3.2 years). The study was carried out using a structured questionnaire that included questions about age, marital status, methods of contraception, reproductive plans, and reproductive and medical history. The questions were supplemented and combined into sections: personal data (age, nationality, education, social status, place of residence), complaints at the time of treatment, concomitant gynecological diseases, past somatic diseases, and infectious anamnesis, as well as socio-biological and family-domestic status.

The gynecological history included the following information: age of menarche, features of the formation of menstrual function (regularity, presence of pain, and blood volume loss), and rhythm of menstruation. Reproductive function analysis included the age of onset of sexual activity, the outcome of the first pregnancy, the number of births, abortions, miscarriages, and missed and ectopic pregnancies. The presence of complications in the postpartum and post-abortion periods was analyzed. In the absence of pregnancies during the first year with regular sexual life without contraception, the duration of infertility, the fertility of the sexual partner, the results of previous examinations, and treatment of sexual partners were determined..

Statistical analysis was performed using STATISTICA 6.1 software package (Stat-Soft Inc., USA). The frequencies of categorical variables were compared using Pearson's chi-squared test or Fisher's exact test (2-tail), when appropriate. A value of $P<0.05$ was considered significant.

Results

An anonymous interview with 85 HIV-infected respondents revealed that most of the women (82.0%) had contracted HIV through heterosexual contact, while in the men, the parenteral route of HIV infection predominated (57.1%) ($P=0.0126$), which coincided with the all-Russian statistical data.(16) Sexual contacts between men were reported in 2.5% of the respondents. History of parenteral drug use was reported by 18% of the women, and 5% reported that they had taken intravenous drugs in the last 6 months.

Eighteen percent of the women and 5.7% of the men did not drink alcohol ($P>0.05$); 14% of the women and 77.1% of the men ($P<0.0001$) used alcohol regularly and more often than 1-2 times a week; 68% of the women and 85.7% of the men ($P>0.05$) smoked, and 18% and 37.1% of them, respectively, smoked more than 10 cigarettes per day; 36% of the women and 48.6% of the men ($P>0.05$) reported regular drug use.

No significant differences were found when comparing the level of education: 70% of the women and 71.4% of the men had secondary or secondary specialized education, and 17% of the women and 14.3% of the men had higher education. The most common professions among both the women and men were employment in a private enterprise. The unemployed were 30% of the women and 17.1% of the men. According to all-Russian studies, in the general population of Russians aged 15 to 64, 88.7% had secondary education, 27.3% had incomplete higher education, 17% had higher education, and the level of education in women was higher than in men (21% and 13%, respectively; $P<0.05$).[16]

The marital status of the interviewed women and men was as follows: single (women 42% and men 5.7%), divorced (18% and 5.7%), unregistered marriage (28% and 48.6%), and registered marriage (12% and 42%). Statistically significant differences were found between the rate of single women and men: 21(42%) women versus 2(5.7%) men ($P<0.001$) (Table 1).

Among 50 women and 35 men, regular sex (≥4 times per month) was reported by 40% of the women and 60% of the men ($P>0.05$). The main reasons for irregular sex were "lack of a permanent sexual partner" and "fear of revealing one's HIV-positive status." About 41% of respondents did not know or did not indicate the HIV status of their regular sexual partner. Of the respondents who knew the HIV status of their

partner, 48.6% of the men and 38% of the women had an HIV-negative sexual partner at the time of the study.

The obstetric history of HIV-infected women was as follows: childbirth, miscarriage, medical abortion, and ectopic pregnancy. It was found that at the time of the study, 11(56%) women had infertility (lack of pregnancy for 1 year with regular sexual activity without contraception), while 8(20%) of them noted complications after a medical abortion.

An analysis of contraceptive behavior revealed statistically significant differences: 26% of the women did not use any methods of contraception with regular or irregular sexual life, citing the fact that "pregnancy still does not occur"; and in 8.6% of the men, their partners also did not use contraceptive methods (P<0.05). The majority of respondents used a barrier method of contraception. Hormonal contraceptives (HC) were used by 8% of the women and by 5.7% of the partners of the men surveyed. Intrauterine contraceptives (IUC) were used by 8% of the women and 5.7% of the female partners of the men surveyed. Coitus interruptus was used by 10% of the women and 17.1% of the men (Table 1).

Table 1.

Socio-demographic portrait of a patient living with HIV

Socio-demographic indicator	Men n=35	Women n=50
Family status		
Registered marriage	14 (40%)	6 (12%)
Unregistered marriage	17 (48.6%)	14 (28%)
Divorced	2 (5.7%)	9 (18%)
Single **	2 (5.7%)	21 (42%)
Contraceptive method		
None *	3 (8.6%)	13 (26%)
HC	2 (5.7%)	4 (8%)
IUC	2 (5.7%)	4 (8%)
Barrier contraception	22 (63%)	24 (48%)
Coitus interruptus	6 (17.1%)	5 (10%)
Pregnancy planning		
In the coming year	5 (14.3%)	2 (4%)
In future**	13 (37.1%)	40 (80%)
No, because there is a child**	13 (37.1%)	3 (6%)
Definitely not	4 (11.4%)	5 (10%)

**- P<0.05; **- P<0.001*

Forty-two percent of the women did not undergo periodic medical examinations by a gynecologist or other specialists, and 44% of them indicated the reason for the lack of visits to the doctor as an unsatisfactory attitude of medical personnel towards them.

The questions regarding the intention to give birth showed that the vast majority of women (80%) had a desire to have children in an indefinite future, as opposed to 37.1% of the men (P<0.001) The reasons for not wanting to have children were as follows: in 6% of the women and 37.1% of the men (P<0.001), "the presence of one child in the family"; 11.4% of men and 10% of women (P>0.05) definitely did not plan pregnancy in the future (Table 1), mainly because of their unsatisfactory financial situation and the presence of a current HIV infection.

Conclusion

Despite the fact that HIV is a barrier to pregnancy and childbirth, motherhood has most often been perceived as something positive. About 80% of the women expressed a strong desire to be pregnant and give birth, to feel like a "real woman," to feel whole and complete. Motherhood and raising a child would mean a new chance in the lives of the women interviewed–the beginning of something new. The pregnancy itself and subsequent motherhood were even explained as salvation. Being a mother and meeting the needs of a child was associated with care and unconditional love, as well as hope for the women interviewed.

The need for social support was very strong among HIV-infected couples. Women stated that they needed professional support from health professionals for specific and objective information about family planning, medical risks, and preventive interventions during pregnancy. The need for practical information on how to take action to eliminate the transmission of HIV to a child was very important.

The results obtained are important for the development of optimal medical care that alleviates the burden of HIV infection. In addition to medical care, health care providers must consider social and psychological needs to help HIV patients improve their health, including their sexual and reproductive health.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Occupational Health

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The Influence of Sustained Mercury Exposure on Prothrombin Time and Partial Thromboplastin Time among Sudanese Gold Mining Workers

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Abstract

Background: Gold mining is the world's leading source of anthropogenic mercury pollution, negatively impacting not only miners but also the surrounding inhabitants; it has many effects on human health, especially cardiovascular problems, which lead to coagulation disorders and an increase in morbidity and mortality rate. The present study aimed to determine the prothrombin time (PT) and partial thromboplastin time (PTT) among Sudanese gold mining workers exposed to mercury.

Methods and Results: This cross-sectional study was carried out among mining workers in the Red Sea state. A total of 50 mining workers were enrolled in the case group, and 50 non-mining apparently healthy subjects were the control group. About 5 ml of whole blood samples were collected in 3.2% sodium citrate blood collection tubes. Platelet poor plasma (PPP) for prothrombin time (PT) and partial thromboplastin time (PTT) measurements was obtained by using high-speed centrifugation. PT and PTT tests were performed using a standard method (Practical-Haemostasis.com.) with a Helena C2 coagulometer (Germany) and reagents manufactured by the Bio-med trademark (China). The mean age of miners was 33.5±11.5 years and occupation time - 1.94±2.1 years. The mean value of PTT was greater in the case group than in the control group (42.43±6.18 sec vs. 37.76±5.33 sec, *P*=0.000). In the age subgroup <40 years, the PT level was longer than in the age subgroup >40 years: 14.04±1.38 sec vs. 13.15±1.35 sec (*P*=0.045), respectively. The correlation analysis revealed a significant, direct correlation between PTT and occupation time (r=0.357, *P*=0.011).

Conclusion: Prolonged coagulation time, notably PTT, has been revealed among mining workers, implying that these workers may have a clinically silent state of coagulation abnormalities.(**International Journal of Biomedicine. 2022;12(2):251-255.**)

Key Words: Mercury • mining workers • prothrombin time • partial thromboplastin time • platelet poor plasma

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Abbreviations

INR, international normalized ratio; **MV,** microvesicle; **PT,** prothrombin time; **PTT**, partial thromboplastin time; **PPP**, platelet poor plasma; **PS**, phosphatidylserine.

Introduction

Mercury is a heavy metal of known toxicity, noted for inducing public health disasters,[1,2] and human exposure to mercury is still a major public health concern; exposure to it is the second most common cause of toxic metal poisoning. The clinical impact of smaller mercury exposures remains controversial.[3,4]

Sudan's gold mining activities are largely conducted in the so-called "informal" economy, during which participants operate unapproved or without legal authorization.[5] Thus, ensuring efficient regulation of mercury emissions is incredibly challenging. These miners significantly contribute to the regional and global sectors of the economy, producing 15% to 25% of the world's gold,[6] so even though each individual mining activity may be limited, the procedure is widespread. The burden on human health is incredible as a result of the remarkable quantities of mercury handled directly by miners and discharged into the atmosphere.[7]

Human toxicity varies with the form of mercury, the dose, and the rate of exposure. The target organ affected by inhaled mercury vapor is the brain, while mercurous and mercuric salts chiefly damage the gut lining and kidneys, and methyl mercury is widely distributed throughout the body. Toxicity varies with dosage: large acute exposures to elemental mercury vapor induce severe pneumonitis, which in extreme cases can be fatal.[8,9] Mercury vapor (an extremely toxic form to humans) inhaled by miners results in impaired cognitive function, neurological damage, kidney damage, and several other health problems. In some cases, amalgams are processed near the home or in gold shops in villages or cities, so the mercury vapor generated in the process affects non-miners living in these areas.[10]

Numerous toxic effects of mercury have been demonstrated in vitro and in animal and human studies. Mercury has a high affinity for sulfhydryl groups, various enzymes and amino acids, N-acetyl cysteine, alpha lipoic acid, and glutathione, which provide about 10% to 50% of the plasma protein antioxidant capacity[11,12] and protect against oxidative stress and inflammation. Mercury increases free-radical production and inactivates antioxidant defenses,[13-15] increases lipid peroxidation[16,17], endothelial dysfunction,[18] and platelet aggregation, production of Factor VIII, platelet factor 4,[19,20] and thrombin.[17,21] All of these abnormalities have the potential to increase the risk for CVD.[15,20,22-26]

Mercury's well-documented hemolytic and anemia-inducing effects suggest that the erythrocyte may be an appropriate priority of mercury. Minimal Hg2+ exposure has been shown to induce phosphatidylserine (PS) translocation to the erythrocyte cell membrane via modulation of a clotrimazole-sensitive potassium ion (K+) channel.[27] Nevertheless, no mention was made of the role of PS-externalized erythrocytes in procoagulant stimulation and subsequent cardiovascular diseases. Another study found that changes to the erythrocyte membrane, such as PS exposure and PS-bearing microvesicle (MV) formation, could make erythrocytes procoagulant, allowing erythrocytes to actively participate in thrombosis MVs derived from deformed erythrocytes via vehiculation, and could also contribute to acceleration of the coagulation cascade and via strong procoagulant activity by representing as a rich source of PS.[28,29] The present study aimed to determine the PT and PTT among Sudanese gold mining workers exposed to mercury.

Materials and Methods

This cross-sectional study was carried out among mining workers in the Red Sea state (Mooch and Arbaate). All adult male mining workers were involved in the study. Exclusion criteria: diseases that affect blood coagulation, and alcohol intake.

A total of 50 mining workers were enrolled in the case group, and 50 non-mining apparently healthy subjects were the control group. About 5 ml of whole blood samples were collected in 3.2% sodium citrate blood collection tubes. Platelet poor plasma (PPP) for prothrombin time (PT) and partial thromboplastin time (PTT) measurements was obtained by using high-speed centrifugation. PT and PTT tests were performed using a standard method **(**Practical-Haemostasis.com.) with a Helena C2 coagulometer (Germany) and reagents manufactured by the Bio-med trademark (China). Laboratory work was conducted at Al Shifa Medical Center (Sudan).

Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Baseline characteristics were summarized as frequencies and percentages for categorical variables. For descriptive analysis, results are presented as mean ± standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. 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.

The study was approved by the Ethics Committee of the department of the Faculty of Medical Laboratory Sciences, Alzaeim Alazhari University (Khartoum, Sudan). Written informed consent was obtained from each research participant.

Results

All 50 mining workers (Case group) were divided into 2 age subgroups: >40 years - 14(28%) participants and ≤40 years - 36(72%) participants. Regarding the duration of mining work, the majority of miners (86%) have less than 5 years of work experience. The mean age of miners was 33.5±11.5 years and occupation time - 1.94±2.1 years (Tables 1 and 2).

Table 1.

Demographic data of study participants

	Case group n (%)	Control group n (%)
Age subgroup		
≤ 40 years old	36 (72.0)	29 (58.0)
>40 years old	14 (28.0)	21 (42.0)
Work experience		
≤ 5 years	43 (86.0)	-
> 5 years	7 (14.0)	-

Parameters of PT, PTT, and INR of subjects in both groups are presented in Table 3. The mean value of PTT was greater in the case group than in the control group (42.43±6.18 sec vs. 37.76±5.33 sec, $P=0.000$). The mean level of PT in the 2 groups

did not differ significantly (*P*=0.078). In the age subgroup <40 years, the PT level was longer than in the age subgroup >40 years: 14.04±1.38sec vs. 13.15±1.35sec (*P*=0.045), respectively. The levels of INR and PTT did not differ between the age subgroups (Table 4).

Table 2.

Age and work experience of mining workers

Variable	Minimum	Maximum	Mean ±SD
Age, yrs	16.0	61.0	33.5±11.5
Work experience, yrs	0.05	7.52	1.94±2.09

Table 3.

Mean levels of the study parameters in the case and control groups

Parameters	Case group	Control group	*P*-value
PT, sec	13.79±1.41	13.38±0.82	0.078
PTT, sec	42.43±6.18	37.76±5.33	0.000
INR	1.13±0.24	1.07±0.06	0.090

Table 4.

Mean levels of the study parameters in the age subgroups of mining workers

Parameter	≤40 years n=36	>40 years n=14	*P*-value
PT, sec	14.04±1.38	13.15±1.35	0.045
PTT, sec	42.80±5.98	41.48±6.81	0.503
INR	1.12±0.21	1.14±0.31	0.794

The correlation analysis (Figure 1-3) revealed a significant, direct correlation between PTT and occupation time (r=0.357, *P*=0.011).

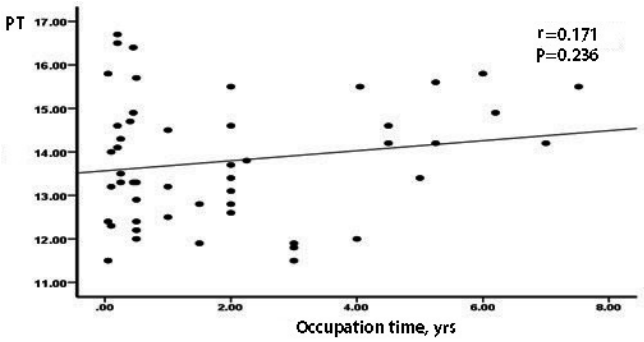

Fig. 1. *Correlation between PT and occupation time*

Discussion

Coagulation studies are one of the most important tools in investigating and monitoring the toxicity of chemicals and their effects on human health. The impact of mercury on human health ranges from moderate to severe, depending on many factors. Exposure to dust from mining can lead to many pathological effects, depending on mineralogical composition, size, shape and levels, and duration of exposure.

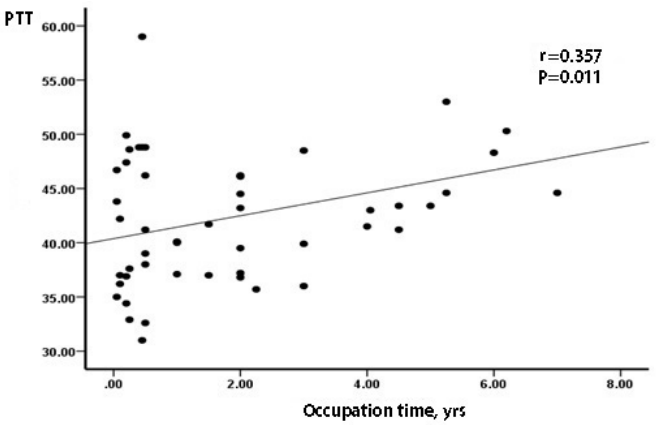

Fig. 2. *Correlation between PTT and occupation time.*

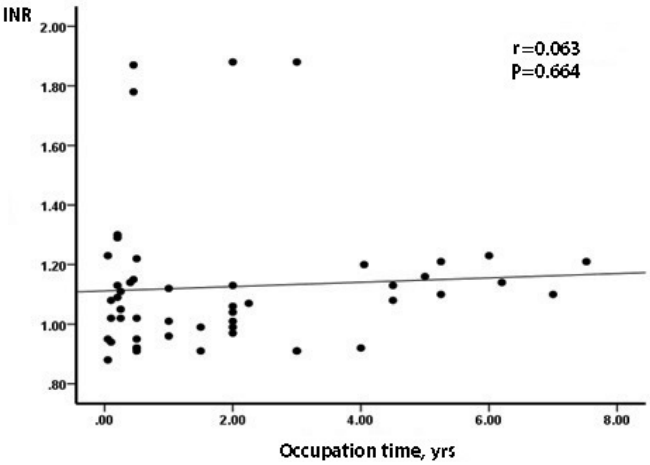

Fig. 3. *Correlation between INR and occupation time.*

Little is known about the current health status of miners, in part because no health surveillance systems exist for this population, but some studies have been developed with the concern about heavy metal exposure and related complications. Hence, the current study aimed to determine PT and PTT among Sudanese mine workers exposed to mercury. Our study showed that, there was a significant increase in PTT (42.43±6.18, *P*<0.000) when compared with healthy individuals (37.76±5.33 sec). Dhanapriya et al.[30] described a case of a patient with unknown substance poisoning who developed acute kidney injury and disseminated intravascular coagulation. The patient's coagulation profile showed PT of 18 sec, INR of 1.6, and APTT of 60 sec. Renal biopsy showed acute tubular necrosis. Later, the consumed substance was proven to be mercuric chloride. Bai et al.[31] described a case of acute severe mercuric chloride in a 38-year-old woman who orally took about 50g of $HgCl_2$ powder. The coagulation tests displayed that PT was 26.7 sec, APTT - 45.7 sec, INR - 2.36, and fibrinogen - 0.63 g/L. The patient was diagnosed with acute oral $HgCl_2$ poisoning, multiple organ dysfunction syndrome (MODS), and digestive tract hemorrhage. At the

same time, Lim et al.[32] demonstrated that mercury could provoke procoagulant activity in erythrocytes through protein-thiol depletion-mediated PS exposure and MV generation, ultimately leading to enhanced thrombosis.

The interaction of mercury ions with hemoglobin or plasma proteins might result in mercury diffusion, which could be distributed in various tissues and organs, such as the liver, large intestine, and small intestine, particularly the kidney, via blood circulation. As a consequence, patients with acute mercury poisoning commonly have multiple organ function failures. Furthermore, the combination of mercury ion and sulfhydryl enzyme in the body may cause degeneration and necrosis of the renal proximal convoluted tubule, tubule blockage, and renal parenchymal lesion, likely to result in oliguric acute renal failure. Routine follow-up using a coagulation profile, especially PTT, must be applied. Furthermore, mercury ions may inhibit the activity of cytochrome oxidase and pyruvate kinase, as well as affect functional groups, negatively impacting cell biological activities and standard metabolism and ultimately leading to cell degeneration and necrosis.

Limitation of the Study and Prospective

A deep investigation of the intrinsic pathway of coagulation factors must be done (VIII, IX, XI, XII). Routine medical assessments for mining workers in order to minimize the risks they potentially face through their duties should be applied.

Conclusion

Prolonged coagulation time, notably PTT, has been revealed among mining workers, implying that these workers may have a clinically silent state of coagulation abnormalities.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Burnout and Stress among Healthcare Workers at Primary Healthcare Centers: The Role of COVID-19 Pandemic

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Abstract

Background: Healthcare workers (HCWs) faced numerous job-related hazards during the COVID-19 pandemic outbreak, such as job-related stress and burnout, which are considered to be the paramount burdens. The aim of the present study was to assess the extent of burnout and stress among HCWs during COVID-19 in primary healthcare centers.

***Methodsd and Results*:** This cross-sectional study was conducted at five primary healthcare centers in Port Said governorate (Egypt). The study sample consisted of 250 HCWs (physicians, nurses, pharmacists, paramedical personnel, and administrative staff). Measurement tools included Maslach Burnout Inventory (MDI) and Perceived Stress Scale (PSS). Regarding degree of burnout, the current study results concluded that, two-thirds of HCWs had high occupational exhaustion, around three-quarters had high depersonalization, and the majority of them had low personal accomplishment assessment. Regarding stress levels, almost half of HCWs had high stress, and more than one-third had moderate stress. There was a statistically significant, positive correlation between degrees of occupational exhaustion, depersonalization, and stress level.

***Conclusion*:** This research can inform healthcare leaders and enable them to improve HCWs' experience by addressing levels of stress and burnout; managers must support staff who provide care and service to decrease the harmful effects of the COVID-19 pandemic.(**International Journal of Biomedicine. 2022;12(2):256-264.**)

Key Words: healthcare workers • burnout • stress • COVID-19

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Abbreviations

DP, depersonalization; **EE**, emotional exhaustion; **HCWs,** healthcare workers; **PA**, personal accomplishment

Introduction

The World Health Organization started announcing the COVID-19 outbreak on March 11, 2020.(1) This new coronavirus produced a global health crisis in that year, with over 100 million people infected and 2 million people dying worldwide.(2) This virus creates significant challenges, causing higher rates of mental health problems among healthcare workers (HCWs). These consequences have been illustrated in studies from China and Italy, and warnings of long-term consequences have been issued.(3,4)

HCWs faced numerous job-related hazards during the COVID-19 pandemic outbreak, such as job-related stress and burnout, which are considered to be the paramount burdens. Burnout among healthcare providers was recognized at the beginning of the 1970s.(1) It is regarded as a significant occupational health hazard among HCWs and has a great negative influence on patients, healthcare providers, and health institutions.(5) High time pressure, workload, and a lack of

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organizational support have all been linked to burnout.[6]

Burn-out is defined in ICD-11 as follows:[7]

Burn-out is a syndrome conceptualized as resulting from chronic workplace stress that has not been successfully managed. It is characterized by three dimensions:

-feelings of energy depletion or exhaustion;

-increased mental distance from one's job, or feelings of negativism or cynicism related to one's job; and

-reduced professional efficacy.

Burnout syndrome is a serious condition caused by excessive work, resulting in bodily or psychological sickness.[8] Organizational burnout manifests itself in decreased quality of healthcare performance, feelings of negativism about one's job, increased personnel turnover, and high service costs. Occupational burnout is considered an important risk factor for reduced quality of life and diminished health of HCWs, especially as the epidemic spreads.[9]

All healthcare providers make massive efforts at their highly critical and stressful workplaces each day. Moreover, HCWs are more susceptible to sickness than the general public. Even in the absence of significant stressful situations, such as epidemics or conflicts, healthcare personnel are subjected to increased stress due to the nature of their employment.[10] According to the literature, healthcare personnel who manage COVID-19 patients have greater psychological concerns or worries than the general public; they are likely to become infected and spread the virus to their families and surroundings.[11,12]

Stress is an inherent aspect of life. Workplace stress is defined as emotional, perceptual, behavioral, and physiological responses to negativism at work.[13] It is hypothesized that working in such a high-stress, high-vigilant condition for an extended period of time will have severe psychological consequences for HCWs. Also, stress can decrease the employees' productivity and limit their creativity and innovation during working hours.[14]

Significance of study

The COVID-19 pandemic has presented additional challenges worldwide; the consequences of those challenges for HCWs are working in a highly infectious environment.[15] Also contributing to burnout are fears of becoming sick or infecting a relative, lack of proper personal protective equipment, lack of access to up-to-date information, communication, limited time with family and friends, and increasing demands from childcare and domestic tasks.[16] Healthcare providers recognize possible symptoms, such as irritation, anger, demotivation, powerlessness, sadness, depression or being overwhelmed, poor concentration, and feeling tired.[17] Finally, this pandemic puts an extra bodily and psychological burden on HCWs, so it is essential to identify the level of stress and burnout among them.

The aim of the present study was to assess the extent of burnout and stress among HCWs during COVID 19 in primary healthcare centers.

Specific objectives

- To determine the levels of burnout among HCWs during COVID-19

- To identify the levels of stress among HCWs during COVID-19

- To explore the relationship between job burnout and stress among HCWs during COVID 19 in primary healthcare centers

Subjects and Methods

Research design

This cross-sectional study was conducted at primary healthcare centers in Port Said governorate (Egypt). Universal Health Insurance (UHI) in Port Said is accessible every day from 9 a.m. to 10 p.m. There are five districts located in Port Said: Elzhour district, Eldawahey district, Elarab district, Elmanakh district, and Port Fouad district. One primary health care center had been chosen randomly from each district: Elgwhara health care center, El Qabouty health care center, Elarab health care center, Elkuwait health care center, and Port Fouad health care center.

The study sample consisted of 250 HCWs (physicians, nurses, pharmacists, paramedical personnel, and administrative staff) from the last-mentioned setting (Table 1). A convenience sampling technique[18] was used from the total population (n=714), and the final calculated sample size was 250 with a confidence level of 95%, based on the following equation: $n = Z^2 P (1–P) / d^2$,

where n is the sample size, Z is the statistic corresponding to level of confidence, P is expected prevalence (that can be obtained from same studies or a pilot study conducted by the researchers), and d is precision (corresponding to effect size).

Measurement tools

Maslach Burnout Inventory (MDI).[19] The Arabic version of (MBI) was translated by Al-Dubai and Rampal.[20] Burnout was measured using the emotional exhaustion (EE) (9 items), depersonalization (DP) (5 items), and personal accomplishment (PA) (8 items) subscales that are parts of the 22-item MBI. The Arabic version has confirmed a high internal consistency, based on Cronbach's alpha coefficient, for the three MBI subscales (EE: α=0.88; DP: α=0.78; and PA: α=0.89), which indicated a valid and reliable instrument for measuring burnout.

Scoring system: The scoring system is measured with a 7-point Likert scale ranging from never having those feelings to having those feelings a few times a week (0=never, 1=a few times a year or less, 2=once a month or less, 3=a few times a month, 4=once a week, 5=a few times a week, 6=every day). Responses were tabulated into three tiers (low, moderate, or high) based on the reference ranges, for EE: low (≤17), moderate (18–29), and high (≥30); for DP: low (≤5), moderate (6–11), and high (≥12); for PA: low (<33), moderate (34-39), and high (>40). For EE and DP, a higher score means greater burnout; this is inverse for PA.

Perceived Stress Scale (PSS).[21] The Arabic version was translated by Almadi et al.[22] PSS is considered the most widely used psychological instrument for measuring the perception of stress. PSS is used to measure the degree to which situations are appraised as stressful. It is composed of ten items. The Arabic PSS has been shown to have adequate reliability and validity. The Chronbach's coefficient for the PSS was α =0.82.

Scoring system: Scoring system is measured with a 5-point Likert scale ranging from never having those feelings to having those feelings very often (0=never, 1=almost never, 2=sometimes, 3=fairly often, 4=very often). Scores ranging

from 0-13 would be considered low stress; scores ranging from 14-26 would be considered moderate stress; scores ranging from 27-40 would be considered high perceived stress.

In addition, we used a Structured Interview sheet. This structured sheet was developed by the researchers in the Arabic language. It was used to collect data about the personal characteristics of the studied subjects. It comprised personal characteristics such as age, sex, marital status, job status, educational level, residence, years of experience, current job, working days per week, and working site at the center.

Table 1.
Personal characteristics of the study group (n=250)

Variable	n	%
Age, years		
>20	10	4.0
20 - >25	38	15.2
25 -> 30	64	25.6
30 - >35	105	42.0
35 - >40	24	9.6
> 40	9	3.6
Mean±SD	33.5±8.92	
Marital status		
Single	59	23.6
Married	173	69.2
Divorced	14	5.6
Widowed	4	1.6
Resident		
Rural	110	44.0
Urban	140	56.0
Educational level		
Diploma education	17	6.8
Technical education	64	25.6
Bachelor degree	169	67.6
Gender		
Male	47	18.8
Female	203	81.2
Years of experience		
Less than 5 years	45	18.0
5-10 years	117	46.8
More than 10 years	88	35.2
Years of experience at this place		
Less than 5 years	131	52.4
5-10 years	73	29.2
More than 10 years	46	18.4
Current job		
Staff nurse	104	41.6
Pharmacist	20	8.0
Physician	57	22.8
Paramedical personnel and administrative staff	69	27.6
Working days per week		
Three days	52	20.8
Four days	135	54.0
Five days	58	23.2
Six days	5	2.0
Salary		
Enough	220	88.0
Not-enough	30	12.0
Overtime work		
Yes	90	36.0
No	160	64.0
Number of patient contacted per days		
Less than 10	64	25.6
10-20	88	35.2
21-30	71	28.4
More than 30	27	10.8
Persons working with you in the clinic		
1	165	66.0
2	85	34.0
Working site at the center		
Vaccination	20	8.0
Family planning	39	15.6
Antenatal care	34	13.6
Clinics	46	18.4
Laboratory	14	5.6
Reception – triage	8	3.2
Pharmacy	20	8.0
Clerical offices	69	27.6

Pilot study

•In January 2021, a pilot study was conducted before performing the main study. The questionnaire was tested on a sample of HCWs that represent 10% of the total subjects. They were randomly selected and excluded from the total population, after the development of the tool and before starting data collection.

•The aim was to determine the applicability of the designated data collection tool, test its feasibility and suitability, assess the clarity of language, estimate the time needed to fill in the questionnaire, and identify potential obstacles and problems that may be encountered during the period of data collection.

•Data obtained from the pilot study was analyzed, and some modifications were done. Completion of the total sheet ranged between 15 and 20 minutes**.**

Field work

•An initial interview: In every primary healthcare center, the research team met with medical and nursing directors to explain the nature and purpose of the study to gain their permission and cooperation; then the research team initiated communication, explained the aim of the study for studied HCWs individually, and obtained their agreement.

•Verbal consent was obtained from studied HCWs, then researchers distributed the questionnaire format and started to collect data from them at their workplace, in the presence of the researchers for any clarification. The researchers assured that information was kept secret and used only for the research purpose.

•The researchers visited the previously mentioned settings at Port Said governorate according to the available time, during two shifts and three days per week.

•The whole process of data collection went on from March 2021 until August 2021.

Administrative design

Written official permission was obtained from the Dean of Faculty of Nursing, Port Said University, to carry out this study in the selected settings. The researchers sent the official letters for permission to collect the data from the last-mentioned settings. The medical and nursing director of each center was contacted and informed in order to obtain permission to include HCWs in the study, then the aim of the study was explained and data collected.

Ethical consideration

Approval was obtained from the Ethics Committee of the Faculty of Nursing, Port Said University. Verbal consent was obtained from all the participants before collecting any data. The aim of the study was explained in a simple and clear manner. All data were considered confidential and not used outside this study's purpose. Participants were informed about their right to withdraw from the study at any time without giving any reason.

Statistical analysis was performed using statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Categorical variables were analyzed using the chi-square test with the Yates' correction. Kruskal-Wallis H test with Bonferroni correction was used to compare differences between 3 or more independent groups. 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.

Results

Table 1 shows that 42% of the HCWs were aged between 30 and <35 years. More than two-thirds of them were married and had bachelor's degrees. More than half resided in an urban area and had less than five years' experience. The majority of HCWs were females and nearly half of them were staff nurses. More than half of them worked four days per week. Finally, 36% had overtime work.

About 60% and 74.8% of HCWs had high tiers for EE and DP, respectively. The majority of HCWs (81.2%) had low PA assessment (Fig.1).

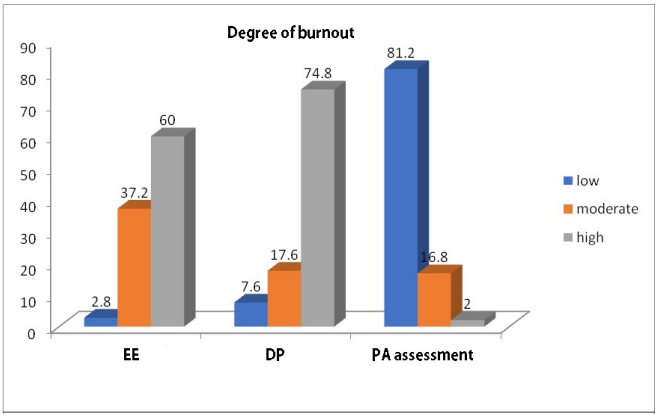

Fig. 1. *Degree of perceived burnout among HCWs*

Table 2 shows that high EE represents 70% of pharmacists, followed by 65.4% of nurses. High DP represents 81.2% of paramedical personnel and administrative staff, followed by 76.9% of nurses. The majority of nurses (87.5%) had low levels of PA, followed by the majority of paramedical and administrative staff (79.7%). Finally, there was no significant difference between perceptions of HCWs regarding the degree of burnout in all dimensions.

Almost half of the HCWs (46%) had a high level of stress, 40% - a moderate level, and only 14% - a low level of stress (Fig.2).

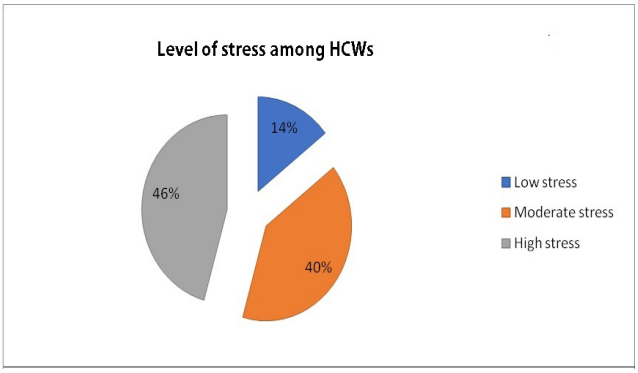

Fig. 2. *Total level of perceived stress among all HCWs*

Approximately half of the nurses (46.1%) and pharmacists (45%) had a moderate level of stress and more than half of the paramedical and administrative staff (56.6%) had a high level of stress. Finally, there was no significant difference between levels of perceived stress among all HCWs (Table 3).

Table 3.
Levels of perceived stress among HCWs

Level of stress	Low		Moderate		High		Statistics
	n	%	n	%	n	%	
Nurses	14	13.5	48	46.1	42	40.4	H=5.769 *P*=0.123
Pharmacists	3	15.0	9	45.0	8	40.0	
Physicians	12	21.1	20	35.1	25	43.8	
Paramedical and administrative staff	7	10.1	23	33.3	39	56.6	

Table 2.
Perceptions of HCWs regarding the degree of burnout

Degree of burnout	Low		Moderate		High		Statistics
	n	%	n	%	n	%	
Occupational/ Emotional exhaustion (EE)							
Nurses	3	2.9	33	31.7	68	65.4	H=2.4401 *P*=0.4862
Pharmacists	1	5.0	5	25.0	14	70.0	
Physicians	2	3.5	27	47.4	28	49.1	
Paramedical personnel and administrative staff	1	1.4	28	40.6	40	58.0	
Depersonalization / loss of empathy (DP)							
Nurses	7	6.7	17	16.3	80	76.9	H=1.6138 *P*=0.6563
Pharmacists	1	5.0	8	40.0	11	55.0	
Physicians	6	10.5	11	19.3	40	70.2	
Paramedical personnel and administrative staff	5	7.2	8	11.6	56	81.2	
Personal accomplishment (PA) assessment							
Nurses	91	87.5	11	10.6	2	1.9	H=0.6916 *P*=0.8752
Pharmacists	13	65.0	6	30.0	1	5.0	
Physicians	44	77.2	12	21.1	1	1.8	
Paramedical personnel and administrative staff	55	79.7	13	18.8	1	1.4	

Table 4.

Relation between personal characteristics of healthcare workers and the level of perceived stress

Variable	Low stress		Moderate stress		High stress		Statistics
	n	%	n	%	n	%	
Age, years							
>20	4	40.0	4	40.0	2	20.0	H=10.979 *P*=0.052
20 - >25	5	13.2	19	50.0	14	36.8	
25 -> 30	4	6.3	29	45.3	31	48.4	
30 - >35	21	20.0	38	36.2	46	43.8	
35 - ≥40	1	4.2	9	37.5	14	58.3	
>40	1	11.1	1	11.1	7	77.8	
Marital status							
Single	5	8.5	28	47.5	26	44.1	H=9.1796 *P*=0.027 P_{M-W}=0.0038
Married	29	16.8	64	37.0	80	46.2	
Divorced	1	7.1	8	57.1	5	35.7	
Widowed	1	25.0	0	0	3	75.0	
Resident							
Rural	13	11.8	47	42.7	50	45.5	$\chi 2$=1.275 *P*=0.529
Urban	23	16.4	53	37.9	64	45.7	
Educational level							
Diploma education	5	29.4	7	41.2	5	29.4	H=4.271 *P*=0.118
Technical education	4	6.3	26	40.6	34	53.1	
Bachelor degree	27	16.0	67	39.6	75	44.4	
Gender							
Male	7	14.9	17	36.2	23	48.9	$\chi 2$=0.363 *P*=0.834
Female	29	14.3	83	40.9	91	44.8	
Years of experience							
Less than 5 years	10	22.2	8	17.8	27	60.0	$\chi 2$=20.201 *P*=0.000
5-10 years	22	18.8	51	43.6	44	37.6	
More than 10 years	4	4.5	41	46.6	43	48.9	
Years of experience at this place							
Less than 5 years	19	14.5	56	42.7	56	42.7	$\chi 2$=7.238 *P*=0.124
5-10 years	15	20.5	26	35.6	32	43.8	
More than 10 years	2	4.3	18	39.1	26	56.5	
Working days per week							
Three days	8	15.4	21	40.4	23	44.2	H=7.205 *P*=0.066
Four days	15	11.1	52	38.5	68	50.4	
Five days	13	22.4	25	43.1	20	34.5	
Six days	0	0	2	40.0	3	60.0	
Salary							
Enough	31	14.1	91	41.4	98	44.5	χ^2=1.421 *P*=0.491
Not-enough	5	16.7	9	30.0	16	53.3	
Overtime work							
Yes	20	22.2	29	32.2	41	45.6	χ^2=8.102 *P*=0.017
No	16	10.0	71	44.4	73	45.6	
Number of patient contacted per day							
Less than 10	24	37.5	17	26.6	23	35.9	χ^2=44.822 *P*=0.000
10-20	9	10.2	44	50.0	35	39.8	
21-30	3	4.2	30	42.3	38	53.5	
More than 30	0	0	9	33.3	18	66.7	
Persons working with you in the clinic							
1	22	13.3	65	39.4	78	47.3	χ^2=0.726 *P*=0.696
2	14	16.5	35	41.1	36	42.4	
Working site at the center							
Vaccination	3	15.0	9	45.0	8	40.0	H=12.141 *P*=0.096
Family planning	12	30.8	13	33.3	14	35.9	
Antenatal care	1	2.9	17	50.0	16	47.1	
Clinics	8	17.4	20	43.5	18	39.1	
Laboratory	1	7.1	6	42.9	7	50.0	
Reception – triage	1	12.5	3	37.5	4	50.0	
Pharmacy	3	15.0	9	45.0	8	40.0	
Clerical offices	7	10.1	23	33.3	39	56.5	

We found a statistically significant, positive relation between the level of perceived stress and marital status ("widowed" vs. "married"), years of experience, having overtime work, number of patients contacted per day and working site at the center (Table 4). Table 5 showed a statistically significant, positive relation between the mean scores of EE/DP and the level of experience, days worked per week, and overtime work. There was a statistically significant relation between the DP score and marital status, educational level and working site at the center. A statistically significant, positive relation was also found between the mean scores of DP/PA and age, and number of patients contacted per day (Table 5).

Table 5.

Relationships between personal characteristics of healthcare workers and scores of burnout

Variable	EE	DP	PA
Age	H=9.113 *P*=0.258	H=16.592 *P*=0.019	H=17.760 *P*=0.017
Resident	χ^2=3.102 *P*=0.159	χ^2=2.614 *P*=0.271	χ^2=0.064 *P*=0.969
Marital status	H=10.361 *P*=0.110	H=14.650 *P*=0.023	H=6.203 *P*=0.169
Educational level	H=6.751 *P*=0.150	H=10.248 *P*=0.036	H=2.672 *P*=0.503
Gender	χ^2=0.114 *P*=0.945	χ^2=1.497 *P*=0.473	χ^2=0.846 *P*=0.655
Level of experience	H=43.781 *P*=0.001	H=25.147 *P*=0.001	H=0.609 *P*=0.548
Days worked per week	H=25.707 *P*=0.001	H=16.864 *P*=0.010	H=6.139 *P*=0.219
Salary	χ^2=3.024 *P*=0.220	χ^2=4.235 *P*=0.147	χ^2=5.366 *P*=0.068
Patient contacted per day	H=47.467 *P*=0.001	H=74.664 *P*=0.001	H=11.677 *P*=0.044
Work experience in years	H=5.455 *P*=0.148	H=1.502 *P*=0.220	H=1.360 *P*=0.335
Current job	H=6.340 *P*=0.237	H=8.612 *P*=0.130	H=7.267 *P*=0.269
Overtime work	χ^2=7.780 *P*=0.021	χ^2=8.674 *P*=0.015	χ^2=0.764 *P*=0.664
Persons working with you in the clinic	χ^2=6.745 *P*=0.034	χ^2=7.893 *P*=0.025	χ^2=3.645 *P*=0.160
Working site at the center	H=17.068 *P*=0.195	H=30.053 *P*=0.003	H=18.074 *P*=0.248

A statistically significant, positive correlation between degrees of EE, DP, and stress levels was found; meanwhile, there was no statistically significant correlations between PA assessment and stress level (Table 6).

Table 6.

Correlation between degrees of burnout and stress levels among HCWs

Burnout degree	Stress	EE	DP
EE	R=0.230 *P*=0.001	-	R=0.501 *P*=0.001
DP	R=0.301 *P*=0.001	-	-
PA assessment	R=0.097 *P*=0.126	R=0.181 *P*=0.040	R=0.204 *P*=0.001

Discussion

Burnout is a common work-related issue that has been viewed as a pandemic of present-day culture that requires expanding consideration and further contemplation around the world.[23] Universally, healthcare providers are facing unusual conditions resulting from the pandemic of COVID-19. Some evidence has appeared about conditions that affect healthcare personnel in many dimensions, such as high workload and extreme stressors that are considered threats to emotional health and lowered quality of life.[24] Consequently, the study aimed to assess the extent of burnout and stress among healthcare providers during COVID-19 in healthcare centers

The current study results identified degrees of burnout: two-thirds of HCWs had high EE, and around three-quarters of them had high DP; meanwhile, the majority of HCWs had low PA. These results may be because of the commonly stressful working environments during crises globally. In addition, a high workload increases liability to increased demands on performance, making it challenging to balance between specialized standards and patient prospects.

This result was consistent with similar study results conducted in Korea and revealed greater risks for burnout in all three aspects (EE, DP, and PA) during the COVID-19 outbreak.[25] In the same line, a study in Italy showed high scores on EE and depersonalization subscales and a low mean score on the PA subscale that correspond to higher degrees of experienced burnout.[26] Conversely, a study in Ghana among HCWs indicated lower levels of burnout and lower to moderate values for EE and physical exhaustion.[27]

The present study determined degrees of burnout among HCWs and showed that the majority of pharmacists and more than two-thirds of nurses had high EE; in addition, the majority of nurses had high DP and, at the same time, low PA. These findings may be due to the prevalence of burnout among staff nurses with different work types.

Moreover, the profession of nursing is linked to a higher grade of burden. Since the beginning of the pandemic, HCWs have experienced worsening working circumstances with high workloads and changing duties and responsibilities. In addition, job burnout seriously affects one's physical and psychological well-being.[28] These findings are in line with a study conducted in Ethiopia among 412 nurses who experienced burnout, and their results indicated that more than two-thirds had high EE, and the majority had high depersonalization and low values of personal achievements.[29] Also, research done about frontline nurses giving care to cases with new coronavirus in China stated that around half of the studied nurses suffered from burnout, more than two-thirds of them had EE, and less than half had DP.[30]

The current study revealed that almost half of the HCWs had high stress; moreover, more than one-third of them had moderate stress, and a lower percentage had low stress. The high prevalence of stress among the studied subjects may be because of direct interaction with patients infected by COVID-19, absenteeism owing to personal or family illness, or a change away from their major roles as well as specific demographic factors as the majority of them are females and

more than two-thirds of them are married. Therefore, they had responsibilities toward their families and worried about infecting their family members. This result may also be explained by other studies, which showed that being female at a young age leads to high psychological stress.[31]

This finding was supported by research carried out on Palestinian healthcare personnel within hospitals and primary healthcare centers, which discovered that a significant group of the study subjects suffered from stress.[32] This result is supported by a systematic review, which concluded that stress is a common syndrome among healthcare personnel in primary healthcare centers.[12]

A study conducted by Arafa et al.[33] on 426 HCWs (physicians, nurses, and other care providers) from Egypt and KSA on the front line of defense against COVID-19 stated that the majority of the studied subjects experienced stress and its related negative consequences, and research done in China showed that about one-third of participants had stress.[34]

Moreover, research done in Spain found that more than two-thirds of healthcare personnel suffered from stress during this pandemic.[29] In a study conducted in Saudi Arabia about healthcare providers working in primary health centers (regular and fever clinics; clinics specialized in managing patients with COVID-19 symptoms), the results affirmed that HCWs in fever clinics exhibited significantly more stress and role conflict and ambiguity than those who were working in regular primary healthcare centers.[35] The results of the current study publicized that almost half of nurses and pharmacists had a high level of stress; meanwhile, nearly half of the physicians and more than half of the secretaries and employees had a moderate level of stress. A possible acceptable explanation for the high level of stress among nurses is that female nurses tend to have more family responsibilities in their daily lives, as well as worry about becoming infected and infecting relatives, work-related concerns about the quality of patient care, changing health team responsibilities, and lack of personal protective equipment. In line with the foregoing, a study on 406 nurses in Xinjiang, China [3] exhibited high levels of stress, which agrees with study results conducted on healthcare providers across the U.S.[36] Thus, among 288 providers, two-thirds (n=184) reported increased stress, and one-third (n=96) reported increased anxiety or depression related to care provision during the COVID-19 epidemic.

The high level of stress among pharmacists may be due to fewer pharmacists being employed per shift, increased workload, increased infection rates, and worry about getting infected; in addition, the pharmacists did not receive sufficient education about epidemics, so the media was their primary source of knowledge about COVID-19. Fears of the pandemic lasting for too long, and increased working hours were factors contributing to increased emotional distress. Similar to the current study results, a study conducted by Hawari et al.[31] revealed that pharmacists experienced greater distress during the COVID-19 lockdown period.

The current study demonstrated a statistically significant positive relationship between the level of perceived stress and age, years of experience, having overtime work, the number of patients contacted per day, and the working site at the center. In the same line, findings of a study about stress and burnout among HCWs during the COVID-19 pandemic showed that younger age was the strongest predictor of perceived stress.[37] Also, our study is consistent with a study of HCWs in Singapore,[38] which showed that working longer hours than usual appeared to be strongly and consistently associated with stress, anxiety, and job burnout. On the other hand, a study of HCWs by Maraqa et al.[32] showed that age, job title, experience, marital status, and type of healthcare setting showed no significant relation with high-stress levels

Results of the present study revealed a statistically significant positive relationship between EE and the level of experience, days worked per week, the number of patients contacted per day and overtime work. Similarly, a study conducted among medical and administrative staff at a tertiary hospital in Italy during the COVID-19 pandemic showed that the factors affecting the levels of EE are the length of working experience and increased workload.[39] The findings of the present study are in congruence with meta-analysis research that reported a negative relation in that greater experience through years worked is one of the factors that decreased HCWs' risk of adverse psychological outcomes during virus outbreaks.[40]

The present study result clarified that DP has a statistically significant, positive relation with days worked per week, the number of patients contacted per day, overtime work, and working site at the center. This DP may be due to feelings of treating patients as objects rather than human beings and becoming more callous toward patients, extra workload, change in work responsibilities, change in the worksite, and less work environment satisfaction. The result aligns with a study conducted in Saudi Arabia,[41] which also found that DP levels are higher when working more than eight hours during the COVID-19 pandemic, when performing on-call duties, and when job duties are changed. In addition, a study among health professionals in Italy stated that work hours were found to be one of the predictors of depersonalization during the COVID-19 pandemic.[42]

The focus of the current study was to identify the correlation between burnout and stress among HCWs. The results revealed a statistically significant, positive correlation between degrees of EE and DP with stress levels. The supplementary analysis may be due to burnout leading to physical stress and sickness in addition to stress. HCWs feared infecting their loved ones with COVID-19 due to their exposure to the virus.[43]

Besides, since the onset of the pandemic, HCWs have experienced worsening work conditions and higher than usual rates of sickness and death among patients and colleagues due to complications related to COVID-19. In line with this hypothesis, the findings from other studies documented that work-related stress is associated with a range of adverse health outcomes, including EE and psychiatric disorders.[44] Similarly, a study among 488 Chinese nurses that investigated occupational stress, job burnout, and quality of life of surgical nurses in Xinjiang (China) clarified a positive correlation between occupational stress and job burnout.[3] A study in Turkey among HCWs showed that high, moderately positive

correlations were found between stress, trait anxiety, and burnout.[45]

Conclusion

Regarding degree of burnout, the current study results concluded that, two-thirds of HCWs had high occupational exhaustion, around three-quarters had high depersonalization, and the majority of them had low PA assessment. Regarding stress levels, almost half of HCWs had high stress, and more than one-third had moderate stress. There was a statistically significant, positive correlation between degrees of occupational exhaustion, depersonalization, and stress level.

We recommend:

•Design and implement health education programs to reduce symptoms of stress, and the impact and risk of burnout.

•Design and implement stress reduction and psychological support programs for HCWs to reduce stress.

•Conduct counseling services in response to the COVID-19 outbreak in different healthcare settings for HCWs.

•Introduce supportive administrative practices in order to reduce the stress and anxiety levels of healthcare professionals.

This research can inform healthcare leaders and enable them to improve HCWs' experience by addressing levels of stress and burnout; managers must support staff who provide care and service to decrease the harmful effects of the COVID-19 pandemic. Finally, organizational managers must provide a safe and comfortable working environment that improves staff satisfaction and countermands burnout and stress. The organization will benefit because employees with low levels of burnout and stress will engage in their jobs and have high achievements.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Sports Medicine

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Saliva Crystallization Features in Young People with Different Levels of Physical Activity

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Abstract

The aim of the study was to estimate the features of dehydration structuring of saliva in untrained people.

***Methods and Results*:** The study included 35 untrained students and 38 people who regularly exercise, who do not have any chronic diseases. The mean age of participants was 17-18 years. The crystallogenic activity and initiatory potential were evaluated for each sample of biological fluid. The crystallization of mixed saliva was studied using the method of classical crystalloscopy, and the initiating properties were studied by the method of comparative tezigraphy. A 0.9% sodium chloride solution was used as the base substance in the tezigraphic test.

The conducted crystalloscopic studies have demonstrated significant differences in the crystallogenic and initiating properties of mixed saliva in people who regularly engage in physical training, compared with untrained individuals. They manifest themselves in a significant qualitative and quantitative transformation of the crystalloscopic picture of the biological fluid, including single-crystalline and dendritic components, as well as in the representation of amorphous bodies in micro-preparations of the biological medium. In the tezigraphic test, it was found that the initiatory potential of mixed saliva also undergoes significant shifts. These shifts are realized in the activation of the initiating ability of biological fluid and optimization of textural characteristics of tezigrams (reduction of cellular density and increase in uniformity) in combination with a decrease in the degree of destruction of structural picture elements and a moderate expansion of the marginal zone of micro-preparations. **(International Journal of Biomedicine. 2022;12(2):265-268.)**

Key Words: saliva • physical training • metabolism • biocrystallomics

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Introduction

Regular physical training is a common way to increase the adaptive capabilities of the body.[1-5] At the same time, numerous publications are devoted to optimizing the regime and the peculiarities of these training routines,[5-7] while the issue of monitoring the human condition in the dynamics of their use remains controversial.[2,4,7-9] An informative criterion for assessing the functional status of body systems is by testing hemodynamic parameters, including heart rate variability.[4,7,10]

Little attention is paid to the study of the metabolic features of people engaged in physical training.[3,7-9,11] Rather, these studies relate to the biochemical aspects of muscle activity itself.[11,12] In contrast, the effect of regular metered physical activity on metabolic processes in the whole body has not been fully disclosed. In this regard, it is of considerable interest to select and evaluate the capabilities of innovative laboratory diagnostic technologies that allow monitoring the metabolic status of people with constant sports activity.[2,3,11,13] In addition, an important advantage of such methods is to ensure the non-

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invasiveness of the study. In this regard, salivary diagnostics based on the study of parameters of mixed human saliva attracts attention.[9,13-15] It should be noted that this technology can be implemented outside the laboratory/medical facility, which further emphasizes its prospects.

Over the past few decades, the study of crystallogenic properties, including an assessment of their own crystal-forming ability and initiatory potential, has been actively used for the integral analysis of the physicochemical characteristics of biological fluids.[13-16] A similar approach has been used in recent years to study the component composition and physicochemical parameters of mixed human saliva;[9,14,15] however, its capabilities and diagnostic informativeness in sports medicine and adaptive biomedicine have been considered only in isolated publications. In particular, some features of the free crystallogenesis of athletes' saliva were established by us earlier,[8,9] but further research is required to obtain a full understanding of the transformation of the body's biocrystallome in the dynamics of physical training.

The aim of the study was to estimate the features of dehydration structuring of saliva in untrained people.

Materials and Methods

The study included 35 untrained students (Group 1) and 38 people who regularly exercise (Group 2), who do not have any chronic diseases. The mean age of participants was 17-18 years. The exclusion criterion was also the presence of dental pathology in the examined persons. Samples of mixed saliva were obtained from all subjects once by spitting into clean, dry test tubes after twice rinsing the oral cavity with distilled water.

The crystallogenic activity and initiatory potential were evaluated for each sample of biological fluid.[8,9,16] The crystallization of mixed saliva was studied using the method of classical crystalloscopy,[8,9] and the initiating properties were studied by the method of comparative tezigraphy.[16] A 0.9% sodium chloride solution was used as the base substance in the tezigraphic test.

The description of the crystalloscopic picture was carried out morphologically by isolating and calculating the density of individual structural elements of the micro preparation (the average value for three fields of view). All structures were grouped into three main categories: single crystals, polycrystalline (dendritic) elements, and amorphous bodies. Tezigraphic pictures were studied using quantitative (the main tezigraphic coefficient Q, the zonal coefficient P) and semi-quantitative (the severity of cellular density, the uniformity of the distribution of elements, the degree of the picture destruction, and the formation of the marginal zone of the micropreparation) indicators.

Statistical analysis was performed using the Statistica 6.1 software package (StatSoft Inc, USA). A probability value of $P<0.05$ was considered statistically significant.

Results

Evaluation of the results of crystalloscopic and tezigraphic analysis of mixed saliva samples allowed us to establish that in persons who regularly engage in physical training, the physicochemical properties and component composition of the biological fluid vary significantly, relative to untrained people. Thus, according to the crystalloscopic picture of the biosubstrate, qualitative and quantitative rearrangements were found (Table 1). Morphostructural features of the single-crystal picture component of the mixed saliva in Group 2 were a significant decrease in the quantitative representation of elements such as "rectangle" and "prism" against the background of the disappearance of octahedral structures from crystallograms and the appearance of "pyramids" that were absent in Group 1. Concerning the dendritic component of micro-preparations, a significant increase in the density of linear and rectangular polycrystalline elements was recorded in Group 2. At the same time, only representatives of Group 1 reveal figures of the "moss," "onion," and "cross" types, and a distinctive characteristic of the Group 2 representatives is that elements of the "horsetail" type were detected in crystalloscopic pictures.

Table 1.

The crystalloscopic picture of the human saliva, qualitative and quantitative rearrangements in study groups (M±m)

Structure element / Parameter	Untrained students	Trained s tudents
Single crystals		
Rectangle figures	4.0±0.1	2.2±0.1*
Prismatic figures	2.1±0.1	0.6±0.1*
Pyramidal figures	0.9±0.1	–
Octahedral figures	–	1.2±0.1
Dendritic (polycrystal) structures		
Linear dendrites	0.8±0.2	1.9±0.1*
Rectangle dendrites	0.5±0.1	1.2±0.1*
Moss-like and onion-like dendrites	2.7±0.2	–
Cross-like dendrites	0.3±0.1	–
Horsetail-like dendrites	–	6.1±0.2
Rose-like dendrites	–	–
Amorphous bodies		
Size	middle	middle
Number	moderate	many

** – $P<0.05$ between groups*

In order to determine the direction of the trends identified on the basis of crystalloscopic analysis, the results of the latter were compared with the data of the tezigraphic test (Figures 1-3). It was found that according to the main parameter characterizing the initiatory potential of mixed saliva (the main tezigraphic coefficient Q), the activation of the structuring of the basic substance in the presence of biological fluid was a feature of the training people's tezigrams (Figure 1). In addition, the representatives of this group are

characterized by a significant expansion of the marginal zone of tezigraphic pictures, which led to a significant increase in the zonal coefficient by 1.30 times (P<0.05), relative to untrained individuals.

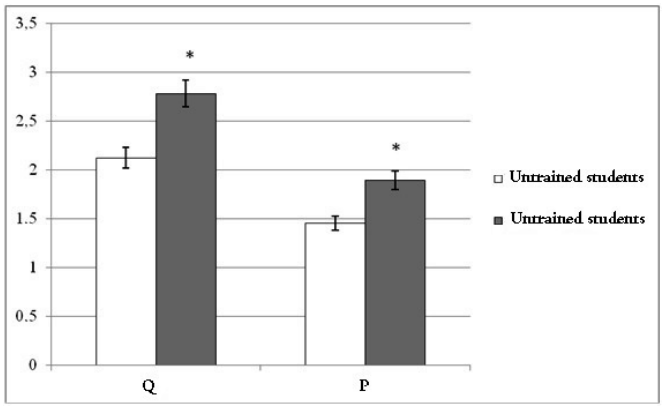

Fig. 1. *The levels of the main tezigraphic coefficient Q and zonal coefficient P in the trained and untrained students (* - P<0.05 between groups)*

The analysis of additional indicators of initiated crystallogenesis–the cellularity and uniformity of the distribution of structural elements in the micropreparation–allowed us to establish that regular physical training contributed to a moderate decrease in the number and size of "crystal islands" in the tezigraphic pictures (Figure 2). Similar changes detected in individuals of this group are clearly correlated with a pronounced increase in the uniformity of texture and spatial distribution of crystalline and amorphous figures in the dehydrated sample, as evidenced by a sharp increase (by 1.75 times, P<0.01) in the corresponding indicator R. The resulting integral parameters reflecting the direction of shifts in the initiated crystallogenesis of mixed saliva formed during physical training are the degree of picture destruction and the clarity of the marginal zone of micro-preparations (Figure 3).

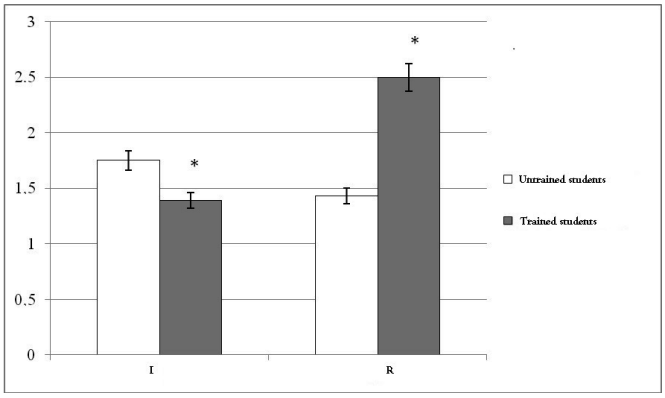

Fig. 2. *The levels of cellularity (I) and uniformity of the distribution of structural elements (R) in the tezigraphic pictures of mixed saliva in the trained and untrained students (* - P<0.05 between groups)*

Similarly, with the indicators of the previous parametric data of tezigraphic pictures, statistically significant differences were observed between the values of trained and untrained people (Figure 3). The degree of picture destruction was markedly reduced in Group 2, (by 1.40 times, P<0.05) compared to Group 1, while the clarity of the marginal zone in representatives of Group 2 was revealed at an increased level, compared to Group 1 (by 1.26 times, P<0.05). At the same time, taking into account the physicochemical nature of the degree of picture destruction,[8-10] its decrease should be regarded as a positive trend and the effect of regular controlled physical activity on the metabolic status of the subjects' bodies should be positively characterized.

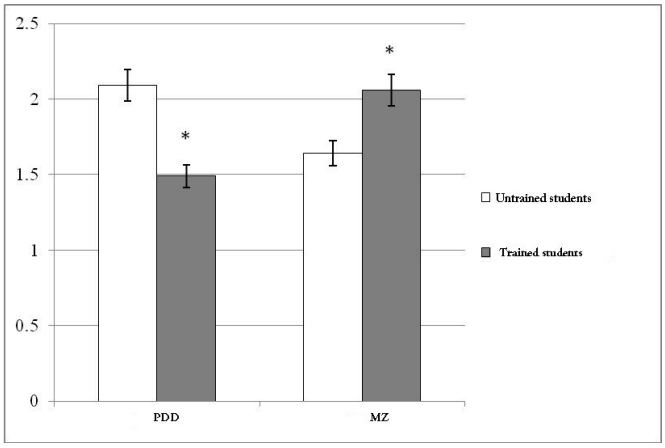

Fig. 3. *The degree of picture destruction (PDD) and the clarity of the marginal zone (MZ) of micro-preparations in the trained and untrained students (* - P<0.05 between groups)*

It is known that the components of the proteome, which have preserved the physiological structure and conformation, are concentrated in the marginal zone of micro-preparations of biological fluids.[8,9,13-16] In this regard, the expansion of the marginal area of tezigraphic pictures observed in Group 2 indicates the optimization of the protein composition of mixed saliva due to regular physical training. Together, the revealed rearrangements of the initiated crystallogenesis of the biological substrate indicate the normalization of its organo-mineral balance.

Conclusion

In general, the conducted crystalloscopic studies have demonstrated significant differences in the crystallogenic and initiating properties of mixed saliva in people who regularly engage in physical training, compared with untrained individuals. They manifest themselves in a significant qualitative and quantitative transformation of the crystalloscopic picture of the biological fluid, including single-crystalline and dendritic components, as well as in the representation of amorphous bodies in micro-preparations of the biological medium. In the tezigraphic test, it was found that the initiatory potential of mixed saliva also undergoes significant shifts. These shifts are realized in the activation of the initiating ability of biological fluid and optimization of textural characteristics of tezigrams (reduction of cellular density and increase in uniformity) in combination with a decrease in the degree of destruction of structural picture

elements and a moderate expansion of the marginal zone of micro-preparations. All of the above suggests that controlled regular physical activity contributes to the normalization of the metabolic status of the body.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Dermatology

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Erbium YAG Laser in the Treatment of Androgenetic Alopecia

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Abstract

The article presents data on the effectiveness of the 2940 nm wavelength erbium-doped yttrium aluminum garnet laser (Er:YAG) in the treatment of androgenetic alopecia (AA) in 85 men. All AA patients underwent a video trichodermatoscopy examination of the scalp using an Aramo-SG video camera (Korea) with ×60 and ×200 lenses, and the Trichoscience diagnostic program. The results of the study were evaluated by videotrichodermatoscopy by the presence of atrophied HF before and after 3 months of laser therapy. A 3-month erbium laser treatment using a 2940-nm Er:YAG laser along with the conventional therapy showed a high efficiency, which was expressed in a decrease in microfibrosis around the hair follicles and an increase in hair growth in the anagen stage. (**International Journal of Biomedicine. 2022;12(2):269-272.**)

Key Words: androgenetic alopecia • erbium laser • microfibrosis • trichoscopy

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Abbreviations

AA, androgenetic alopecia; **EL**, erbium laser; **HF**, hair follicles.

Introduction

One of the most common causes of hair loss is androgenetic alopecia (AA), which dramatically reduces the quality of life, since the awkward appearance can lead to psychological maladaptation. According to some authors, up to 70% of men and 40% of women experience AA. AA is a progressive baldness caused by the action of androgens on the hair follicles.[1,2] The main reason for the development of AA is the activation of the androgen receptor, resulting in a shortening of the anagen, or growth phase, in the normal hair-growth cycle. Circulating free testosterone either binds to intracellular androgen receptors in the hair bulb and dermal papilla[3-5] or is metabolized to dihydrotestosterone by the enzyme 5-α-reductase.[6]

In recent years, lasers and various light sources have been widely used in medicine, in particular in dermatocosmetology. It is well known that Low Level Laser Therapy and the 1550 nm Erbium Glass Fractional Laser are highly effective in treating all forms of hair loss by stimulating hair follicles. But today, we also have the 2940 nm wavelength erbium-doped yttrium aluminum garnet (Er:YAG) laser, which is able to penetrate into the dermis to a depth of 3-4 mm, and energy absorption occurs in the area of the dermal papilla, resulting in an increase in blood circulation of the papilla and activation of metabolism in the hair follicle.[7-9]

Effects of using the 2940 nm wavelength Er:YAG in AA:[10-12]

- ✓ Activation of mitosis, proliferation in the cells of the hair follicles, which leads to a lengthening of the anagen phase and the growth of new hair.
- ✓ Resorption of microfibrosis of hair follicles (HF) because EL generates radiation in the form of microfractional "columns" that penetrate the dermis and cause tissue heating, which leads to a powerful anti-inflammatory effect and a decrease in fibrosis of the scalp skin. The epidermis receives minimal damage, which disappears after a few days and which is one of the indisputable

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advantages of this method in the treatment of AA.

- ✓ Anti-inflammatory effect (reducing the infiltration of HF by lymphocytes). Also, one of the likely mechanisms of action of erbium laser (EL) is the stimulation of apoptosis of T-lymphocytes.
- ✓ Activation of signaling pathways (Wnt, BMP, Shh, and FGF). β-catenin can induce the transition of the hair-growth cycle from the telogen phase to the anagen phase. It has also been reported that Wnt 10b can induce a telogen-to-anagen transition via canonical Wnt signaling pathways to promote hair follicle growth.[13-17]

The purpose of this study was to evaluate the effectiveness of the 2940 nm wavelength Er:YAG laser in the treatment of male AA.

Materials and Methods

We examined 105 men with AA aged 18 to 41 years. The main group consisted of 85 AA patients who received external laser therapy along with traditional therapy, including vitamin therapy, microelements, angioprotectors and specific blockers of hair follicles. The main group patients underwent laser therapy using the 2940-nm Fractional Er:YAG (Alma Harmony XL), handpieces 7×7mm, pulse energy 800-1200 mJ/cm^2, pulse mode - Long, 1-2 passes along the partings of the scalp with an interval of 3 weeks for 3 months. The comparison group consisted of 20 AA patients who received only traditional therapy. The control group consisted of 35 healthy individuals.

The distribution of patients with AA according to the duration of the pathological process was as follows: disease duration of 1 to 5 years - 51.6%, up to 1 year - 24.8%, and from 5 up to 10 years - 23.6%. The results of a hormonal test for testosterone showed an increase in its concentration in the blood in only 4% of AA patients. Provoking factors for hair loss were stressful situations (62%), chronic diseases of the nose and throat (43%), and the use of hormonal drugs (28%).

All AA patients underwent a video trichodermatoscopy examination of the scalp using an Aramo-SG video camera (Korea) with ×60 and ×200 lenses, and the Trichoscience diagnostic program. The main detail of the study was a phototrichogram, which allows one to differentiate AA from other forms of alopecia. For this study, an area of pronounced hair thinning was determined, usually in the parietal region of the scalp, and the hair was shaved with a trimmer in an area of 8x8mm. After 2 days, when among the shaved hair it was possible to detect hair that had grown by 1 mm (anagen) and the remaining hair of the same size (telogen), the areas were tinted with an ammonia-free hair dye and entered into a computer program (Trichoscience) using an ×60 lens.

The results of the study were evaluated by videotricho-dermatoscopy by the presence of atrophied HF before and after 3 months of laser therapy.

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. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. A probability value of $P<0.05$ was considered statistically significant.

The study protocol was approved by the Ethics Committees of the Center for the Development of Professional Qualification of Medical Workers.

Results and Discussion

According to the BAsic and SPecific (BASP) classification of alopecia, in the main group, 36(42.3%) patients had type C2-C3, in which there was hair loss in the frontal and temporal areas of the scalp; 26(30.5%) patients had type M3, in which there were pronounced bald patches in the fronto-parietal region; and 23(27.2%) patients had U1, in which the bald patches of the frontal and parietal regions merged and only the hair of the occipital region of the scalp remained intact. A characteristic feature of the phototrichogram, carried out in the parietal zone, was an increased amount of velus hair and thinning hair (more than 45%) (Fig.1).

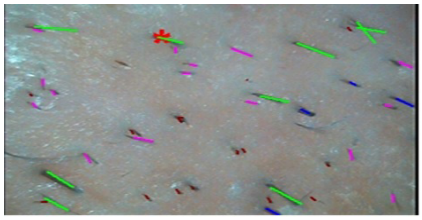

Fig. 1. *Phototrichogram: AA type C2-C3.*

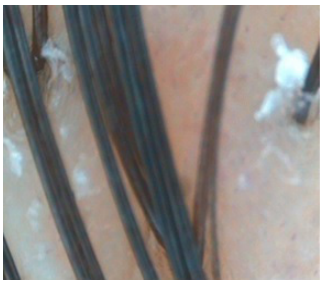

Fig. 2. *Dermoscopy: oily seborrhea*

Fig. 3. *HF in the telogen stage.*

When examining the scalp under the ×200 lens, oily, sometimes dry seborrhea was more often noted; HF of the examined hair were mostly in the telogen stage (Fig. 2 and Fig. 3).

A study of the anagen and telogen phases in the main group patients showed a highly significant decrease in the number of hairs in the anagen phase and an increase in the number of hairs in the telogen stage in the parietal region, while the ratio of growth and resting phases in the occipital regions was not significant in type C2-C3 and significantly decreased in types M3 and U1. So, in AA patients with types C2-C3, M3 and U1 (Table 1), the number of hairs in the growth stage in the parietal region was 58.4±3.1%, 41.7±1.1%, and 32.1±0.8%,

respectively, while in the control group it was 87.1±4.3%. At the same time, the number of hairs in the resting stage was 41.6±1.8%, 58.3±2.4%, and 67.9±2.0% in C2-C3, M3, and U1 types, respectively.

Table 1.

The number of hair (%) in the growth and resting stages in the control group and main group

Scalp region/ hair growth cycle		Control group (n=35)	Main group: AA type		
			C2-C3 (n=36)	M3 (n=26)	U1 (n=23)
Parietal region	Anagen	87.1±4.3	58.4±3.1**	41.7±1.1**	32.1±0.8**
	Telogen	12.9±1.9	41.6±1.8**	58.3±2.4**	67.9±2.0**
Occipital region	Anagen	85.4±3.9	81.7±1.1	79.2±3.2	72.1±1.8**
	Telogen	14.6±2.3	18.3±2.4	20.8±1.4*	27.9±2.5**

** - P<0.05,** - P<0.01 - relative to the control group*

Trichoscopic criteria for atrophied HF in the form of "white" dots after EL therapy are shown in Table 2. Thus, in type C2-C3, the number of atrophied HF significantly decreased, compared to the initial level, and amounted to 11.4±2.17% versus 45.2±3.58%, with M3 type – 28.3±3.46% and 71.7±6.2%, respectively, and with U1 type – 54.8±711% and 88.6±5.91%, respectively.

Table 2.

Atrophied HF (%) in the form of «white» dots after EL therapy

AA type	Before treatment	*P*-value	After treatment
C2-C3 (n=36)	45.2±3.58	<0.001	11.4±2.17
M3 (n=26)	71.7±6.2	<0.001	28.3±3.46
U1 (n=23)	88.6±5.91	<0.001	54.8±7.11

Table 3.

The effectiveness of the therapy in the study groups with AA

Effectiveness of the therapy	Comparison group (n=20)		Main group (n=85)	
	n	%	n	%
Clinical recovery	-	-	-	-
Significant improvement	-	-	41	48.2
Improvement	7	35	27	31.8
No changes	11	55	16	18.8
Worsening	2	10	1	1.2

The effectiveness of the therapy in the study groups was evaluated on the basis of the following criteria (Table 3):

- ✓ Clinical recovery: full hair growth in the foci of hair loss
- ✓ Significant improvement: a growth of a large amount of hair (50%-70%) in the foci of hair loss
- ✓ Improvement: moderate hair growth in the foci of hair loss (25%-50%)
- ✓ No changes: lack of hair-growth dynamics
- ✓ Worsening: the appearance of new foci of hair loss and the absence of hair growth in old foci.

"Significant improvement," "Improvement," and "No changes" were found in 48%, 32%, and 19% of patients, respectively, against the background of EL therapy, and 0%, 35%, and 55%, respectively, in the control group.

Conclusion

A 3-month erbium laser treatment using a 2940-nm Er:YAG laser along with the conventional therapy showed a high efficiency, which was expressed in a decrease in microfibrosis around the hair follicles and an increase in hair growth in the anagen stage.

Competing Interests

The authors declare that they have no competing interests.

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Association between Hypodontia of the Permanent Lateral Incisors and other Dental Anomalies in School Children Aged 12-16 Years in Kosovo

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Abstract

Background: Hypodontia of lateral incisors (LI) is frequently associated with other dental anomalies**.** The objective of this study was to determine the association of LI with other dental anomalies by comparing the two groups: Group 1 with hypodontia of the maxillary LI (MLI1) and Group 2 with hypodontia of the mandibular LI (MLI2), in secondary school education students in Kosovo.

Methods and Results: A total of 3306 secondary school students aged 12-16 years, regardless of gender, were included in this prospective study. The abnormalities investigated were recorded by RTG-panoramic and dental charts. The teeth were recorded as a congenital absence when the mineralization of the crown, identified by panoramic tomography, was absent. In a sample of 3306 subjects, 77(2.3%) subjects were diagnosed with hypodontia. The highest percentage of hypodontia was found in the upper left LI in 20.4% of cases, in the upper right LI in 18.4% of cases, while the percentage of hypodontia of the lower left LI was 0.7% and 2.0% on the right side. A lower percentage of 0.7% or just one missing tooth was found in teeth 13, 32, and 46. The prevalence of LI hypodontia was as follows: MLI1 (Group 1) included 36 cases (92.3%) and MLI2 (Group 2) included only 3 cases (7.6%), which indicates a much higher percentage of cases with hypodontia of MLI1. In Group 1, 21(58.3%) cases of LI hypodontia were bilateral and 15(41.7%) unilateral; in Group 2, 2(66.7%) cases were unilateral and 1(33.3%) case – bilateral.

Among dental anomalies, the occurrence of rotation was found in 19(48.7%) cases with LI hypodontia: 47.2% cases in Group 1 and 66.7% cases in Group 2. The prevalence of dental inclination anomaly was 30.77% of all cases with hypodontia of LI: 27.8% of cases in Group 1 and 66.7% of cases in Group 2. The prevalence of ectopy was in 17.9% of cases of all hypodontia cases of LI: 16.7% of cases in Group 1 and 33.3% of cases in Group 2. Crown anomalies were evident in 7(17.9%) patients of all hypodontia cases of LI, all of which were in Group 1. Other anomalies such as microdontia were evident in two patients in Group 1. Transposition, bodily movement, and superposition were present in one patient in Group 1; in Group 2, one patient had transposition. At the same time, the frequency of dental transposition was significantly higher in Group 2 than in Group 1 (P=0.0209). Other dental anomalies of crown and root and infraposition were not present in both groups. There were no significant differences in the other dental anomalies between the two groups.

Conclusion: The consequences of hypodontia in dental arches are obvious. Knowing the prevalence of hypodontia and its association with other dental anomalies helps classify the need for further treatment for the patients, whether orthodontic, prosthetic, or surgical.(**International Journal of Biomedicine. 2022;12(2):273-278.**)

Key Words: hypodontia • lateral incisors • dental anomalies

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Introduction

Hypodontia is one of the most common developmental anomalies in humans,[1,2] which is characterized by the absence of one or more deciduous or permanent teeth, as well as on both upper and lower dentitions.[3] Hypodontia can cause masticatory dysfunction, impact speech, create aesthetic problems with psychological implications affecting self-esteem,[4] and cause the need for orthodontic treatment and prosthetic compensation in some cases. Hypodontia of lateral incisors (LI) is frequently associated with other dental anomalies**.**

A variety of terminology describes this anomaly in the literature, such as tooth aplasia, congenitally missing teeth, missing teeth, dental agenesis, oligodontia, or anodontia.

The appearance of dental agenesis in permanent dentition varies. Polder et al., using meta-analysis, found that the prevalence of missing permanent teeth varies from 2.2% to 10.1%, excluding third molars, which are missing in about 20% of the general population.[5] This anomaly has multifactorial etiology, including genetic factors, phylogenetic and environmental factors,[6,7] and has variability in the size of the teeth, most often characterized by microdontia.[8]

The absence of anterior teeth and disorders regarding the position of the other teeth cause malocclusions that directly affect both function and aesthetics.[9-11]

The appearance of unilateral hypodontia in LI is, in most cases, accompanied by changes in the shape and size (peg-shaped) of the same tooth on the opposite side of the jaw. This means that both abnormalities deviate from the same genes, but the difference lies in changes in gene expressiveness. In people with permanent dental hypodontia, the persistence of a deciduous tooth is often seen as a result of the absence of a permanent tooth. This includes the second deciduous mandibular molars and deciduous maxillary canines. In the absence of a permanent upper lateral incisor, the canine migrates forward and emerges between the central incisor and the deciduous canine.[12]

Hypodontia in deciduous teeth is a relatively rare phenomenon. The prevalence is from 0.1% to 0.9%.[13] This anomaly is more often localized in the maxilla than in the mandible, while the missing teeth are usually the upper LI. Hypodontia of a deciduous tooth in primary dentition can also be associated with hypodontia of the same tooth in permanent dentition.[14]

The persistence of deciduous teeth often occurs in association with hypodontia. As a result, when a tooth is missing, it is thought that there is a lack of impulse for resorption of the root of the deciduous tooth, which results in the tooth remaining in the jaw for a long time. We often notice the persistence of deciduous canines in cases with hypodontia. As a result of hypodontia, deciduous teeth are prevalent because their root resorption is not helped due to the missing permanent teeth. According to some authors, the retention of primary teeth prevents the vertical development of the alveolar process.[15,16]

The prevalence of hypodontia of permanent maxillary LI ranges from 6% to 8% in different ethnic groups, and molecular genetics has identified shared genetic mutations in families with tooth agenesis.[17] Hypodontia of permanent maxillary LI is also associated with other dental anomalies, while the same genetic mutation may have a variable phenotypical expression.[18]

There have also been suggestions of various inheritance models related to this anomaly, including dominant autosomal incomplete penetration, recessive autosomal, gender-linked inheritance, and polygenic inheritance model. These genes are associated with about 120 syndromes, such as cleft lip, cleft palate, and ectodermal dysplasia, as well as Down, Rieger, and Book Syndromes.[19]

Hypodontia of LI is not an isolated phenomenon because they are often reported associated with other dental anomalies, such as LI in the form of a peg, transposition, delayed tooth development, ectopic eruption, stagnant deciduous teeth, inclination, infraposition, and other anomalies in the size and shape of the teeth.[12] A study of orthodontic patients with at least one canine in the palatal ectopic position showed that LI near these canines were missing in a high percentage of cases.[20]

Peg-shaped maxillary LI were found in 5.5% of family members (probands) with hypodontia, compared with a frequency of 1.7% of the general population.[21] In a study by Alvesalo and Portin, the frequency and inheritance models were researched, and it was observed that the peg-shaped upper LI and the mesiodistal reduction of the upper LI are strongly different expressions of the autosomal dominant gene with reduced penetration.[22]

Hypodontia has a strong connection with microdontia. Congenital absence of LI is often associated with a reduced tooth on the contralateral side with genetic etiology that, combined with hypodontia, can result in multiple diastemas and rotations of adjacent teeth.[23] Previous studies have reported an association between hypodontia of maxillary incisors and other dental anomalies such as transposition,[24] displacement of palatal canines,[25] and premolar rotation.[26]

The studies about the differences between hypodontia of maxillary and mandibular LI regarding their association with other dental anomalies are scarce. In this study, we aimed to investigate whether hypodontia of the maxillary LI has a higher prevalence in the presence of other dental abnormalities compared to the hypodontia group of mandibular LI. This research may provide further evidence on the field-specific genetic control of tooth development.

The objective of this study was to determine the association of LI with other dental anomalies by comparing the two groups: Group 1 with hypodontia of the maxillary LI (MLI1) and Group 2 with hypodontia of the mandibular LI (MLI2), in secondary school education students in Kosovo.

Materials and Methods

A total of 3306 secondary school students aged 12-16 years, regardless of gender, were included in this prospective study. All students attended their respective secondary schools throughout Kosovo. The selection of schools and participants was random, involving all regions of the country, both rural and urban. Examinations were performed by orthodontics and dentistry specialists. The abnormalities investigated were recorded by RTG-panoramic and dental charts. The teeth were recorded as a congenital absence when the mineralization of the crown, identified by panoramic tomography, was absent. The excluding criteria were a history of tooth loss from trauma, caries, periodontal disease, or orthodontic extractions.

The order of patients, based on age, was therefore selected by taking into account the delayed development of second mandibular premolars in boys[24] and according to the dental stage classification developed by Björk.[25]

Inclusion criteria were children of both genders from all regions and secondary schools throughout the country, subjects with all teeth present, students who have no previous history of trauma according to the anamnesis.

Exclusion criteria were students who provided data showing a previous history of tooth loss due to trauma, caries, periodontal disease, or orthodontic extractions; subjects with hypodontia associated with congenital syndromes or systemic diseases; all suspected cases in medical history and clinical examination.

The sample selection was made through the "cluster sampling" technique. This was based on the radiography verification of hypodontia, which was completed for all teeth, excluding the third molars from the study. The file for each subject was reviewed for medical histories, dental and family histories, and study models of maxillary and mandibular dental arches.

Statistical analysis was performed using statistical software package SPSS version 20.0 (Armonk, NY: IBM Corp.). Baseline characteristics were summarized as frequencies and percentages for categorical variables. The frequencies of categorical variables were compared using Pearson's chi-squared test or Fisher's exact test (2-tail), when appropriate. A two-proportion z-test was used to determine whether the two proportions were different from each other. A value of $P<0.05$ was considered significant.

This study was approved by the Ethics Committee of the Faculty of Medicine, the University of Prishtina, supported by the Ministry of Science and Education and the University Dental Clinical Center of Kosovo. Written informed consent was obtained from the parent/guardian/relative of each patient.

Results

In a sample of 3306 subjects aged 12-16 years, 77(2.3%) subjects were diagnosed with hypodontia (Table 1).

Table 1.

Presentation of cases frequency by gender, hypodontia and hypodontia in jaw, including LI hypodontia

Gender	Number of cases, n (%)	Hypodontia frequency	Hypodontia in jaw			Hypodontia of LI	
			Maxillary	Mandibular	Both	Yes	No
Female	1566 (47.4%)	46 (2.9%)	26 (56.5%)	13 (28.3%)	7 (15.2%)	23 (1.47%)	23 (1.47%)
Male	1740 (52.6%)	31 (1.8%)	20 (64.5%)	8 (25.8%)	3 (9.7%)	16 (0.92%)	15 (0.86%)
Total	3306	77 (2.3%)	46 (59.7%)	21 (27.3%)	10 (13.0%)	39 (1.18%)	38 (1.15%)

Regarding the gender groups, 46(2.9%) were female and 31(1.80%) male. There were significant differences between the gender groups (z=2.2, P=0.0278). Among all 77 hypodontia cases, the presence of jaw-based hypodontia was 46(59.7%) cases in the maxillary jaw and 21(27.3%) cases in the mandibular jaw. Hypodontia of both jaws was present in 10(13.0%) cases. There were no significant differences between groups based on gender and jaw hypodontia. The prevalence of LI hypodontia was 1.18% or 39 cases in the total sample. The presence of LI hypodontia was 39(50.6%) of overall hypodontia cases, of which 23(58.9%) were female and 16(41.3%) male.

The highest percentage of hypodontia was found in the upper left LI in 20.4% of cases, in the upper right LI in 18.4% of cases, while the percentage of hypodontia of the lower left LI was 0.7% and 2.0% on the right side (Table 2). A lower percentage of 0.7% or just one missing tooth was found in teeth 13, 32, and 46.

Table 2.

Frequency of affected teeth (FDI notation) in hypodontia subjects

Affected teeth	Number of cases	Percentage
12	27	18.4
13	1	0.7
14	10	6.8
15	6	4.1
22	30	20.4
23	4	2.7
24	11	7.5
25	5	3.4
31	3	2.0
32	1	0.7
34	7	4.8
35	12	8.2
41	4	2.7
42	3	2.0
44	10	6.8
45	12	8.2
46	1	0.7
Total	147	100

The prevalence of LI hypodontia was as follows: MLI1 (Group 1) included 36 cases (92.3%) and MLI2 (Group 2) included only 3 cases (7.6%), which indicates a much higher percentage of cases with hypodontia of MLI1 (Table 3). In Group 1, 21(58.3%) cases of hypodontia were bilateral and 15(41.7%) unilateral; in Group 2, 2(66.7%) cases were unilateral and 1(33.3%) case – bilateral.

Among dental anomalies, the occurrence of rotation was found in 19(48.7%) cases with LI hypodontia: 47.2% cases in Group 1 and 66.7% cases in Group 2. The prevalence of dental inclination anomaly was 30.77% of all cases with hypodontia of LI: 27.8% of cases in Group 1 and 66.7% of cases in Group 2. The prevalence of ectopy was in 17.9% of cases of all hypodontia cases of LI: 16.7% in Group 1 and 33.3% of cases in Group 2. Crown anomalies were evident in 7(17.9%) patients of all hypodontia cases of LI, all of which were in Group 1. Other anomalies such as microdontia were evident in two patients in Group 1. Transposition, bodily movement, and superposition were present in one patient in Group 1; in Group 2, one patient had transposition. Other dental anomalies of crown and root and infraposition were not present in both groups. At the same time, the frequency of dental transposition was significantly higher in Group 2 than in Group 1 (P=0.02088). There were no significant differences in the other dental anomalies between the two groups.

Table 3.
The presentation of the frequency and distribution of dental anomalies in the group with hypodontia of MLI1 and MLI2

Dental anomaly	Group 1 (Hypodontia of the MLI1)								Group 2 (Hypodontia of the MLI2)					
	Uni-lateral		Uni-lateral		Bila-teral		Total		Uni-lateral		Bila-teral		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
	6	16.7	9	25.0	21	58.3	36	100	2	66.7	1	33.3	3	100
	12		22		12-22				42		32-42			
Rotation	4		6		7		17		2		0		2	
Inclination	3		3		4		10		2		0		2	
Bodily	0		0		1		1		0		0		0	
Superposition	0		1		0		1		0		0		0	
Infraposition	0		0		0		0		0		0		0	
Ectopia	2		4		0		6		1		0		1	
Transposition	0		1		0		1		1		0		1	
Crown anomaly	3		3		1		7		0		0		0	
Root anomaly	0		0		0		0		0		0		0	
Microdontia	1		0		1		2		0		0		0	

Table 4.
Distribution and prevalence of dental anomalies in groups MLI1 and MLI2

Dental anomaly	Group 1 (MLI1) (n=36)	Group 2 (MLI2) (n=3)	Statistics	
	n (%)	n (%)	z-score	*P*-value
Rotation	17 (47.2)	2 (66.7)	-0.6474	0.5157
Inclination	10 (27.8)	2 (66.7)	-1.4022	0.16152
Bodily	1 (2.8)	0	0.2924	0.77182
Superposition	1 (2.8)	0	0.2924	0.77182
Infraposition	0	0	-	-
Ectopia	6 (16.7)	1 (33.3)	-0.7227	0.47152
Transposition	1 (2.8)	1 (33.3)	-2.3053	0.02088
Crown anomaly	7 (19.4)	0	0.8432	0.4009
Root anomaly	0	0	-	-
Microdontion	2 (5.6)	0	0.4191	0.67448

Discussion

The data of our study, with a higher prevalence among females than males, follows the studies of Gokkaya & Kargul[27] and Badrov et al.[28] Also, the findings of the ratio of male to female, which was 1:1.4 in our study, is similar to the studies done by Baceti [26] and Muller et al.[29] but differ from the findings of Albashaireh & Khader,[30] Maklin et al.,[31] Rölling,[32] and Aasheim & Ogaard.[33]

The most common missing teeth are the LI of the upper jaw on the left side in 20.5% of cases and the right side in 17.8% of cases, followed by the second premolars of the lower and upper jaw (10%), which is consistent with some studies.[11,34] The frequency of LI hypodontia in our study was higher in 50.6% of all cases with hypodontia, and similar results were found in the studies by Pinho et al.,[35] Celikoglu et al.,[36] and Silve Meza R.[37] The prevalence of hypodontia of maxillary LI was 46.7% of the total sample with hypodontia and is similar to the findings of a study by Al-Abdallah et al.[38]

In our study, the prevalence of uni- and bilateral maxillary LI hypodontia was 1.09% of the total sample and is consistent with other reported studies ranging from 0.3% to 11.3%.[3,37,39] According to a meta-analysis, the prevalence of agenesis of permanent teeth in the general population, excluding third molars, ranges from 3.2% to 7.6%.[34] This prevalence varies according to the tooth type. For example, hypodontia of maxillary LI was 1.7% of all samples, which is similar to our findings.

Bilateral agenesis of the maxillary LI occurs more frequently than unilateral agenesis.[40-43] Our study also shows a higher frequency of agenesis of bilateral maxillary LI in 56.4% of cases, but other studies by Pinho et al.[35] and Delli K et al.[44] showed less frequency of bilateral than unilateral agenesis.

A study by Celikoglu et al.[36] found significantly increased prevalence rates for ectopic eruption, transposition, and transmigration of the maxillary canines and reduced or peg-shaped maxillary LIs in their study sample. This is similar to the findings in our study, where the results indicate a high prevalence of dental anomalies, such as rotation, inclination, ectopic, and corona anomalies, in the group of patients with hypodontia of LI. Rotation of premolars was significantly associated with congenitally missing maxillary LI in the study by Baccetti.[26] This result was similar to the findings in our study, where we found a high prevalence of the dental rotation anomaly [19(48.7%) cases with LI hypodontia].

The lateral incisor of the maxilla was the tooth most often missing congenitally, as in the study of Augard & Gayard.[45] However, this is not consistent with the study of Al-Mulla et al.[46] who found that the second premolar of the mandibula is the most frequently missing tooth.

In a study about hypodontia, a critical issue is the patient's age at the time of the diagnosis, which tells us that the visibility of the dental germ on radiography depends on the stage of tooth mineralization.[43] The stages of tooth development are more closely related to tooth mineralization than the chronological age of tooth eruption.[47] Unilateral hypodontia is often associated with dysmorphia or microdontia corresponding to the contralateral tooth.[48] In another study by Pinho et al.,[49] the findings indicate that microdontia of the maxillary LI may represent a presentation of the molecular changes that lead to a developing defect of the maxillary lateral incisors. Therefore, considerable emphasis should be placed on the clinical diagnosis or family history where we may suspect missing teeth, and the treatment option for closing the spaces becomes unreal from the orthodontic aspect.[50]

Considering that the possibilities of treating patients with hypodontia are wide during treatment planning,[51] it is necessary to know the number of missing teeth, especially in the intercanine sector, the size of the teeth, the condition

of the teeth present, the condition of the periodontium, the position of the teeth, alveolar bone mass, general and local health status (soft tissue, lip line, gingival aesthetic condition, malocclusions, patient's age, the attitude of the child and parents towards the anomaly, occlusion, etiological factors, technical and financial possibilities of solving the problem).

Recognizing the prevalence and different models of hypodontia, strategies are created to treat this anomaly, which is not only statistically important but also important for the formation of an interdisciplinary professional team to cooperate in planning successful treatment.

The consequences of hypodontia in dental arches are obvious. Depending on the models of hypodontia, the need for the treatment ranges from simple to intensive. The problems that can occur in these patients are extensive, such that each case needs to be considered uniquely, in terms of approach and treatment. Knowing the prevalence of hypodontia and its association with other dental anomalies helps classify the need for further treatment for the patients, whether orthodontic, prosthetic, or surgical.

In our study, there were dental abnormalities associated with dental agenesis, which should be sought and investigated in patients with agenesis. It is particularly necessary, for those with agenesis of upper lateral incisors, and especially in young children for whom it is crucial that the orthodontist intercept these anomalies as early as possible, in order to establish proper treatment. The time-consuming and financial cost of extensive treatments of this anomaly is of interest to numerous clinical, basic science, and public health fields, such as orthodontics, pediatric dentistry, prosthodontics, periodontics, maxillofacial surgery, anatomy, anthropology, and even to the insurance companies.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Dentistry

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Correction of the State of Enamel Mineral Metabolism in Persons with Decompensated Caries at Various Times after Oral Cavity Sanitation

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Abstract

The aim of the study was to determine the effectiveness of brushite exposure to correct the marginal permeability of composite restorations in persons with a decompensated form of carious process activity; this study also examined the penetrating ability of brushite in the micro-cracks of the enamel-composite border.

Methods and Results: The study included 120 patients (age 18-40 years) with a decompensated course of the carious process, who underwent complete oral cavity sanitation with the direct composite restoration of 300 teeth. The obtained results were analyzed and interpreted in vivo (the clinical effectiveness of the restoration was assessed according to the Ryge criteria immediately after treatment and 1, 12, and 36 months after treatment) and in vitro (scanning electron microscopy of the enamel-composite joint after applying the composite and after treatment with the studied means). According to the purpose and objectives of the study, two groups of patients were formed: Group 1 (control, n=58): the prevention of recurrent caries after sanitation was carried out using a standard personal hygiene product containing fluorine; Group 2 (experimental, n=62): the prevention of recurrent caries after sanitation was carried out using a natural two-component complex for strengthening and remineralization of enamel RemarsGel. During statistical processing of the obtained data, immediately after sanitation and one month after it, the differences in the indicators of the Ryge criteria in Groups 1 and 2 were statistically insignificant. Twelve months after the sanitation, the Ryge restoration quality indicators in Group 2 were higher than in Group 1 ($P \leq 0.001$). After 36 months, the statistical significance of the difference in the restoration quality indicators increased ($P \leq 0.0001$). These results were confirmed by the data of scanning electron microscopy. Based on the results obtained, stating the state of the quality of the enamel-composite joint, it can be concluded that brushite crystals are highly effective in preventing violations of the integrity of the enamel-composite joint, which has a direct and immediate impact on the quality of restoration.

Conclusion: We consider it necessary to add to the list of main indications for the use of the RemarsGel system one more, a scientifically based indication of the targeted use of the system in the presence of a significant amount of adhesive, both direct and indirect restorations in the oral cavity, especially in the decompensated course of the carious process.(**International Journal of Biomedicine. 2022;12(2):279-283.**)

Key Words: caries • composite material • brushite crystal • individual oral hygiene product • Ryge criteria

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Abbreviations

RC, Ryge criteria; **SEM**, scanning electron microscope.

Introduction

In modern clinical dentistry, the most used method of restoring defects in hard tooth tissues is restoration with composite materials. Moreover, according to a number of domestic and foreign researchers, the indications for the use of composites have expanded significantly in recent years, which is associated with the improvement of their aesthetic and physical and mechanical properties. But despite this, the main problem of composite restoration – violation of the marginal tightness of the enamel-composite connection – remains relevant.[1-8]

Solutions to this problem are developed in two directions: first, the development of innovative techniques for constructing restorations, and second, the modification and development of new restoration materials and adhesive systems with the required physical and chemical characteristics.[2,8-15]

One of the leading causes of depressurization is that the method of preparing the hard tissues of the tooth for adhesive is imperfect – the agent applied to the surface of the enamel is sprayed with air. As a result, under the action of the airflow, the adhesive is displaced to places subject to the lowest air pressure, i.e., on the edges of the cavity, where there is a matrix on one side and tooth tissues on the other. Thus, a "side" is formed at the enamel border, which dissolves with oral fluid over time and, which, in turn, causes depressurization of the enamel-composite joint with subsequent demineralization of the enamel.[1,2,4,5,8,16-19]

In addition, there are a sufficient number of reports in the literature stating that after the preparation and filing of the carious cavity, Ca^{2+} is lost by the tooth enamel, which, according to various sources, occurs from 1 month to 1 year after treatment. To correct these disorders, remineralization therapy is widely used. This problem is especially acute in persons with a decompensated form of activity of the carious process.[4,8,20-23]

The aim of the study was to determine the effectiveness of brushite exposure to correct the marginal permeability of composite restorations in persons with a decompensated form of carious process activity; this study also examined the penetrating ability of brushite in the micro-cracks of the enamel-composite border

Materials and Methods

The basis for obtaining scientific data was a contingent of 120 patients with a decompensated course of the carious process, who underwent complete oral cavity sanitation with the direct composite restoration of 300 teeth. The obtained results were analyzed and interpreted in vivo (the clinical effectiveness of the restoration was assessed according to the Ryge criteria (RC) immediately after treatment and 1, 12, and 36 months after treatment) and in vitro (scanning electron microscopy of the enamel-composite joint after applying the composite and after treatment with the studied means). The material for scanning electron microscope (SEM) was 20 planned extractions of 3 molars of the upper and lower jaws.

The inclusion of patients in the study and further processing of the results obtained was carried out on the basis of voluntary informed consent. The inclusion criteria were the presence of a decompensated form of the carious process (CEE≥16, where C – caries and restored teeth, E – endodontic treated teeth, E – extraction teeth), the absence of removable and conditionally removable orthopedic structures, complete sanitation of the oral cavity, age 18-40 years.

According to the purpose and objectives of the study, two groups of patients were formed: Group 1 (control, n=58): the prevention of recurrent caries after sanitation was carried out using a standard personal hygiene product containing fluorine; Group 2 (experimental, n=62): the prevention of recurrent caries after sanitation was carried out using a natural two-component complex for strengthening and remineralization of enamel RemarsGel.

After sanitation of the oral cavity, all patients were recommended a preventive program of individual oral hygiene, according to group membership. For patients of Group 1, a hygiene product was chosen according to the results of the literature data of marketing analysis of the most used toothpaste. These patients were recommended to use Colgate Total 12 professional cleaning paste two times a day (morning and evening) after learning the technique of brushing their teeth. The participants of Group 2 were recommended to use RemarsGel daily also two times a day (morning and evening).

The prophylactic course of application of RemarsGel consisted of 28 procedures. The criterion for the effectiveness of the course was the absence of damage to the enamel-composite border visible to the naked eye. With visible damage, RemarsGel was used every day until the integrity of the border was restored, and then, according to the prophylactic scheme. RemarsGel prophylactic course was carried out 4 times a year. The rest of the time, all patients used individual oral hygiene products identical to Group 1.

The clinical quality of the restorations was determined using the Ryge criteria immediately after debridement, 1, 12, and 36 months after treatment. This assessment was recommended by the International Organization for Quality Standardization (Protocol PN-EN No. 4049\2003). Scanning electron microscopy was carried out to understand the mechanism of the protective action of brushite. For statistical processing of the obtained results, we used the cluster analysis.

Results and Discussion

The quality of the performed restorations was assessed according to the Ryge criteria for each tooth at various times after the oral cavity was sanitized (Table 1).

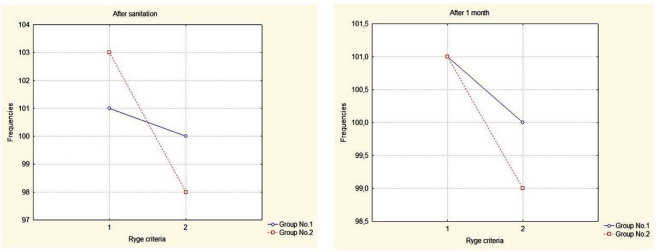
Table 1.

Comparative characteristics of the quality of restorations in the study groups, points.

Timing observations	Group	Ryge score				Total
		0	1	2	3	
Absolute values [n=150] for every group						
After sanitation	Group 1	78	72	–	–	150
	Group 2	77	73	–	–	150
After 1 month	Group 1	77	73	–	–	150
	Group 2	77	73	–	–	150
After 12 months	Group 1	40	83	27	–	150
	Group 2	65	83	2	–	150
After 36 months	Group 1	17	27	91	15	150
	Group 2	41	93	14	2	150
Relative values [n=100%]						
After sanitation	Group 1	52.0%	48.0%	–	–	100%
	Group 2	51.33%	48.67%	–	–	100%
After 1 months	Group 1	51.33%	48.67%	–	–	100%
	Group 2	51.33%	48.67%	–	–	100%
After 12 months	Group 1	26.66%	55.33%	18.01%	–	100%
	Group 2	43.33%	55.33%	1.34%	–	100%
After 36 months	Group 1	11.33%	18.01%	60.66%	10.0%	100%
	Group 2	27.33%	62.0%	9.33%	1.34%	100%

0 points – "ideal" restoration (code 1); 1 point – good restoration (code 2); 2 points – restoration in need of delayed replacement (code 3); 3 points – restoration in need of immediate replacement (code 4).

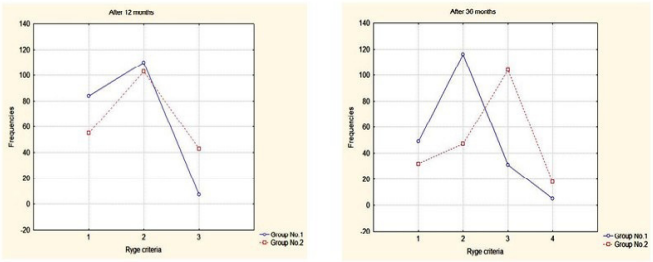
During statistical processing of the obtained data, immediately after sanitation and one month after it, the differences in the indicators of the RC in Groups 1 and 2 were statistically insignificant (P=0.488 and P=0.563, respectively, Fig.1. A-B).



(A) (B)

Fig. 1. *Comparative characteristics of the study population in terms of Ryge indicators immediately after sanitation (A) and 1 month later (B). NewVar0 – Group 1; NewVar1 – Group 2.*

Twelve months after the sanitation (Fig.2A), the Ryge restoration quality indicators in Group 2 were higher than in Group 1 (P≤0.001). After 36 months, the statistical significance of the difference in the restoration quality indicators increased (P≤0.0001) (Fig.2.B).



(A) (B)

Fig. 2. *Comparative characteristics of the study population in terms of Ryge indicators 12 (A) and 36 (B) months after sanitation. NewVar0 - Group 1; NewVar1 - Group 2.*

The results of cluster analysis are shown in Figure 3. In cluster analysis, the K-means method was used with a sequential increase in the number of clusters from two to 5 and the number of iterations from 10 to 20. The choice of the final number of clusters was determined based on the situation when a large number of clusters only detailed the obtained clinical results (RC) without highlighting new features of the identified dynamics of the process.

The mean value of the variable in Cluster 1 (Group 2) was noticeably lower than in Clusters 2 and 3 (Group 1), and the range of dispersion of the mean values of the variable in the groups of patients was not significant (P<0.1) and does not affect objectively estimated parameters. Such a difference in the average values of the variable can be associated with the quality of the enamel-composite joint according to the RC (visual control) since immediately after treatment, the mean values of the variable in different groups differed slightly; in this variation series not only the mean values of the variables in different groups of patients changed, but also these values differed significantly from each other. In addition, Cluster 2 (95 cases) was identified, in which initially high values of variables at 36 months after treatment corresponded to those of Cluster 1. The results of statistical processing clearly confirm the previously obtained clinical results of the Ryge parameters.

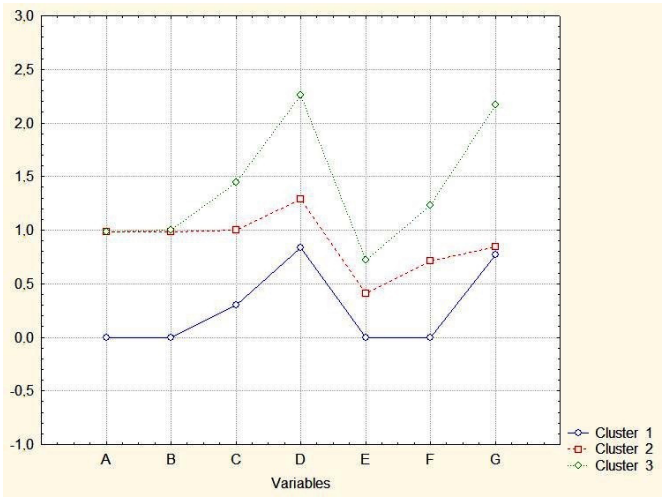


Fig. 3. *Cluster analysis*

Variable A – RC after sanitation; Variable B – RC (visual control), 1 month after sanitation; Variable C – RC (visual control), 12 months after sanitation; Variable D – RC (visual control), 36 months after sanitation; Variable E – RC (instrumental control), 1 month after sanitation; Variable F – RC (instrumental control), 12 months after sanitation; Variable G – RC (instrumental control), 36 months after sanitation.

In the SEM study of samples obtained in Group 1 and Group 2 immediately after sanitation, where the restoration was carried out using an identical adhesive system and composite, there were no visual differences in the quality of the state of the enamel-composite joint (Figures 4, 5).

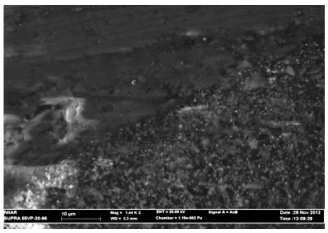


Fig. 4. *SEM of an enamel-composite joint immediately after treatment (Group 1).*

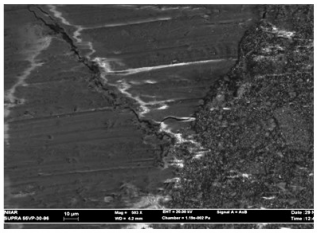


Fig. 5. *SEM of an enamel-composite joint immediately after treatment (Group 2).*

After the sample was treated with a hygienic agent in Group 1, when scanning in the area of the enamel-composite joint, we noted the ability of the hygienic agent to be adsorbed on its surface, especially in areas with a pronounced prismatic enamel microstructure (with an increase in x1500, an insignificant number of particles of a hygienic agent of a non-crystalline structure was noted, Fig. 6).

After processing the sample in Group 2 with the RemarsGel system and scanning in the area of the enamel-composite joint, we noted the presence of a continuous film of brushite crystals covering the entire field of study, including

the enamel-composite joint itself. The photograph shows the adhesive nature of the fastening of brushite crystals (the crystals did not collapse, and their connection with the enamel was not disturbed, even during the intermediate processing of the sample). This pattern was observed both along the entire perimeter of the enamel-composite joint, and over the entire surface of the enamel of the test sample, regardless of the microstructure (Fig.7).

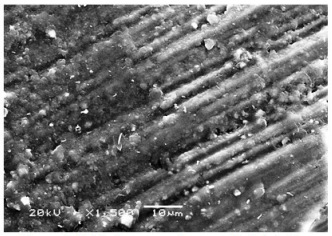


Fig. 6. *SEM of an enamel-composite joint after treatment with Colgate Total 12 (Group 1).*

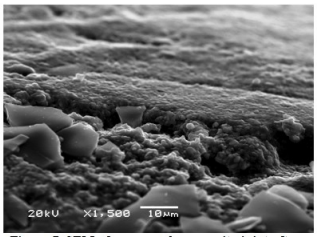


Fig. 7. *SEM of an enamel-composite joint after treatment with RemarsGel (Group 2).*

In the course of the study, using laboratory research methods, it was established that the unique physical and chemical properties of brushite crystals (adhesiveness, high adaptability, and strength) provide the RemarsGel system with a number of unique properties that determine its advantage in maintaining the tightness of the enamel-composite enamel connection, compared to Group 1, and, consequently, increasing the durability of the consistency of the restoration.

Thus, the above problems determine the relevance of permanent protection of the enamel-composite border, including the use of personal hygiene products, which, in addition to maintaining the required level of oral hygiene, would have pronounced remineralizing properties. In particular, this applies to brushite crystals. Brushite has unique adhesive properties and an affinity for enamel surface hydroxyapatite crystals. [9,17,24-30]

The system consists of two components (Tube 1 and Tube 2) which are used strictly alternately without mixing. According to the developers, the compounds that make up the RemarsGel have the ability to gradually penetrate into the enamel of the teeth and replace the lost calcium. RemarsGel actually "repairs" the area of tooth enamel that has lost its strength, strengthens it, and helps restore the natural protection of the tooth.[1,2,5,6,8,31]

From a scientific point of view, the principal mechanism of the system's action is a chemical reaction that occurs during the sequential use of reagents in the oral cavity (calcium nitrate from Tube 1 is mixed with ammonium hydro phosphate from Tube 2). As a result of this reaction, which is safe for humans, a brushite crystal is formed on the surface of the enamel, similar in composition to the main structural substance of tooth enamel, the hydroxyapatite crystal. Due to its small size (40-50 nm), the brushite crystal penetrates deeply into the tissues of the tooth, restores enamel damaged by caries, closes micro-cracks, and quickly relieves tooth sensitivity (even after professional whitening).[3,5,9,10,32-35]

Currently, there are reports that a brushite crystal acting directly on the focus of demineralization not only densifies it, that is, replaces a structural defect, but also forms new mineral compounds, i.e. exhibits chemical activity. In terms of abrasiveness, the complete absence of the aggressive effect of the complex has been demonstrated, which makes it possible to prevent enamel abrasion during cleaning and to effectively use the drug with increased tooth sensitivity.[1,2,9]

Conclusion

Based on the results obtained, showing the state of the quality of the enamel-composite joint, it can be concluded that brushite crystals are highly effective in preventing violations of the integrity of the enamel-composite joint, which has a direct and immediate impact on the quality of restoration. We consider it necessary to add one more to the list of main indications for the use of the RemarsGel system: a scientifically based indication of the targeted use of the system in the presence of a significant amount of adhesive, for both direct and indirect restorations in the oral cavity, especially in the decompensated course of the carious process.

Competing Interests

The authors declare that they have no competing interests.

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Prevalence and Characteristics of Impacted Third Molars in a Kosovar Population: A Cross-Sectional Study

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Abstract

Background: The goal of this randomized cross-sectional study was to determine the prevalence of impacted third molars in the Kosovar population and to assess the site of impaction using the Winter and the Pell and Gregory classifications.

Methods and Results: A total of 5515 digital panoramic radiographs (PANs) were reviewed to determine the number of impacted third molars, their angulation, and depth. The age range was from 18 to 77 years (29.3±12.8 years). The prevalence of impacted third molars in the sample of the Kosovar population was 73.7%. Of all PANs, one or more impacted third molars were observed in a total of 710 PANs, including 296(41.7%) in men and 414(58.3%) in women, with a sex ratio of 1:1.4; this difference was not significant (P=0.616). The mandible exhibited a substantially greater prevalence of impacted third molars than the maxilla 62.1% vs. 37.9% (P=0.001). In the mandible, significantly higher frequencies of third molar impaction were noted in the distoangular and vertical positions (36.9% and 33.5%). In the maxilla, the most common angulation of impacted third molars was mesioangular (52.8%). Most impacted third molars recorded a Class C depth of impaction (62.3%) followed by Class B (34.9%), and only 2.8% recorded a Class A depth of impaction.

Conclusion: The samples of third molar impaction in the Kosovar population are characterized by a high prevalence of impaction, especially in the mandible. The most common angulation was the mesial in the maxilla as well as in the mandible. The most common level of impaction in the maxilla and mandible was level C.(**International Journal of Biomedicine. 2022;12(2):284-288.**)

Key Words: impaction • panoramic radiograph • dental radiograph

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Abbreviations

OPG, orthopantomogram; **PAN**, panoramic radiograph; **TMI**, third molar impaction.

Introduction

Tooth impaction is a condition in which a tooth fails, or is not expected, to erupt into the dental arch, based on clinical and radiographic findings. Failure of these teeth to erupt may be a consequence of local or systemic factors. Local factors consist of the presence of supernumerary teeth, early loss of deciduous teeth, discrepancies between the size of the tooth and the dental arch, tumors or cysts acting as mechanical barriers, and space deficiencies in the size of the dental arch due to micrognathia.[1-3] Systemic factors related to tooth impaction include childhood diseases, ankylosis of the temporomandibular joint, hereditary factors, cleft palate, syphilis, and cleidocranial dysostosis.[4,5]

Several studies have reported tooth impaction as a common phenomenon with a wide range in the incidence of impaction.[3,6-8] The last tooth to develop and erupt is the third molar, so it is the tooth that most frequently fails to erupt into the dental arch.[2-4] Therefore, impaction of the third molar is

the most commonly reported in the literature, and the incidence of TMI seems to be increasing in adults.[9,10]

Studies report a variable prevalence of impacted third molars, with a range of 15.2%–94.8%.[10-13] Most studies underscore that mandibular TMI exhibits a high prevalence prevalence, compared with its counterpart in the maxilla.[3,10,14,15]

Given their relationship with various diseases and other serious clinical conditions, such as caries and root resorption of the neighboring tooth to the impacted third molar, impacted third molars are a common health issue.[5,16-18] TMI is linked to the formation of cystic lesions and tumors, but the frequency is limited (2.77%).[19] As a result, extraction is one of the most common treatments [20,21] and is based on an assessment of the degree of impaction, angulation, and interaction with the mandible's anterior ramus.[22,23] The Winter and the Pell and Gregory categorization methods, which employ the connection between the longitudinal axis of the tooth, the occlusal surface of the neighboring tooth, and the ascending ramus of the mandible vigilant,[22,23] are the most reliable in usage.

Information about all teeth in the maxilla and mandible, including their surrounding anatomical structures provided by OPGs, is widely used in dentistry to diagnose impacted teeth and is the main method for evaluation in epidemic research due to its economic and rational characteristics for use.[5,6]

Therefore, given that the prevalence of TMI has not been determined in the Kosovar population and that its prevalence varies in different regions, this study aimed to evaluate the prevalence of TMI in the Kosovar population and their angulation while using panoramic radiographs (PANs). The findings of this study will contribute to expanding current epidemiological knowledge of the pathology of TMI. Moreover, these data might be useful for all dental health workers, specifically those involved in oral health care and preventive dentistry, via the development of efficient prevention approaches.

Materials and Methods

In this cross-sectional study, 5515 PANs were randomly obtained from the radiology unit at the University Dentistry Clinical Center of Kosovo (UDCCK), which were obtained as part of patients' random clinical treatment between 2011 and 2015. Of these 5515 OPGs, 2724 belonged to men and 3620 to women. The age range was from 18 to 77 years. OPGs were retrieved as digitalized images from the UDCCK database using Sidexis Next Generation imaging software, version 2.4®, integrated with the I-Max Touch Line (Owandy, Champs-sur-Marne, France)® at 220-240 V-7A 50/60 Hz with a maximum exposure time of 15 sec.

Patient data, such as date of birth and sex, were collected. The study inclusion criteria for PAN were records of patients aged 18 years or older and both males and females of Kosovo origin with all forms of impaction. Exclusion criteria were OPGs of patients under 18, absence of birth records, incomplete third molar root formation, and recorded congenital anomalies.

This study was approved by the Ethics Committee of UDCCK, the Faculty of Medicine, the University of Prishtina, and the University of Zagreb. Written informed consent was obtained from each patient.

An impacted third molar was defined as the third molar with fully formed roots that did not erupt into the dental arch and had no functional occlusion because it was impeded in its eruption path by another tooth, bone, or soft tissue (Figure 1).[5]

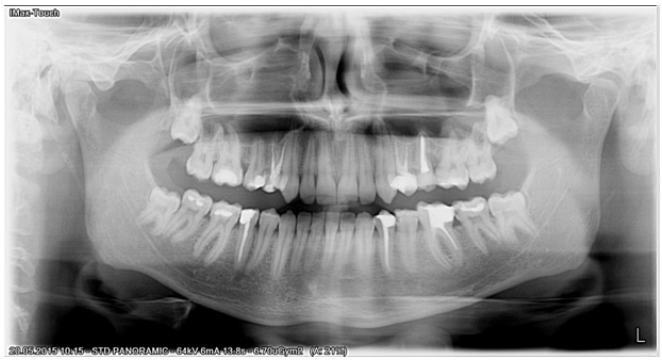

Fig. 1. *OPG of a 22-year-old female patient. Impacted third molars in the maxilla and third right molar in the mandible.*

Winter's classification, which is based on the angle created between the longitudinal axis of the second and third molars, was used to identify the angulation of an impacted third molar in the current investigation. Corel DRAW was used to examine the angulations on the digital photos (Graphics Suite X7, U.S.). The tools 'Magnify' and 'Ruler' were utilized during the analysis. Angulation of the impacted third molar was determined by evaluating the angle formed between the intersected longitudinal axes of the impacted third molar and the adjacent second molar: vertical impaction (10° to -10°), mesioangular impaction (11° to 79°), horizontal impaction (80° to 100°), distoangular impaction (-11° to -79°), other impaction (111° to -80°), and buccolingual impaction (tooth oriented in a buccolingual direction with the crown overlapping the roots).[22]

Classification by Pell and Gregory is extensively used to predict the difficulty of extracting impacted third molars. The level of difficulty is measured by the thickness of the overlying bone, and the level of difficulty increases as the depth of the impacted tooth increases. Pell and Gregory's assessments were used in this study to measure the depth of impacted third molars in relation to the occlusal plane.[23]

The Pell and Gregory classification (Class I to III) was exclusively used for impacted mandibular third molars. The classification is based on the spatial relationship of the third molar to the ascending ramus of the mandible and the second molar.

Statistical analysis was performed using statistical software package SPSS version 18.0 (Chicago: SPSS Inc.). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Continuous variables with normal distribution were presented as mean±standard deviation (SD). The frequencies of categorical variables were compared using Pearson's chi-squared test or Fisher's exact test (2-tail), when appropriate. A value of $P<0.05$ was considered significant.

Using Kappa statistics, the validity of the OPG readings was verified for 99 impacted third molars detected on 50 randomly selected OPGs.[24] Without the ability to assess age or sex, all OPGs were evaluated in a blind manner. The average of the coefficients derived for the study's key variables was significant (0.756).

Results

The prevalence of impacted third molars in the Kosovar population was 73.7%. In our total sample, 42.9% of participants were males and 57.1% were females. The percentage of female participants was significantly higher than that of males (P<0.001). Of all PANs, one or more impacted third molars were observed in a total of 710 PANs, including 296(41.7%) in men and 414(58.3%) in women, with a sex ratio of 1:1.4 (Table 1); this difference was not significant (P=0.616).

Table 1.

Distribution of impacted third molars according to sex and in PANs

Number of impacted third molars	Male n (%)	Female n (%)	Total n (%)
1	155 (52.4)	202 (48.8)	357 (50.3)
2	91 (30.7)	138 (33.3)	229 (32.3)
3	32 (10.8)	41 (9.9)	73 (10.3)
4	18 (6.1)	33 (8.0)	51 (7.2)
Total	296 (100)	414 (100)	710 (100)

Pearson Chi-Square value = 1.79, df = 3, P = 0.616

The age range was from 18 to 78 years (29.3±12.8 years). The mean age of men was greater than that of women (32.1±14.6 years vs. 27.2±10.9 years; P<0.001). Six working-age groups were included in this research: 18–20, 21–30, 31–40, 41–50, 51–60, and > 60 years. TMI was most prevalent in the two younger age groups (27.5% in the group of 18–20 years and 42.3% in the age group of 21–30; total =69.8%). However, in the older age group (31–78), men had a higher number of TMI than did women, while in the younger age groups (18–30), TMI were more common in women than in men, resulting in a statistically significant difference (P<0.001).

In both jaws, a total of 1310 impacted third molars were observed. The distribution of impacted third molars between the maxilla and the mandible is shown in Table 2. The mandible exhibited a substantially greater prevalence of impacted third molars than the maxilla 62.1% vs. 37.9%; P=0.001). A total of 814 of the 1310 impacted third molar teeth were found in the mandible, and 496 in the maxilla. As a result, impacted third molars were 1.64 times more likely to occur in the mandible than in the maxilla (Table 2).

Based on Winter's classification, the most common angulations of impacted third molars were mesioangular impactions (P=0.000) (Table 3). In the mandible, significantly higher frequencies of TMI were noted in the distoangular and vertical positions (36.9% and 33.5%). On the other hand, in the maxilla, the most common angulation of impacted third molars was mesioangular (52.8%).

Table 2.

Distribution of impacted third molars according to jaw location and sex

Jaw	Male n (%)	Female n (%)	Total n (%)
Maxilla	227 (43.1)	269 (34.4)	496 (37.9)
Mandible	300 (56.9)	514 (65.6)	814 (62.1)
Total	527 (100)	783 (100)	1310 (100)

Pearson chi-square value = 10.18, df = 1, Fisher's exact test P = 0.001

Table 3.

Distribution of Winter's angulations according to jaw location

Winter angulations	Maxilla n (%)	Mandible n (%)	Total n (%)
Mesial	262 (52.8)	181 (22.2)	443 (33.8)
Distal	53 (10.7)	300 (36.9)	353 (26.9)
Vertical	80 (16.1)	273 (33.5)	353 (26.9)
Horizontal	76 (15.3)	18 (2.2)	94 (7.2)
Bucco-lingual	25 (5.0)	42 (5.2)	67 (5.1)
Total	496 (100)	814 (100)	1310 (100)

Pearson Chi-Square value = 272.10, df = 4, P = 0.000

Most impacted third molars recorded a Class C depth of impaction (62.3%), and only 2.8% recorded a Class A depth of impaction. No significant difference was noted between the sexes related to the ethical depth of impaction. However, a statistically significant difference in depth of impaction was noted between the maxilla and mandible (P=0.000) (Table 4).

Table 4.

Distribution of Pell-Gregory depth classes of impacted third molars according to jaw location

Pell-Gregory	Maxilla n (%)	Mandible n (%)	Total n (%)
Class A	36 (7.2)	1 (0.1)	37 (2.8)
Class B	113 (22.8)	344 (42.7)	457 (34.9)
Class C	347 (70.0)	469 (57.6)	816 (62.3)
Total	496 (100)	814 (100)	1310 (100)

Pearson Chi-Square value = 96.61, df = 2, P = 0.000

In the mandible, most impacted third molars [390(47.9%)] were classified with a Class II position followed by Class I [258(31.7%)] and Class III [166(20.4%)].

Discussion

The frequency of impacted teeth was estimated to be 73.7% in this research of 5515 PANs of Kosovar patients, with 710 PANs having at least one impacted tooth. The current study's prevalence of TMI is similar to that of Morris and Jerman,[25] who reported a frequency of 66% in a study of 5000 participants in the United States.[10] In a survey of 1000 PANs in the Singapore Chinese community, a frequency of 69% was discovered. Impacted third molars were found in 72.09% of

cases by Obiechina et al.,[26] whereas impaction in Scandinavian cultures varied from 22% to 76.1% (Figure 2).[27-29]

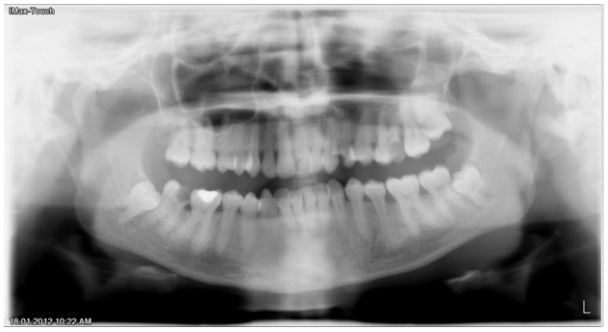

Fig. 2. *OPG of a 24-year-old male patient. Impacted third molars are located in the maxilla and the mandible.*

Our results outperform those of Eliasson et al. (33%),[17] Hashemipour et al. (44.3%),[6] and Pillai et al. (50.20%).[9] The results are considerably higher than those of Hattab et al.,[12] who investigated 232 PANs in Jordanians and found a 28.2% frequency of impacted third molars, whereas Hellman found a 15.3% incidence of TMI in 433 Columbia University students.[30]

However, the results were significantly lower than those reported by Gisakis et al.[4] in a Greek population (91.6%). Kramer and Williams[13] reported an incidence of 94.8% from 3,748 radiographs (a survey at Harlem Hospital, NY, U.S), and Kazemian et al.[31] reported an incidence of 95.6% from 10,000 participants in Iran's population. The documented variability in the TMI prevalence might be due to a variety of factors, such as genetic and ethnic characteristics, differences in sampling, quantity of samples, age group studied, radiographic criteria, or definition of impaction.

The distribution of impacted third molars in the mandible was 62.1%, which differed from that noted in the maxilla (37.9%). The results are similar to those of Quek et al.,[10] who reported that impacted third molars occurred 1.33-fold more frequently in the mandible than in the maxilla. However, some studies have discovered that the maxilla has a higher percentage of impacted third molars than the mandible. Dachi and Howell (21.9%),[32] Hattab et al. (54%),[12] and Kramer and Williams (63%)[13] all verified a maxillary prevalence.

More female patients than male patients were included in our study. Pedro et al.[2] and Quek et al.[10] also reported a female preponderance. Despite the higher incidence of female patients, this study found no statistically significant difference (P=0.616) between males and females in terms of TMI. Hattab et al.,[12] Kramer and Williams,[13] Dachi and Howell,[32] and Ayranci[33] also found no sex difference. After the third molars erupt, female jaws cease developing but male jaws continue to expand.[6,14,31] In addition, contrary to our findings, Hashemipour et al.[6] and Quek et al.[10] discovered a larger percentage of impacted third molars in females, whereas Haidar and Shalhoub[11] found that males (34%) had a greater rate of TMI than females (29%).

Winter's categorization of angulation of impacted third molars revealed that among 1310 affected third molars, mesioangular impaction was the most common (33.8%). The results are compatible with conclusions reported by Obiechina et al.(48.20%)[26] and Hattab et al.(50%)[12] However, these findings are incompatible with Pillai et al.,[9] who found that the vertical position of impacted third molars was more common (46.6%), followed by the mesioangular position. Similar results of the predominance of vertical angulation were reported by Haidar and Shalhoub.[11]

Using the Pell and Gregory categorization, the depth of impacted third molars revealed that 816(62.3%) of impacted third molars were classified as Class C (Figure 3). Compared to the mandible, the maxilla had a greater incidence of profoundly impacted third molars, with a Class C frequency of 70.0% versus 57.6%. Similarly, Pillai et al.,[9] Quek et al.[10] Venta et al.,[34] and Kruger et al.[35] found the highest prevalence of profoundly impacted third molars in the mandible. No statistically significant association was noted between impaction level and gender.

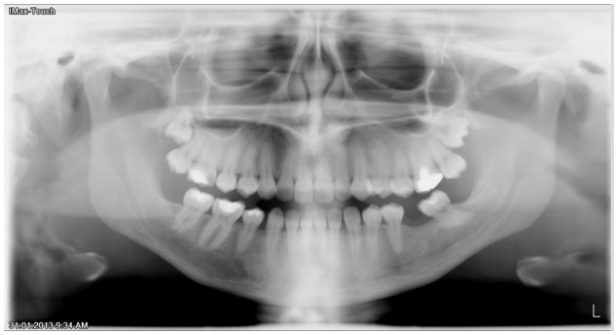

Fig. 3. *OPG of a 22-year-old female patient. Impacted third molars are located in maxilla right side level C and left side level B.*

According to Pell and Gregory, a high majority of impacted third molars in the mandible have half of their crown in the ramus (48%) and are classed as Class II. This conclusion is consistent with the findings of several previous studies that revealed Class II impaction to be the most prevalent for mandibular third molars.[6,20] In the 710 PANs, 357 patients had at least one impacted third molar, 229 had two, 73 had three, and 51 had four. Thus, the most common number of impacted third molars was one per patient. Nanda et al.[36] noted that one impacted third molar per patient was the most common frequency. In contrast, Quek et al.[10] and Pillai et al.[9] reported that the most frequent number of impacted third molars in their retrospective radiographic study of 1000 and 1100 PANs was two per patient.

Conclusion

The samples of TMI in the Kosovar population are characterized by a high prevalence of impaction, especially in the mandible. The most common angulation was the mesial in the maxilla as well as in the mandible. The most common level of impaction in the maxilla and mandible was level C.

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ORIGINAL ARTICLE

Experimental Medicine

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Experimental Evaluation of Hemostatic Agents and Powdered Sorbent Effectiveness on the Dynamics of Blood Aggregate State Regulation using the Method of Thromboelastography

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Abstract

Background: This article presents the results of an experimental study on the effect of hemostatic agents (Hemostatic collagen sponge, Tachocomb, Surgitamp and granular sorbent Molselect G-50) on the system regulating the aggregate state of the blood (SRASB) using piezoelectric thromboelastography (TEG).

Methods and Results: The study involved 18 healthy men aged between 21 and 43 years with normal indicators of the SRASB. In the first series of experiments, TEG of the blood was recorded without the addition of the test material (the control stage). At the second stage of the experiment, the hemostatic properties of the Hemostatic collagen sponge, Tachocomb, hemostatic gauze Surgitamp and granular sorbent Molselect G-50 were studied.

In vitro experimental studies to assess the effect of hemostatic agents and granular sorbent on the SRASB using piezoelectric TEG have shown that the use of Hemostatic collagen sponge, Tachocomb, Surgitamp, and the granular sorbent Molselect G-50 convincingly affects all links of the thrombosis process. However, Surgitamp and the granular sorbent Molselect G-50 show the greatest influence on such important indicators as the time of blood clotting and maximum clot density, which gives reason to conclude they are effective in clinical use in surgical practice.(**International Journal of Biomedicine. 2022;12(2):289-292.**)

Key Words: thromboelastography • aggregate state of the blood • hemostatic agents • granular sorbents

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Abbreviations

MCD, maximum clot density; **SRASB**, system regulating the aggregate state of the blood; **TEG**, thromboelastography; **TCPC**, time of the contact phase of coagulation; **TRTC**, time to reach the thrombin constant; **TBC**, time of blood clotting; **TPC**, time of polymerization of the clot; **TFTC**, time of formation of fibrin-platelet clot; **TOF**, the time of the onset of fibrinolysis.

Introduction

Abdominal trauma remains one of the urgent problems of emergency surgery. Liver injury ranks second among other abdominal injuries. One of the technically difficult issues of hemostasis is the stopping of parenchymal bleeding.[1-3]

Many methods of hemostasis have been proposed to stop bleeding from liver wounds. The main surgical method is wound suturing. The disadvantage of all sutures applied in cases of liver injury is that necrosis zones appear between the sutures. For surface, large-area injuries, various methods of coagulation devices are used (diathermocoagulation, laser coagulation, argon-plasma coagulation). All these methods of coagulation give an unstable hemostatic result, often expanding the necrosis zone in the wound. In large surgical clinics, hepatic arteries are embolized. The main disadvantages

of this method are the high cost and the lack of the necessary equipment in city and district hospitals.[4-7]

In the conditions of conventional clinics for hemostasis in liver wounds, options for wound tamponing are increasingly used, for example, with an omentum and various hemostatic agents (sponges, powders, films). At the same time, there remain problems of bioinertness and the presence of local and systemic reactions.[8,9]

A promising method in the complex treatment of hemorrhagic complications is the use of polymer granular sorbents, which, when swelling, form soft hydrogels that have hemostatic, plastic, and other properties. However, the mechanism of action of granular sorbents in combination with a local hemostatic on the SRASB is not fully understood.[10-16]

The aim of this research was to study the comparative features of the effect of hemostatic agents (Hemostatic collagen sponge, Tachocomb, Surgitamp, and granular sorbent Molselect G–50) on the system regulating the aggregate state of the blood (SRASB) in vitro experiments using modern capabilities of piezoelectric TEG.

Materials and Methods

The present study involved 18 healthy men aged between 21 and 43 years [29.0 (25.0-32.5) years] with normal indicators of the SRASB.

The venous blood of healthy volunteers served as the material for research. In the conditions of the treatment room, the cubital vein was punctured and blood was taken into sterile vacuum tubes containing a 3.8% sodium citrate solution with a volume of 4.5ml, intended for coagulographic studies. The study of the SRASB processes was performed by piezoelectric thromboelastography using the piezoelectric thromboelastograph ARP-01M "Mednord."

In the first series of experiments, TEG of the blood was recorded without the addition of the test material (the control stage). At the second stage of the experiment, the hemostatic properties of the Hemostatic collagen sponge, Tachocomb, hemostatic gauze Surgitamp and granular sorbent Molselect G-50 were studied. To study the SRASB processes, 0.3 ml of citrate blood was injected into the thromboelastograph cuvette installed in the thermostat chamber. Then a fixed amount (1.0mg) of the studied drug was added to the cell of the device. The contents of the cuvette were mixed evenly, an activator solution (0.025 M calcium chloride solution) was added and the study was started.

The results of the studies were analyzed by evaluating the following parameters (time in minutes): the time of the contact phase of coagulation (TCPC), the time to reach the thrombin constant (TRTC), the time of blood clotting (TBC), the time of polymerization of the clot (TPC), the time of formation of a fibrin-platelet clot (TFTC), the time of the onset of fibrinolysis (TOF), and maximum clot density (MCD).

Statistical processing of the results of the study was carried out using STATISTICA Base (License dated 17.12.2010). For descriptive analysis, results are presented as median (Me), lower quartile (Q1) and upper quartile (Q3). A non-parametric Kruskal-Wallis test was used for comparisons of median values among three groups, followed by post-hoc testing using unpaired Mann-Whitney U tests. 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 of Voronezh State Medical University named after N. N. Burdenko. Written informed consent was obtained from all participants.

Results and Discussion

The results of the study are presented in Table 1. When analyzing the TCPC indicator reflecting the adhesive-aggregation activity of platelets and the suspension activity of shaped blood elements, it was found that in the control stage, TCPC was 1.0(1.0-1.05) min. No significant changes in TCPC were observed when using Tachocomb, Surgitamp or Molselect G-50, and for all these drugs this indicator was 1.0(1.0-1.0) min. With the addition of a Hemostatic collagen sponge to the cell of the device, TCPC became elongated (1.0(1.0-2.25) min); however, no statistical significance was observed, compared to the control (P=0.5137). Thus, Hemostatic collagen sponge, Tachocomb, Surgitamp and Molselect G–50 do not have a significant effect on the initial process of blood clotting–adhesion and platelet aggregation.

When studying the TRTC, it was found that in the control stage this indicator was 6.5(4.8-7.6) min. For Tachocomb, TRTC occurred at an earlier time and amounted to 5.4(4.5-5.9) min; however, compared with the control, the differences were statistically insignificant (P=0.0780). Applications of Hemostatic collagen sponge reduced TRTC to 2.9(2.2-4.2) min (P=0.0001), Surgitamp to 2.9(2.2-3.4) min (P=0.0000). The best result of achieving the thrombin constant was shown by the granular sorbent Molselect G-50 – 2.6(2.2-3.3) min (P=0.0000). Thus, the Hemostatic collagen sponge, Surgitamp and Molselect G-50 begin to show their hemostatic activity precisely at the stage of achieving the thrombin constant, possibly potentiating the activity of prothrombinase.

Analyzing TBC, the key SRASB indicator, reflecting the transition of liquid blood states to gel-like, it was found that in the control stage, TBC occurred at 12.9(12.1-13.9) min. The use of all the agents we studied led to a statistically significant reduction in TBC: for Hemostatic collagen sponge – 9.5(8.9-10.1) min (P=0.0000), for Tachocomb – 6.4(6.1-6.9) min (P=0.0000), for Molselect G-50 – 4.3(3.9-5.1) min (P=0.0000). Thus, the use of all the drugs we studied significantly reduce the time of blood transition from a liquid to a gel-like state, which reflects their hemostatic potential and determines the effectiveness of their clinical use.

In the study of TPC, an indicator characterizing the process of frontal and lateral assembly of fibrin monomers and the formation of protofibrils with their subsequent polymerization and cross-linking, it was found that in the control stage, TPC was 23.5(22.1-24.4) min. The use of Hemostatic collagen sponge reduced TPC to 19.8(18.6-24.6) min, but the difference compared to the control was statistically insignificant (P=0.0887). For Tachocomb, TPC was 16.5(15.7-17.1) min (P=0.0000). The best indicators of TPC were observed with the use of Surgistamp – 14.7(13.8-15.5) min (P=0.0000) and for Molselect G-50 – 14.7(14.0-15.7) min (P=0.0000).

Table 1.

Effects of hemostatic agents (Hemostatic collagen sponge, Tachocomb, Surgitamp, and granular sorbent Molselect G–50) on the SRASB in vitro experiments

TEG indicators	Control stage	Hemostatic collagen sponge	Tachocomb	Surgitamp	Molselect G-50	*P*-value
TCPC, min	1.0(1.0-1.05)	1.0(1.0-2.25)	1.0(1.0-1.0)	1.0(1.0-1.0)	1.0(1.0-1.0)	0.0196
P-value		0.513723	0.887386	0.197808	0.127688	
TRTC, min	6.5(4.8-7.6)	2.9(2.2-4.2)	5.4(4.5-5.9)	2.9(2.2-3.4)	2.6(2.2-3.3)	0.0000
P-value		0.0001	0.0780	0.0000	0.0000	
TBC, min	12.9(12.1-13.9)	9.5(8.9-10.1)	6.4(6.1-6.9)	4.7(4.1-5.1)	4.3(3.9-5.1)	0.0000
P-value		0.0000	0.0000	0.0000	0.0000	
TPC, min	23.5(22.1-24.4)	19.8(18.6-24.6)	16.5(15.7-17.1)	14.7(13.8-15.5)	14.7(14.0-15.7)	0.0000
P-value		0.0887	0.0000	0.0000	0.0000	
TFTC, min	31.3(39.9-35.7)	28.7(28.1-29.0)	29.2(28.9-29.5)	29.1(28.6-29.5)	28.3(27.9-28.7)	0.0000
P-value		0.0000	0.0000	0.0000	0.0000	
TOF, min	29.5(28.7-29.6)	-	-	-	-	
MCD, U	399.5(339.5-427.0)	430.5(418.0-488.0)	533.5(516.0-577.0)	452.0(423.5-500.5)	556.5(535.0-573.5)	0.0000
P-value		0.0145	0.0001	0.0023	0.0001	

Analyzing the final stage of the thrombosis process (the formation of a fibrin-platelet clot), we found that TFTC in the control was 31.3(39.9-35.7) min. The use of all studied substances led to a reduction in TFTC index: for Tachocomb – 29.2(28.9-29.5) min (*P*=0.0000), for Surgistamp – 29.1(28.6-29.5) min (*P*=0.0001). The best results for TFTC showed Hemostatic collagen sponge – 28.7(28.1-29.0) min (*P*=0.0000) and Molselect G-50– 28.3(27.9-28.7) min (*P*=0.0000).

The stability of the formed fibrin-platelet clot was assessed by the time of the beginning of fibrinolysis. In the control stage, in 4 observations, the beginning of clot lysis was observed on 29.5(28.7-29.6) min. Analyzing the effect of Hemostatic collagen sponge, Tachocomb, Surgitamp and Molselect G-50, no clot lysis was observed in any observation, which indicates the pronounced antifibrinolytic activity of the substances we studied.

A qualitative indicator of the clot formation process is the maximum clot density (MCD), reflecting the stability of the fibrin-platelet thrombus to external influences. In the control stage, MCD was 399.5(339.5-427.0)U. The use of Hemostatic collagen sponge increased MCD up to 430.5(418.0-488.0)U (*P*=0.0145), Surgitamp – 452.0(423.5-500.5)U (*P*=0.0023), Tachocomb – 533.5(516.0-577.0)U (*P*=0.0001). The best result of MCD was shown by the granular sorbent Molselect G-50 – 556.5(535.0-573.5)U (*P*=0.0001).

Conclusion

In vitro experimental studies to assess the effect of hemostatic agents and granular sorbent on the SRASB using piezoelectric TEG allowed us to state that the use of Hemostatic collagen sponge, Tachocomb, Surgitamp, and the granular sorbent Molselect G-50 convincingly affects all links of the thrombosis process. However, Surgitamp and the granular sorbent Molselect G-50 show the greatest influence on such important indicators as TBC and MCD, which gives reason to conclude they are effective in clinical use in surgical practice.

Competing Interests

The authors declare that they have no competing interests.

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Antibacterial Control of an Extremely Low Frequency Electric Field on *Escherichia coli*

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Abstract

The aim of this study was to investigate the electric field frequency and the time of exposure that causes maximum inhibition of *Escherichia coli* (*E. coli*) growth.

Methods and Results: Bacterial suspensions were subjected to an extremely low frequency electric field (ELFEF) with a 0.1 Hz interval between 0.2 Hz and 0.4 Hz. The bacterial growth was observed through optical density (OD) readings. OD values were taken every hour for four hours to monitor bacterial growth in both exposed and unexposed samples. The antibiotic susceptibility test was done to determine the difference between the susceptibility of both exposed and unexposed bacterial samples. Structural changes in the exposed bacterial samples were monitored by transmission electron microscope (TEM). The bacterial growth curve revealed a highly significant growth inhibition after being exposed to 0.2 Hz at 2 hours' exposure time. *E. coli* suspension exposed to ELFEF at inhibition frequency 0.2 Hz showed a significant increase in susceptibility to antibiotics Keflex, meropenem, and piperacillin-tazobactam.

Conclusion:The current data suggest that treating E. coli with 0.2 Hz for 2 hours is an effective, prospective, and novel technique for reducing cellular growth and dramatic alteration in the cell membrane. TEM clarified the great destruction of the bacteria cell wall. (**International Journal of Biomedicine. 2022;12(2):293-298.**)

Key Words: extremely low frequency electric field • *E. coli* • optical density • transmission electron microscope

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Abbreviations

ELFEF, extremely low frequency electric field; **OD**, optical density; **TEM**, transmission electron microscope.

Introduction

There is an interest in applications of a pulsed electromagnetic field with different frequencies in the medical field, such as pain control, enhancing the efficacy of anticancer drugs, cancer cell proliferation and apoptosis, and transdermal delivery of low permeant drugs.[1-4] Previous studies on extremely low frequency electromagnetic waves succeeded in controlling the growth of Ehrlich tumors.[5,6] It was shown that extremely low frequencies from 6 Hz up to 500 Hz radiation can process wound repair,[7,8] skin, and bones.[7-10] Cellular activity of microorganisms has recently been controlled using

extremely low electromagnetic waves with a very low field strength that resonate with bioelectric signals created during a specific metabolic activity.[11,12] The exposure of Agrobacterium tumefaciens (the common cause of crown gall disease in plants) to 1 Hz square amplitude modulating waves for 90 minutes changed its cellular activity and DNA structure, inhibiting growth and affecting virulence.[13]

Extremely low frequency electric field (ELFEF) effects on bacteria have been investigated. Depending on the bacterial strain and the physical parameters such as frequency and field strength, it has been demonstrated that ELFEF can negatively or positively affect cell growth, viability, and bacterial antibiotic sensitivity.[14,15]

The category of microorganisms includes a massive range of organisms, including bacteria, fungi, viruses, algae, archaea, and protozoa.[16] Some microorganisms that are seen to be beneficial to health are termed probiotics and are available as dietary supplements, or food additives.[17] But some microorganisms, particularly bacteria, are harmful to plants, animals, or humans by attacking their cells and causing disease, for example, Salmonella, E. coli, and MRSA.[18] There is a type of bacteria that can be harmful or useful for the human body, such as E. coli, which normally dwell in the intestines of humans. It can also be found in the intestines of some animals.[19]

The majority of E. coli strains are safe and even beneficial to the digestive system. Some strains like enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), and Shiga toxin-producing E. coli (STEC), can cause breathing issues, pneumonia, diarrhea, and urinary tract infections. E. coli is responsible for 75 to 95% of urinary tract infections.[20] Healthy persons who have been infected with E. coli typically feel better within a week. However, some people have a significant kidney issue known as hemolytic uremic syndrome.[21] The elderly and children are more likely to be affected.[22] The traditional and effective method to destroy bacteria and other harmful microorganisms in the body is antibiotics, such as Amoxicillin, Doxycycline, Cephalexin, Ciprofloxacin, Clindamycin, Gentamycin, and Azithromycin.[23]

Bacteria often develop resistance to antibiotics; therefore, there is a constant search for alternative ways to combat harmful bacteria. The application of a pulsed electric field will help in preventing bacterial resistance. It has been reported that stimulation or inhibition of bacterial growth is dependent on the field strength of the electric field radiation and types of bacteria.[24-26]

Therefore, this research studied the effects of electric field frequencies on bacterial growth at different time exposures, as well as the effects of the chosen range of electric field frequency (0.2-0.4H z) on bacteria. The optical density (OD), which is a measure of the quantity of light absorbed by a bacterial suspension, was used to determine the growth in the organism's cell mass. The TEM examined the ultrastructure of the bacteria.

Materials and Methods

Throughout the study, test organism E. coli (ATCC 27853) was obtained from the plant and microbiology department, Faculty of Science, Cairo University, Giza, Egypt. The bacterial strain was subcultured on nutrient agar plates.

Preparation of bacterial suspension

To prepare broth subculture, two cultured colonies of bacteria grown on a nutrient agar plate were inoculated into 5ml sterile nutrient broth of pH 7.4±0.2 (Lab M Limited, UK) in a test tube and incubated at 37°C for 24 hours (incubator of plue pard, PH050A, Italy). After adjusting the bacterial cell concentration to the 0.5 McFarland standard,(27) a standardized bacterial suspension of 20µl (1.5×108 CFU/ml) was distributed into 1.5ml broth media tubes: 1.5ml of broth medium (organism-free) was used as a blank tube (negative control), 1.5ml of broth media seeded with the microorganism was used as a positive control, and a corresponding number of test tubes were produced to be subjected to various frequencies; each experiment was performed in triplicate, with the average taken into account. The tubes were cultured for 24 hours at 37°C in a shaking (20 rpm) incubator (Orbital/ Reciprocal Shaking Water Bath, Boekel). Spectrophotometer measurements of OD at 560nm per hour were used to create growth curves for bacterial cell cultures (Humalyzer Primus manufactured for Human GmbH. Germany).

Experimental setup

The exposure system was constructed at the Biophysics Department, the Cairo University, to develop an electric field generator with a frequency range of 0.1-20 Hz and frequency intervals of 0.1 Hz. For each frequency, the duty cycle was increased by 10% each time until there was a 100% variation. When 12V was utilized as an input source, the microcontroller was automated to manage a power supply circuit (Venus Scientific INC, NY) that can generate high output voltage up to 1500V of direct current signals. During the exposure duration, two parallel copper electrodes were utilized to conduct the signal, and the sample was positioned between them as shown in Figure 1.

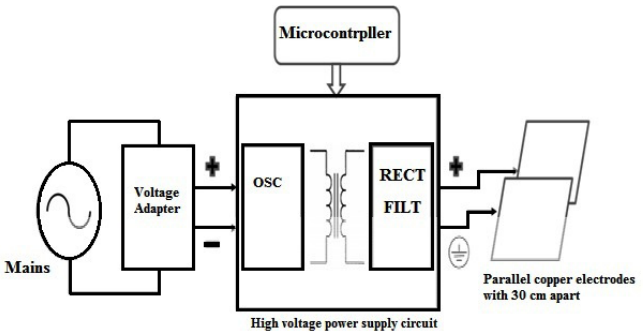

Fig. 1. *Schematic diagram for the exposure device.*

Investigation of the inhibition frequency of the E. coli bacteria

The standardized bacterial suspension (Luria Broth) was used to make the cultured broth medium, which was then incubated at 37°C for 24 hours. Subsequently, the bacteria were divided into two groups: a control group that has not been exposed and a group that has been exposed to ELFEF in the frequency range (0.2-0.4Hz) with an interval frequency of 0.1 Hz. OD values were taken every hour for four hours

to monitor bacterial growth in both exposed and unexposed samples.

Determination of the optimal exposure time

The standardized bacterial suspension was exposed to an electric field at 0.2 Hz for different exposure time ranges from 0.5 h to 2.5 h with an interval of 0.5 h. The samples were incubated at 37°C at the end of the exposure duration. The samples were shaken every hour and the absorbance was recorded.

Antibiotic susceptibility test

Antimicrobial Drugs

Susceptibility testing was determined by three distinct antibiotics: Keflex (K), piperacillin-tazobactam (PTZ), and meropenem (MEM) against *E. coli* ATCC 27853 bacterial strain.

Antibacterial Tests

The disc diffusion method was used to determine *E. coli* susceptibility to these antibiotics. The fresh incubated *E. coli* was suspended in sterilized saline (0.9% NaCl) until it was adjusted to an 0.5 McFarland turbidity standard, resulting in a suspension of 1.5×10^8 CFU/ml. *E. coli* suspension was separated into two groups, control (unexposed) and exposed, to ELFEF for 2 hours at inhibition frequency 0.2 Hz. After the exposure period (2h), bacterial *E. coli* suspensions of two groups were streaked on Mueller Hinton agar (MHA) plates. With sterile forceps, the appropriate antibiotic discs were placed on the agar surface; mild pressure was applied over the surface of each disc to ensure contact. The plates were incubated for 24 hours at 37°C. After that, each inhibition zone's diameter was measured by millimeters of the growth inhibition zones. All tests were performed in triplicate.

Dielectric relaxation measurements for the bacterial cells

The dielectric measurements were carried out for the samples in the frequency range 10Hz-MHz using a Loss Factor Meter (type: HIOKI 3532 LCR Hi TESTER, version 1.02,1999, Japan), with a sample cell (PW 9510/60, Philips). The sample cell had two squared platinum black electrodes of 0.64 cm^2 area (A), which were separated 1 cm apart (d). The capacitance of the samples was measured at each frequency and the resistance was recorded at room temperature. The absolute dielectric increment (ε_o), the relative permittivity (έ), loss tangent (tan δ), dielectric loss (ε″), conductivity (C) and relaxation time (τ) of the samples were calculated for each frequency using the following relations;

$\varepsilon' = C\,d / \varepsilon_o A$ Eq. (1)

$(\delta) = 1/2\pi f C$ Eq. (2)

$\varepsilon'' = \varepsilon'(\delta)$ Eq. (3)

$\tau = 12\pi f_c$ Eq. (4)

$\sigma = 2\pi f\, \varepsilon''\varepsilon_o$ Eq. (5)

where f_c is the critical frequency corresponding to the mid-point of dispersion curves.

Morphological examination by TEM

TEM (JEM-1400; JEOL Ltd., Akishima, Tokyo, Japan) was used to determine the morphological changes in *E. coli* caused by exposure to an electric field at the most effective frequency (0.2 Hz) for the most effective time (2 h). The bacteria sample was centrifuged and the pellet was passed through some processing according to Demicheli et al.[28] CCD camera model AMT, an optronics camera with 1632×1632 pixels, was used to capture the images for the processed bacterial samples.

Statistical analysis was performed using the statistical software package SPSS version 16.0 (SPSS Inc, Chicago, IL). Variables were presented as the mean (M) and standard deviation (SD). Multiple comparisons were performed with one-way ANOVA. A probability value of $P<0.05$ was considered statistically significant.

Results

Inhibition frequency of the *E. coli* bacteria

The bacterial growth curve of groups subjected to electric pulses of frequencies in the range 0.2-0.4 Hz was compared to that of the control group (Figure 2). After the groups were exposed to 0.2 Hz, the findings revealed a highly significant ($P<0.05$) growth inhibition. The changes in absorbance as a function of the applied frequency between bacterial groups exposed to the range 0.2-0.4 Hz and the control group are shown in Figure 3. When compared to the control, the highest growth inhibition ($P<0.05$) occurred after exposure to 0.2 Hz.

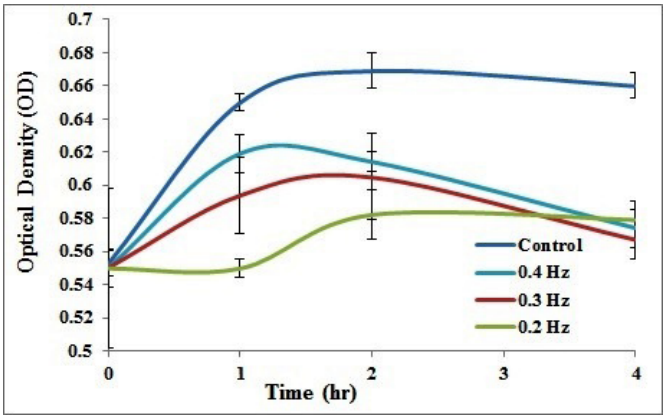

Fig. 2. *The growth curve for control (unexposed group) and exposed bacterial samples for 0.2, 0.3, and 0.4 Hz.*

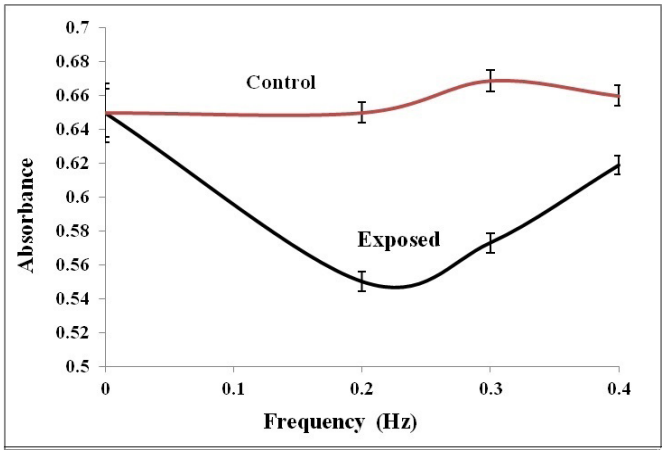

Fig. 3. *The variation in absorbance as a function of frequency after 12 hours of incubation compared to the control.*

Determination of exposure time

The growth inhibition of *E. coli* following exposure to 0.2Hz for various exposure times was studied (Figure 4). Two-hour treatment resulted in the greatest growth inhibition (P<0.05) at the specified frequency (0.2 Hz).

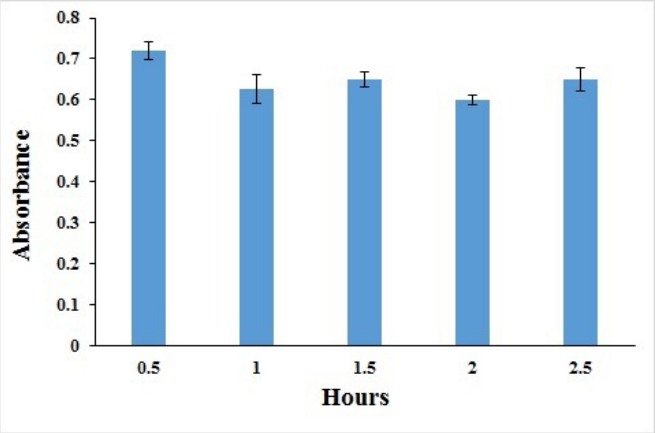

Fig. 4. *The exposure time for inhibition against OD. The samples were exposed for different exposure times ranging from 0.5 h. to 2.5 h with an interval of 0.5 h.*

The antibiotic sensitivity test

Table 1 demonstrates the antibiotic sensitivity for unexposed and exposed *E. coli* to 0.2 Hz for 2 hours, revealing a substantial difference between the unexposed (control) and exposed samples. Samples exposed to 0.2 Hz exhibited high, significant sensitivity (P<0.01) to antibiotics Keflex, Meropenem, and Piperacillin-tazobactam (cell wall synthesis inhibitors) (Table 1). All antibiotics (K, MEM, and PTZ) used in this study showed antibacterial activity against exposed and unexposed test organism *E. coli* (Table 1). On the other hand, the activity of the antibiotics was remarkably increased against *E. coli* exposed to ELFEF, compared to unexposed *E. coli*. Antibacterial activity of Keflex showed activity (21.5 mm and 17.5 mm) against exposure to ELFEF and unexposed *E. coli,* respectively. Meropenem showed activity (20 mm and 10 mm) against exposure to ELFEF and unexposed *E. col;*, finally, Piperacillin-tazobactam showed activity (19.5 mm and 16.0 mm) against exposed and unexposed *E. coli,* respectively.

Table 1.
The average diameter in mm of the inhibition zones of antibiotics against unexposed and exposed E. coli to ELFEF (0.2 Hz for 2 h)

Test organism	Mean of inhibition zone diameter (mm)		
	Antibiotics		
	K	MEM	PTZ
E. coli	17.50±0.27	10.00±0.10	16.00±0.24
Exposed *E. coli* to 0.2 Hz ELFEF	21.50±0.22	20.00±0.19	19.50±0.16

K, Keflex; MEM, Meropenem; PTZ, Piperacillin-tazobactam

The dependence of dielectric properties on ELFEF

Table 2, represented the values of the relaxation time (τ), the dielectric increment (Δέ), and the electric conductivity (σ) for the control and exposed sample. The obtained data indicated a high increase (P<0.05) in the relaxation time, dielectric increment, and conductivity for the exposed sample, compared to the control.

Table 2.
The relaxation time (τ), dielectric increment (Δέ) and conductivity (σ) of control and exposed samples to ELFEF (0.2 Hz for 2 h)

Test organism	Dielectric parameter		
	Relaxation time (τ) ($\times 10^{-6}$sec)	$\Delta\grave{\varepsilon}=(\grave{\varepsilon}0 - \grave{\varepsilon}_{\infty})$	Conductivity (σ) At 1 MHz ($\times 10^{-3}$ S/M)
E. coli control	2.2±0.26	1900.05 ±0.96	47.83 ±0.63
Exposed *E. coli* to 0.2 Hz PEF	4.9±0.67	5159.43 ±0.53	167.44 ±0.33

Investigation of morphological changes by TEM

TEM pictures of control *E. coli* cells are shown in Figure 5 (a,b), demonstrating that the cells' contents are un-damaged and their shape is preserved. The cells have a smooth outer membrane and a well-preserved cell envelope. Furthermore, the cells displayed binary fission, indicating active metabolic processes. Figure 5 (c,d) shows TEM images of cells subjected to 0.2 Hz, which demonstrate breakdown and disintegration of the cell wall as well as aberrant septation.

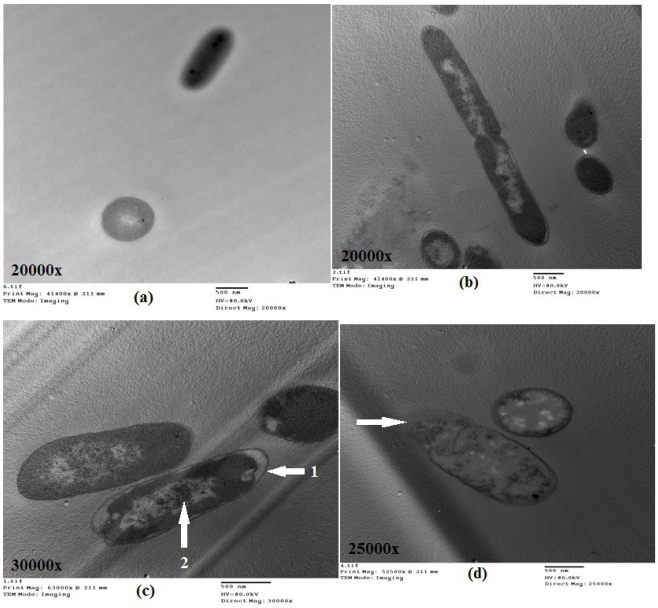

Fig. 5. (a-d) *TEM images of exposed and unexposed E. coli ATCC 27853 to ELFEF (0.2 Hz for 2 h). Transmission electron micrographs of (a) Unexposed (normal) E. coli a normal morphology and had a smooth and compact cell membrane with homogeneous electron density in the cytoplasm, (b) Unexposed E. coli cells undergoing binary fission, (c) Exposed E. coli demonstrating deformation of bacterial cell as the cytoplasm shrinks (arrow 1). The nucleus is completely damaged (arrow 2), (d) Exposed E. coli exhibit cell-wall disruption, damage to the cell membrane, and leakage of intracellular cytoplasmic constituents was observed (arrow).*

Discussion

Plotting cell growth (absorbance) versus incubation time can be used to investigate bacterial growth. It was possible to obtain a sigmoid standard growth curve. Thus, increasing the turbidity of the broth medium indicated an increase in the *E. coli* cell mass. When *E. coli* was placed into a freshly prepared medium, it took time to adapt to the new surroundings. This phase was called the Lag phase, in which cellular metabolism was accelerated, cells grew larger, but the bacteria were unable to multiply, and therefore cell mass was maintained. During the log phase, *E. coli* was growing rapidly. All their metabolic activity was increased, and the bacterial cells duplicated at a consistent pace by binary fission. The maximum amount of growth medium was used, the culture attained its maximum rate of growth, and the number of bacteria increased logarithmically (exponentially). It is known that *E. coli* split every 20 minutes, resulting in a 20-minute generation time. *E. coli* consumes all of the nutrients in the growth medium as the bacterial population grows, resulting in fast multiplication. As a result, waste products, harmful metabolites, and inhibitory chemicals like antibiotics accumulate in the medium. This changes the medium's parameters, such as pH and temperature, making the environment unsuitable for bacterial growth. The bacterium's reproduction rate will drop down until it eventually stops dividing entirely. The cell enters the stationary phase of its life cycle.

The current study presented a novel strategy for inhibiting the growth of hazardous *E. coli* strains by utilizing a low-frequency electric field at an inhibitory frequency. The current findings revealed that exposing *E. coli* to 0.2 Hz for 2 hours resulted in highly significant growth inhibition. The low-frequency, inhibitory impact of the electric field was caused by interference with bioelectric signals created by the bacterial cells' physiological processes, which disrupted the physiological process in progress.[29] It could also be linked to pH changes, antimicrobial agent synthesis, electrophoretic transfer of antimicrobial compounds into the biofilm,[30] hyperoxygenation, or the formation of extra biocide ions. Changes in cellular membrane permeability or structure may result in the loss of cell inter-constituents and/or the penetration of extracellular elements into the cell. The main source of the extremely substantial increase in electric charges is assumed to be changed in the charge distribution on the protein molecules of the cellular membrane, which can be markers of structural changes in the cellular membrane.

Antibiotic sensitivity tests involving inhibition of cell wall synthesis revealed a variation in diameter of the inhibition zone significantly, approving the influence of the inhibitory frequencies on the cell wall. Keflex, Meropenem, and Piperacillin-tazobactam act as cell wall synthesis inhibitors. All antibiotics (K, MEM, and PTZ) used in this study showed antibacterial activity against exposed and unexposed test organism *E. coli* (Table 1). On the other hand, the activity of the antibiotics was remarkably increased against *E. coli* exposed to ELFEF in comparison to unexposed *E. coli*. In previous studies, Meropenem displayed antibacterial activity as cell wall inhibitors against Enterobacteriaceae (*E. coli* and *Pseudomonas aeruginosa*); moreover, Piperacillin/tazobactam was less active against Enterobacteriaceae but not *P. aeruginosa*. Keflex (cephalexin) showed moderate activities against *E. coli* isolated from children with urinary tract infections. According to the results of A. Abduzaimovic et al.,[31] the sensitivity of *E. coli* to cefuroxime was 89.87%, ciprofloxacin - 89.24%, gentamicin - 89.24%, cefalexin - 87.97%. *E. coli* resistance to ciprofloxacin was 9.49%, gentamicin - 8.86%, cephalexin - 8.23%.

TEM images showed the rupture of the cell wall at 0.2Hz, allowing the observed loss of intercellular contents. The results of dielectric properties indicated a pronounced increase in the average values of the dielectric increment, the relaxation time, and the conductivity for exposed samples. The relaxation time and electrical conductivity are directly related to the macromolecular electric dipole moment which in turn is dependent on the size and charge of the macromolecule and thus, the significant changes in dielectric relaxation may be due to the redistribution of cellular and molecular charges. It has been verified that ELF-EMF can affect membrane functions. The frequency range used in the present study was extremely low, compared to the previous studies that used 50Hz with a moderate effect on *E. coli*.[32,33]

Conclusion

The current data suggest that treating *E. coli* with 0.2 Hz for 2 hours is an effective, prospective, and novel technique for reducing cellular growth and dramatic alteration in the cell membrane. It has the feature of being non-invasive, rapid, safe, and inexpensive when compared to conventional therapies, and it may be used to treat human illnesses and to pasteurize and sterilize food products. Future in vivo investigations on the implementation of this approach to assess its suitability as a biophysical therapy for *E. coli* infection will be possible based on the results of this research.

Acknowledgments

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Medicinal Plants

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Potential Anticoagulant Activity of *Thymus atlanticus* Leaves Part *in vitro* on Normal Plasma Samples

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Abstract

The purpose of this study was to evaluate the anticoagulant activity of *Thymus atlanticus* leaves.

Methods and Results: This cross-sectional study was carried out in Khartoum state from March 2021 to June 2021. This study comprised 60 healthy volunteers (40% men and 60% women) aged 19-25 years. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were evaluated in the blood plasma with different amounts of *Thymus atlanticus* leaves using Coatron M1 (TECO Medical Devices). *Thymus atlanticus* leaves considerably lengthened APTT and PT in all tested concentrations. Furthermore, only the APTT test at a concentration of 5.17 µl revealed a significant difference in the anticoagulant activity of *Thymus atlanticus* leaves between males and females. No correlation between age and anticoagulant activity of *Thymus atlanticus* leaves on PT and APTT in the study was found. (**International Journal of Biomedicine. 2022;12(2):299-302.**)

Key Words: activated partial thromboplastin time • prothrombin time • *Thymus atlanticus*

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Introduction

In underdeveloped countries, thromboembolic diseases, such as heart attacks, deep vein thrombosis, and pulmonary emboli, are among the leading causes of death. Furthermore, anticoagulant medications, which inhibit blood coagulation, are a powerful means of preventing thrombosis illnesses.(1) In this regard, numerous studies have found that medicinal herbs are a good source of natural anticoagulant and antioxidant chemicals.(2) The plant kingdom is an important source of antioxidant chemicals, particularly phenolic content.(3) As a result, plant-based diets can help to minimize destruction caused by oxidative stress. Atherosclerosis, on the other hand, is a vascular disease characterized by the formation of atherosclerotic plaques, which involves numerous mechanisms such as endothelial dysfunction, degradation, thrombosis, inflammation, and oxidative stress.(4) As a result, medicinal plants can be used to prevent and treat various illnesses. Thyme, a member of the Lamiaceae family, is a medicinal and aromatic plant of the Mediterranean flora that has been used in traditional medicine to treat a variety of chronic conditions,

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including hypertension, heart disease, and diabetes mellitus. Moreover, various investigations have found that *Thymus* species have anti-inflammatory, antioxidant, and antibacterial properties.[5]

The purpose of this study was to evaluate the anticoagulant activity of *Thymus atlanticus* leaves.

Materials and Methods

Study design

This cross-sectional study was carried out in Khartoum state from March 2021 to June 2021. This study comprised 60 healthy volunteers (40% men and 60% women) aged 19-25 years.

Inclusion criteria: Physically fit individuals who do not have bleeding disorders or thrombosis problems and do not use anticoagulant medications.

Exclusion criteria: Participants with a history of bleeding disorders or thrombosis illness, as well as those on anticoagulant medications.

Sampling

Each subject had 2.5 ml of fresh venous blood collected in a trisodium citrate bottle, and activated partial thromboplastin time (APTT) and prothrombin time **(**PT) were evaluated with different amounts of *Thymus atlanticus* leaves. The anticoagulant activity was assessed using Coatron M1 (TECO Medical Devices). Participants' demographic information was gathered using a structured questionnaire.

Plant Material Collection and Preparation

The leaves of *Thymus atlanticus* were obtained from a local market. They were ground, shade-dried, and ground again into a fine powder for extraction. At the Medicinal and Aromatic Plants Research Institute (MAPRI), the plant components were authenticated, and a voucher specimen was placed in the herbarium.

Preparation of Aqueous Extracts

Each extraction process used the air-dried leaf portion of *Thymus atlanticus* (20g). Plant pieces were ground to fine particles in a blender and mixed with 500 ml of double-distilled water. The sample was filtered after extraction using the techniques indicated; subsequently, the extracts were concentrated to dryness, and the residues were kept at 4°C. Each extraction experiment was repeated three times.[6] In the decoction extracting technique, aqueous preparations of plant components were cooked in water for 30 minutes before being filtered and cooled.

Soxhlet Extraction Method

In the Soxhlet extraction procedure, aqueous preparations of plant components were extracted for 5 hours using Soxhlet extractors, and the resulting boiled preparation was filtered and chilled. The powered plant was placed in a stoppered container holding 500 ml of double-distilled water in the dark for 12 hours at room temperature with constant stirring for this approach.[6]

Phenolic Compounds Determination

The total phenolic composition of the plant was measured using the method outlined by Alem et al.[7] Gallic acid was used to create the calibration curve. The total phenolic compounds were expressed in mg/g dry matter plant as gallic acid equivalent. The determination of the total flavonoid content of a plant was studied using a method proposed by Bammou et al.[8]

In Vitro Anticoagulant Activity

With only minor modifications, in vitro anticoagulant activity was assessed using the methods mentioned by Athukorala et al.[9]) For the APTT experiment, 50 µl of citrated normal human plasma was combined with 25 µl of aqueous extract and incubated for 10 minutes at 37 °C before adding 50 l of APTT reagent and incubating for 3 minutes at 37 °C.

Thus, clotting was induced and time was monitored by adding 50 µl of $CaCl_2$ (0.025 mol/L). 50 µl of citrated normal human plasma was combined with 25 µl of aqueous extract and incubated for 10 minutes in the PT assay. Then, 100 ml PT reagents were added after being pre-incubated for 10 minutes at 37°C, and the clotting time was recorded.

Statistical analysis was performed using the standard Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Continuous variables were presented as mean±standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. A probability value of $P \leq 0.05$ was considered statistically significant.

The study was approved by the Ethics Committee of the College of Medical Laboratory Sciences, Prince Sattam Bin Abdulaziz University. Written informed consent was obtained from all participants.

Results

Thymus atlanticus leaves were examined for blood coagulation effects in normal human plasma and were shown to be extremely significant prolonged APTT and PT of the human plasma ($P \leq 0.001$, Eta squared ($^2\eta$) value between 0.50 and 1.0) (Table 1). Also, there was no significant difference in *Thymus atlanticus* activity on PT according to gender; however, APTT indicated a significant difference ($P \leq 0.05$) between males and females at concentration 5.17 µl (Table 2).

Table 1.

Effects of different concentrations of Thymus atlanticus in the PT and APTT tests

Test	Concentration, µl	Mean±SD	T	*P*	Eta Squared
PT, sec	Control	14.6±1.2			
	0.18	13.4±2.1	-4.01	0.000	0.12
	1.34	25.3±1.8	38.4	0.000	0.93
	5.17	97.4±1.4	138.2	0.000	0.99
APTT, sec	Control	23.2±2.9			
	0.18	35.8±6.8	13.2	0.000	0.56
	1.34	63.3±9.5	31.2	0.000	0.89
	5.17	108.5±6.2	97.1	0.000	0.99

Table 2.

Thymus atlanticus activity on the PT and APTT tests according to gender

Test	Concentration, µl	Male (n=24)	Female (n=36)	T	*P*
PT, sec	Control	14.4±1.2	14.8±1.1	1.28	0.203
	0.18 µl	13.0±1.9	13.6±2.2	0.93	0.355
	1.34 µl	25.2±2.2	25.3±1.5	0.30	0.762
	5.17 µl	97.0±1.3	97.6±1.4	1.68	0.099
PTT, sec	Control	23.0±3.1	23.3±2.8	0.47	0.643
	0.18 µl	34.5±6.8	36.7±6.8	1.21	0.233
	1.34 µl	61.1±8.9	64.8±9.8	1.50	0.139
	5.17 µl	110.5±5.6	107.2±6.3	2.09	0.041

When PT and APTT tests were performed in different concentrations of *Thymus atlanticus* leaves among different age groups using the regression test to find a relationship between increasing the dose and age, it was discovered that no significant relationship exists in all concentrations and age groups (Table 3).

Table 3.

Regression analysis for the PT and APTT test and different concentrations of Thymus atlanticus among different age groups

Test	Concentration, µl	19-21 yrs (n=28)	>22 yrs (n=32)	T	*P*	Eta Squared
PT, sec	Control	14.6±0.88	14.7±1.42	0.272	0.786	0.001
	0.18	13.1±2.1	13.5±2.1	0.715	0.477	0.009
	1.34	25.6±1.4	25.0±2.1	1.20	0.234	0.024
	5.17	97.6±1.3	97.2±1.5	1.09	0.282	0.020
APTT, sec	Control	23.0±2.6	23.3±3.1	0.465	0.643	0.004
	0.18	36.4±6.3	35.3±7.3	0.58	0.564	0.006
	1.34	63.2±9.5	63.4±9.7	0.10	0.921	0.000
	5.17	109.2±5.6	108.0±6.7	0.704	0.484	0.008

Table 4.

Regression analysis for the different concentrations of Thymus atlanticus leaves in the PT and APTT tests

Test	Concentration, µl	B	Beta	R	R^2	F	T	*P*
PT, sec	0.18	0.05	0.089	0.089	0.008	0.46	0.68	0.050
	1.34	0.22	0.33	0.33	0.11	6.84	2.62	0.011
	5.17	-0.011	-0.013	0.013	0.001	0.010	-0.10	0.919
APTT, sec	0.18	-0.011	-0.026	0.026	0.001	0.038	-0.196	0.845
	1.34	0.073	0.241	0.241	0.058	3.58	1.89	0.064
	5.17	0.025	0.053	0.053	0.003	0.165	0.407	0.686

Furthermore, when we conducted the regression test to determine the proportion of the dose with the increase in blood fluidity, we discovered that concentrations of 0.18 µl and 1.34 µl were significantly effective in prolonging the time of the PT test, but had no effect on the APTT test (Table 4).

Discussion

Thrombosis is a major cause of cerebral infarction, acute myocardial infarction, and various types of cardiovascular illness.[10] Anticoagulants are drugs that are widely used to avoid and treat thrombotic disorders.[11] In this context, many epidemiological studies have shown that a diet rich in polyphenol compounds may reduce the development of cardiovascular disease.[12] On the other hand, the search for anticoagulant agents from natural herbal medicine is of intense interest because inhibiting blood coagulation through anticoagulant drugs is an important method of avoiding thrombotic disorders.[13]

In this study, different quantities of *Thymus atlanticus* leaves were tested for in vitro anticoagulant action in normal human plasma using two coagulation tests: APTT and PT. The APTT investigates the endogenous pathway of coagulation, whereas the PT investigates the exogenous pathway of coagulation.[14] According to the findings of our investigation, thyme leaves considerably lengthened coagulation time ($P \leq 0.05$) in a concentrated dependent way at all tested concentrations, as compared to controls in the APTT experiment.

Likewise to the APTT assay, thyme leaves inhibited clotting time in a concentration-dependent way. The current study's data clearly show that *Thymus atlanticus* leaves had a significant inhibitory effect on both intrinsic and extrinsic coagulation pathways. These findings are consistent with a recent study by Khouya et al.,[15] who found that *Thymus* plants increased the APTT and PT in human plasma. As a result, its high polyphenol content could be responsible for these effects. All aqueous extracts have significant antioxidant activity and are high in total polyphenols and flavonoids, although they behave differently in the inflammatory and coagulation processes investigated.

Hmidani et al.[6] undertook a follow-up study to assess and compare the anti-inflammatory, antioxidant, and anticoagulant effects of three thyme species grown in southeastern Morocco in vitro. *Thymus atlanticus* species were discovered to have significant antioxidant, anti-inflammatory, and anticoagulant properties. The bioactive compounds in these plants are responsible for the differences in their activities. The researchers believe that thymes have significant anticoagulant properties due to their high polyphenol content.

The study found no significant variation in coagulation time between males and females, except for APTT at a concentration of 5.17µl, when comparing anticoagulant activities of different concentrations of *Thymus atlanticus* leaves on APTT and PT according to gender. Furthermore, there was no correlation between age and anticoagulant activity of *Thymus atlanticus* leaves on PT and APTT in the study.

Polyphenol identified in *Thymus atlanticus* may be regarded as a potential bioactive compound for medicinal reasons, particularly in thrombosis-related illnesses. More research is needed to better understand the active chemicals found in Thymus plants as well as the processes through which they work. Further research with larger sample size is required to gain a deeper understanding of the population.

In conclusion, *Thymus atlanticus* leaves considerably lengthened APTT and PT in all tested concentrations. Furthermore, only the APTT test at a concentration of 5.17 µl revealed a significant difference in the anticoagulant activity of *Thymus atlanticus* leaves between males and females.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Program Development for Choosing a Surgical Treatment Option and Mathematical Prediction of Findings in Patients with Postoperative Median Abdominal Hernias

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Abstract

Background: The aim of the study was to develop a mobile program for choosing a method of surgical treatment and mathematical prediction of findings in patients with postoperative median abdominal hernias, sized small to large, using a complex of non-linear mathematical models to help a practicing surgeon.

Methods and Results: The level of intra-abdominal pressure was determined indirectly, based on SpO_2. The total electrical activity (TEA) and the degree of fatigue (DF) of the abdominal muscles were determined by electromyography. The experimental data were processed using non-linear programming methods. There has been detected a non-linear dependence of the parameters of the DE and the TEA of the abdominal muscles six months after surgery, based on their preoperative values compared to SpO_2 values on the preoperative day and on the postoperative Day 7, and compared to SpO_2 values under physical simulation of hernia repair and the selected treatment option.

Conclusion: A mobile program has been developed for smartphones that implements the recommended choice of hernioplasty technique and predicts typical features of the patient's postoperative condition.(**International Journal of Biomedicine. 2022;12(2):303-307.**)

Key Words: postoperative median abdominal hernia • hernioplasty • mathematical model • predictive software • iPhone

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Abbreviations

DF, the degree of fatigue; **HO**, hernial orifice; **MM**, mathematical models; **SpO_2**, oxygen saturation; **TEA**, total electrical activity.

Introduction

Currently, surgical interventions due to postoperative median hernias of the anterior abdominal wall are common in medical practice.[(1)] However, the issue of surgical treatment of these hernias is still challenging. These surgeries are quite complicated and dangerous: about every fourth one of them results in complications and, on average, 4% of surgeries are fatal.[(2)] Pathology is common in older patients suffering from chronic diseases that complicate the choice of treatment tactics. For example, almost 50% of elderly and geriatric patients undergo relapses during plastic surgery with their own tissues.[(3)] On the other hand, the number of postoperative hernias of small sizes, which are repaired by local tissue plastic, is increasing;[(4)] this is due to the development of laparoscopic technologies in hernia surgery. Currently, mesh

endoprostheses providing a "tension-free" technique of treating abdominal wall hernias are actively used in the treatment of postoperative median hernias.[5] However, the recurrence rate for these interventions is 30% or more due to the rejection of the endoprosthesis.[6] For large-sized hernia treatment, separation techniques are currently being introduced. Drawbacks of these types of plastic surgery are the lack of knowledge about long-term consequences, high trauma and a long duration of surgical intervention. In patients with ventral hernias, the problem of reconstruction of the anterior abdominal wall is still relevant.[7] However, where feasible, the self-tissue repair is probably the best option for surgical treatment of postoperative median hernias. The disadvantage of this technique is that the immersion of hernia components into the abdominal cavity and the reduction of the hernial orifice (HO) contribute to a sharp increase in intra-abdominal pressure, especially with extensive dimensions of the orifice. The main factors for successful surgical treatment of large postoperative ventral hernias are as follows: 1) a decrease in the intra-abdominal pressure, up to its complete elimination; 2) a decrease in the degree of tension of proper tissues.

Surgical interventions for postoperative ventral hernias, especially large ones, should be accompanied by control of the magnitude and dynamics of changes in intra-abdominal pressure. For this purpose, intra-abdominal pressure is monitored at various stages of treatment by measuring the pressure in the bladder.[8,9] However, this option is painful, poorly tolerated, and is frequently accompanied by complications. Measurement of blood SpO_2 at various stages of patients' treatment to control their intra-abdominal pressure is a more promising non-invasive, mediated technique.[10,11] In the treatment of patients with postoperative ventral hernias, it is important to restore the function of the abdominal wall muscles after hernioplasty.[9]

A common drawback of most publications on statistical modeling in medical research, for example, the condition of patients with burns[12] and peritonitis,[13] and a number of others, is that they have not explored the possibility of creating non-linear mathematical models (MM) to predict the patients' condition. However, non-linear modeling under a wide range of parameter changes appears to be the most important tool for creating adequate predictive models of the behavior of various objects, including the vital signs of patients.[14-17] Importantly for patients with hernias, neural network models have been developed,[17] which allow predicting their vital signs depending on input parameters, including a treatment option. In this regard, the development of an algorithm and a mobile program for the iPhone smartphone, which selects the method of surgical treatment and predicts the vital signs of patients using a complex of non-linear MMs, is relevant.

The aim of the study was to develop a mobile program for choosing a method of surgical treatment and mathematical prediction of findings in patients with postoperative median abdominal hernias, sized small to large, using a complex of non-linear MM to help a practicing surgeon.

Materials and Methods

The study included 55 patients with postoperative median reducible abdominal hernias who were examined and treated in the surgical departments of emergency hospitals No. 1 and No. 10, Voronezh, Russia. All patients were divided into three main groups depending on the width of the hernia orifice and the size of the hernia: 1) 16(29%) patients had small hernias with an HO width up to 5 cm; 2) 20(36%) people had medium-sized hernias with an HO width 5-9 cm; 3) 19(35%) patients had large hernias with an HO width 10 cm to 16 cm. The patients were comparable in gender, age, and comorbidity. In MM, data on gender, age, and concomitant chronic diseases were not taken into account.

The level of intra-abdominal pressure was determined indirectly, based on SpO_2.[9,11] Pulse oximeters OP-31.1 Triton T-31, Bitmos Sat 816, and Armed were used to measure blood saturation. No significant differences were detected in the assessment of SpO_2 values when measured with these devices. Blood SpO_2 level was determined several times – prior to surgery at rest and during physical modeling of the postoperative condition. Physical modeling was implemented by contracting the abdomen and bringing the hernia orifice closer together with the simultaneous immersion of the hernia components into the abdominal cavity by the pelota. A decrease in SpO_2 was recorded on Days 1, 2, and 7 after surgery.[8,9] The total electrical activity (TEA) and the degree of fatigue (DF) of the abdominal muscles were determined before surgery and six months after surgery according to EMG data using the "Neuro-MVP" Neurosoft device. During static loads, the TEA parameters of the muscles of the anterior abdominal wall were recorded first, and then the DF muscle values were calculated.[18] The following surgical treatment options were used for patients with postoperative median reducible hernias of the abdomen: 1) duplication plasty using local tissues (according to Sapezhko); 2) surgical bridge plasty with surgical wound immobilization; 3) instrumental 2-stage technique according to E. N. Lyubykh; 4) posterior separation plasty with retromuscular endoprosthesis; 5) flap endoprosthetics (correction) of the anterior abdominal wall (intramuscular mesh prosthesis).

Statistical data processing and computational experiments were conducted at the Department of Higher Mathematics and Information Technology, Voronezh University of Engineering Technologies. The experimental data were processed using non-linear programming methods: a genetic algorithm and the Hooke-Jeeves configuration technique implemented in the author's interactive identification MM system.[19] Because the developed MMs have a significant nonlinearity of the internal structure, the significance of the coefficients was estimated by the relative error achieved by the model in accordance with the special criterion.(1) In addition, the adequacy of the developed regression models was tested using the Fisher criterion in the absence of parallel experiments.

Results

Table 1 demonstrates the experimental data obtained in patients with large hernias who underwent an instrumental 2-stage technique surgery, endoprosthetics and posterior separation plasty. The HO size was 10-16 cm. The structure of the MM was detected in the process of computational

experiments in an interactive mode. When searching for MM coefficients, the sum of root-mean-square deviations of the calculated patients' findings from the measured ones was minimized:

$$R=\sum_{i=1}^{L_p}\left(1-\frac{P_i^{pac}(\mathbf{A})}{P_i^{экс}}\right)^2 \xrightarrow[\mathbf{A}]{} \min, \quad (1)$$

where L_p - the amount of processed experimental data when modeling the P value;

$P_i^{pac}, P_i^{экс}$ -calculated and experimental P value of the state of the i-th patient with similar plastic surgery;

A – vector of nonlinear MM coefficients.

In this way, the calculated formulas for SpO_2 of patients on Days 1, 2, and 7 after surgery were obtained, as well as DF and TEA six months after surgery.

1. Mathematical modeling of the dependence of saturation measured on Day 1after surgery on the preoperative parameters of the patient:

a) Large hernias, the treatment option is "two-stage instrumental technique": (2)

$$S_1=a_1\cdot S^M+a_2\cdot S^M\cdot 10^{11}\exp\left(-0,3\cdot S^M\right)+a_3\cdot S^M\cdot\ln S^M+a_4\cdot S^M\cdot 10^{-2}\cdot\sqrt{S^0-S^M}\cdot\ln A^0/U^0+$$
$$+a_5\cdot\ln U^0/\ln A^0+a_6\cdot\left(\sqrt{S^0-S^M}\cdot\ln A^0/U^0\right)^{a_7}+a_8\cdot\left(\sqrt{S^0-S^M}\cdot\ln A^0/U^0\right)^{a_9},$$

where S_1- SpO_2 on Day 1 after surgery, %; S^0 - SpO_2 prior to surgery at rest, %; S^M - SpO_2 under physical simulation, %; A^0, U^0 - TEA (mkV) and DF values prior to surgery at rest.

b) Large hernias, the treatment option is "endoprosthetics": (3)

$$S_1=a_1\cdot S^M+a_2\cdot S^M\cdot 10^{10}\exp\left(-0,28\cdot S^M\right)+a_3\cdot S^M\cdot\ln S^M+a_4\cdot S^M\cdot 10^{-2}\cdot\sqrt{S^0-S^M}\cdot\ln A^0/U^0+$$
$$+a_5\cdot\ln U^0/\ln A^0+a_6\cdot\left(\sqrt{S^0-S^M}\cdot\ln A^0/U^0\right)^2.$$

Table 1.

Experimental data of patients with large abdominal hernias (M W3-4 R0)

№№	S^0, %	S^M, %	U^0	A^0, mkV	S_1, %	S_2,%	S_7, %	$U_{0,5}$	$A_{0,5}$, mkV
Treatment option - two-stage instrumental technique									
1	97.0	86.2	2.29	506	84.0	86.3	96.5	1.8	527
…									
8	96.8	86.0	2.35	504	83.7	86.0	96.5	1.78	527
Treatment option - endoprosthetics									
9	96.5	87.3	2.7	421	90.0	91.5	95.3	2.89	414
…									
15	96.8	87.2	2.61	425	90.5	92.1	95.5	2.79	418
Treatment option – posterior separation									
16	97.5	89.5	2.14	507	84.7	85.6	96.0	1.95	518
…									
19	97.5	89.6	2.15	510	84.6	85.8	96.1	1.9	521

c) Large hernias, the treatment option is "posterior separation": (4)

$$S_1=a_1\cdot S^M+a_2\cdot S^M\cdot 10^{10}\exp\left(-0,28\cdot S^M\right)+a_3\cdot S^M\cdot\ln S^M$$

Table 2 demonstrates the MM coefficients (2), (3), (4).

Thus, MMs of saturations were obtained for three surgical techniques of large hernia repair depending on the preoperative parameters of the patient. The relative error of modeling SpO_2 was within ±0.2%.

Table 2.

Model coefficient values (2), (3), (4)

Treatment option - two-stage instrumental technique				
$a_1=-578.36351$	$a_2=2.010195$	$a_3=151.23776$	$a_4=-2452.898$	$a_5=-378.5249$
$a_6=141.52329$	$a_7=2.0$	$a_8=-0.12163317\cdot 10^{-2}$		$a_9=6.0$
Treatment option - endoprosthetics				
$a_1=49.346888$	$a_2=-2.072135$	$a_3=-10.958287$	$a_4=12.828297$	$a_5=269.95193$
$a_6=-0.41083574$				
Treatment option – posterior separation				
$a_1=-12.558293$	$a_2=0.58435778$	$a_3=2.9763028$		

2. SpO_2 modeling on Days 2 and 7 after the operation was performed according to the general formula:

$$S_{2,7}=a_1+a_2\cdot\left(\sqrt{S^0-S^M}\cdot\ln A^0/U^0\right)+a_3\cdot\left(\sqrt{S^0-S^M}\cdot\ln A^0/U^0\right)\times \quad (5)$$
$$\times\exp\left(-a_4\cdot\tau\right)+a_5\cdot S^M\cdot\ln A^0/U^0+a_6\cdot\left(S_1-S^M\right)+a_7\cdot\ln U^0/\ln A^0,$$

where S_2 и S_7 – SpO_2 of the patient on Days 2 and 7 after surgery, respectively; τ – is equal to Day 2 or Day 7.

Table 3 shows MM coefficients (5) for plastic surgery techniques: two-stage, endoprosthetics, and posterior separation. According to the formula (5), the average relative error of SpO_2 modeling was ±0.3%.

Table 3.

MM coefficient values (5)

Treatment option - two-stage instrumental technique				
$a_1=-77.76535$	$a_2=2.5291146$	$a_3=4.0145439$	$a_4=0.61454595$	$a_5=0.3327949$
$a_6=0.080563498$	$a_7=558.03233$			
Treatment option - endoprosthetics				
$a_1=-54.928943$	$a_2=-1.5786892$	$a_3=-0.9298535$	$a_4=-0.07855635$	$a_5=0.38088$
$a_6=0.78321156$	$a_7=446.24765$			
Treatment option – posterior separation				
$a_1=-118.95495$	$a_2=8.4055883$	$a_3=-0.52959447$	$a_4=-0.1922029$	$a_5=0.14555876$
$a_6=-3.303022$	$a_7=615.75785$			

According to formula (5) the average relative error of SpO_2 modeling is ±0.3%.

3. General models of the degree of fatigue of the patient's abdominal muscles and their electrical activity six months after surgery are:

$$U_{0,5}=U^0+a_1\cdot S_7+a_2\cdot S_7^2+a_3\cdot\sqrt{S^0-S^M}\cdot\ln A^0/U^0+a_4\cdot\left(S^0-S^M\right)+a_5\cdot\left(S_7-S^M\right)^2; \quad (6)$$

$$A_{0,5}=A^0+a_1\cdot S_7+a_2\cdot S_7^2+a_3\cdot\sqrt{S^0-S^M}\cdot\ln A^0/U^0+a_4\cdot\left(S^0-S^M\right)+a_5\cdot\left(S_7-S^M\right)^2, \quad (7)$$

where $U_{0,5}$ и $A_{0,5}$ – are DF and TEA values six months after surgery, respectively.

Table 4 demonstrates the coefficients of models (6) and (7) for the studied options of plastic surgery. The average relative error of the DF and TEA modeling calculated by formulas (6) and (7) was within ±2-3 %.

The algorithm for choosing the surgical treatment option is implemented as follows: The HO width determines further analysis of the patient's condition and, depending on the combination of values, the difference between the initial and simulated SpO_2, and terms of hernia, a certain plastic treatment option is selected. The algorithm uses the limiting values of TEA and DF equal to A=500μV and U=2.4, their combinations are crucial to conclude if the abdominal muscle functions are preserved. If $A^0 \geq 500$ mkV and $U^0 \leq 2.4$, then it is assumed that the functions of the abdominal muscles are

preserved. Otherwise, if $A^0 < 500$ mkV and $U^0 > 2.4$, then it is assumed that the functions of the abdominal muscles are lost. Variants with partial preservation of the anterior abdominal wall muscle functions are also considered. In cases of large hernias, when the functions of the muscles are not preserved and the term for which the hernia is carried is 5 years or more, corrective methods of surgical treatment (endoprosthetics) are recommended to the patient. Otherwise, a 2-stage instrumental technique or posterior separation plastic is proposed, depending on the difference between preoperative and simulated SpO_2.

To implement the algorithm, a mobile application for the iPhone smartphone running iOS version 14 and higher has been developed. Objective-C is a programming language, Xcode 12.5.1 is a development environment.

Table 4.

MM coefficient values (6) and (7)

Treatment option - two-stage instrumental technique, MM (6)			
$a_1 = 0.10638154$	$a_2 = -0.10995636 \cdot 10^{-2}$	$a_3 = 0.19294668$	$a_4 = -0.21550095$
$a_5 = 0.6143278 \cdot 10^{-3}$			
Treatment option - two-stage instrumental technique, MM (7)			
$a_1 = 11.718234$	$a_2 = -0.13230293$	$a_3 = 30.811606$	$a_4 = -26.992092$	$a_5 = 1.3228408$
Treatment option - endoprosthetics, MM (6)			
$a_1 = -0.27254978$	$a_2 = 0.2896318 \cdot 10^{-2}$	$a_3 = -0.64509639$	$a_4 = 0.58871533$
$a_5 = -0.018020586$			
Treatment option - endoprosthetics, MM (7)			
$a_1 = 81.043115$	$a_2 = -0.82771741$	$a_3 = 81.783149$	$a_4 = -141.50292$	$a_5 = 8.3268172$
Treatment option – posterior separation, MM (6)			
$a_1 = 0.09674961$	$a_2 = -0.11747287 \cdot 10^{-2}$	$a_3 = 0.15946951$	$a_4, a_5 = 0$
Treatment option – posterior separation, MM (7)			
$a_1 = 0.22938094$	$a_2 = -0.11963044 \cdot 10^{-2}$	$a_3 = 0.56666165 \cdot 10^{-3}$	$a_4, a_5 = 0$

Discussion

Figure 1 shows the results of the program for choosing the surgical treatment options for patients with large hernias and predicting their vital signs.

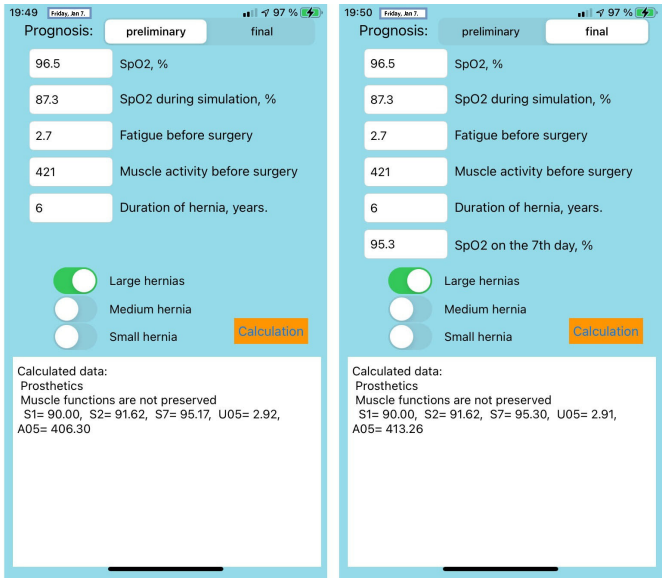

Fig. 1. *Program interface for the surgeon's mobile phone for choosing the plastic treatment option and predicting the patient's condition (a, b - "endoprosthetics")*

First, according to the patient's initial findings, the mobile program selects the plastic technique option in accordance with the programmed algorithm. Then, for the surgical technique selected by the program, a "preliminary" calculation of the patient's postoperative condition is carried out based on the preoperative findings of the patient's condition according to the models (2)-(7). The SpO_2 value is measured on Day 7 after the surgery; it is entered into the smartphone program and substituted into models (6) and (7). In such a way, the "final" calculation of the patient's DF and TEA vital signs is realized six months after the operation. Comparison of the data in Figure 1 and Table 1 (No. 9) evidences a high convergence of the results obtained and potential of the program's application in medical practice.

Conclusion

An algorithm has been developed for choosing the surgical treatment option and a complex of non-linear mathematical models to assess the vital signs of the patient's postoperative condition with median reducible abdominal hernias of various types and sizes. A mobile program that recommends a plastic surgery option and predicts typical features of the patient's postoperative condition has been developed to help the surgeon; it is installed and operates on an iPhone smartphone running iOS version 14 and higher. Objective-C is a programming language, Xcode 12.5.1 is a development environment.

Disclaimer

Views expressed in the submitted article belong to the authors and not to the university and funder.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Novel Technology and Devices

INTERNATIONAL JOURNAL OF BIOMEDICINE

Results of Evaluation of the Physical and Mechanical Properties of a New Suture Material with Miramistin Coating

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Abstract

Background: The imperfection of surgical sutures, from the point of view of insufficient prevention of microbial contamination in the area of suturing, is one of the urgent problems in modern practical healthcare. In this connection, one of the promising areas at the present time is the study of suture material under conditions of its coating with various antiseptics. This study aimed to evaluate the physical and mechanical properties of the suture material impregnated with miramistin for further application in experimental studies on animals.

Methods and Results: By applying a two-layer polymer coating on absorbable polyglycolide threads, the threads were given antimicrobial properties. In the amount of 10% or 20% by weight of the polymer, miramistin was introduced into both coatings.

The breaking load and elongation data in the surgical suture knot were evaluated by immersing the samples in a phosphate buffer solution (pH=7.4), maintaining a constant temperature of 37°C by a thermostat. Threads from the solution were extracted on Days 1, 3, 7, 14, and 21 of the experiment. Further, the studied samples of the suture material were subjected to stretching to rupture on a universal tensile testing machine of the domestic company Metrotest. Physical and mechanical properties were evaluated by the dynamics of the breaking load and elongation at a break in the node after being in a phosphate buffer solution. Coating of polyglycolide thread, which is a suture material, with miramistin at a concentration of 10% and 20% does not lead to loss of strength even in the long term of the experimental study, which, in turn, meets the standards for suture material.

Conclusion: The results obtained allow us to recommend a suture material impregnated with miramistin for further research, the ultimate goal of which will be the possibility of active use of this material in practical medicine, in particular in surgery to prevent microbial contamination in the area of suturing and infection of wounds. (**International Journal of Biomedicine. 2022;12(2):308-310.**)

Key Words: miramistin • suture material • microbial contamination • breaking load • breaking elongation

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Introduction

One of the important directions in modern medicine is the development of surgical suture material. The rate of occurrence of the wound and postoperative complications requires new trends in this area. Quite often, practicing surgeons are faced with imperfect surgical threads, which means low prevention of microbial contamination in the area of suturing.[1-3] This is due to a local disorder of microcirculation of tightened tissues, partial necrosis of cells, and accumulation of inflammatory exudate. To solve this problem and reduce the risk of suture failure, various drugs have been applied to the surface of the suture material, such as proteolytic enzymes, antibiotics, antiseptics, analgesics, etc. Thanks to the development of an antibacterial coating in the area of surgical intervention, it is possible to create an environment that can stop the growth of pathogenic microorganisms and reduce the dose of general antibiotic therapy, which is important for

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immunocompromised patients, as well as elderly patients.[4-6] In practical surgery, both biodegradable (absorbable) suture material and non-absorbable suture material are used. The latter often remains forever in the area of operation and can, in some cases, become a source of chronic inflammation. To date, among domestic modern surgical threads, only a small number can be considered both biodegradable and having an antibacterial component on their surface, which indicates the relevance of scientific research aimed at creating and studying the characteristics of such suture materials.[7-9] Antiseptics remain the most promising substances in this direction, given the frequent side effects that occur when using antibiotics, as well as a decrease in the sensitivity of pathogenic microflora. Miramistin can be regarded as the universal antiseptic. Its wide range of uses in various fields of medicine opens up prospects for the development of surgical suture material.

This study aimed to evaluate the physical and mechanical properties of the suture material impregnated with miramistin for further application in experimental studies on animals.

Materials and Methods

The experimental study was carried out in the department "Experimental Surgery and Oncology of the Research Institute of Experimental Medicine" at the Kursk State Medical University.

By applying a two-layer polymer coating on absorbable polyglycolide threads, the threads were given antimicrobial properties. In the amount of 10% or 20% by weight of the polymer, miramistin was introduced into both coatings. The samples of suture material used in the experiment were provided by Lintex LLC. All experimental material was divided into three groups. An inert suture material was used in Group 1 (control group, n=10), a polyglycolide thread coated with miramistin at a concentration of 10% in Group 2 (comparison group, n=10), and a polyglycolide thread coated with miramistin at a concentration of 20% in Group 3 (experimental group, n=10). The breaking load and elongation data in the surgical suture knot were evaluated by immersing the samples in a phosphate buffer solution (pH=7.4), maintaining a constant temperature of 37°C by a thermostat. Threads from the solution were extracted on Days 1, 3, 7, 14, and 21 of the experiment. Further, the studied samples of the suture material were subjected to stretching to rupture on a universal tensile testing machine of the domestic company Metrotest. Physical and mechanical properties were evaluated by the dynamics of the breaking load and elongation at a break in the node after being in a phosphate buffer solution.

Statistical analysis was performed using Microsoft Excel 2020. The mean (M) and standard error of the mean (SEM) were calculated. Differences of continuous variables were tested by the Mann-Whitney *U*-test. A probability value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

As a result of the study to determine the breaking load in the knot, it was found that the initial breaking load of all samples of suture material met the standards for absorbable surgical sutures. At the same time, it should be noted that the gradual decrease in the strength of the samples is directly proportional to the duration of their immersion in the buffer solution. In particular, by the end of the experiment (on Day 21), the breaking load of the threads decreased, without reaching, at the same time, zero values. The degree of decrease in indicators at all periods of the experiment in the second and third groups was almost equal. At the same time, the lowest indicator of values was noted in the first group of suture material samples, which did not contain an antiseptic coating on their surface. The results obtained are presented in Table 1.

Table 1.

Breaking load in a simple knot of absorbable suture materials (n)

Day	Group 1	Group 2	Group 3
1	13.42±0.254	14.61±0.142*	15.4±0.141* ^
3	12.7±0.15	14.05±0.184*	14.5±0.133* ^
7	11.3±0.142	13.5±0.144*	13.6±0.08* ^
14	10.72±0.072	11.7±0.07*	12.6±0.104* ^
21	6.4±0.154	7.33±0.106*	7.62±0.114*

** - P≤0.05 when compared with Group 1; ^ - P≤0.05 when compared with Group 2*

In the second stage of this work, the breaking elongation of the thread in a simple knot was carried out under the conditions of placing the threads in a buffer solution and keeping them there for Days 1, 3, 7, 14, and 21. The results obtained are presented in Table 2.

According to the data obtained, in all samples of suture materials, there was a gradual decrease in the elongation index when the thread broke in a simple knot. It should be noted that the value of this indicator in the early stages of the experiment was greater in Group 1 on Days 1, 3, and 7 than in Group 2 and Group 3. By Day 21 of the experiment, the elongation of the breaking load of the threads decreased but did not reach zero values. The degree of decrease in indicators at all periods of the experiment was approximately equal in Groups 2 and 3.

Table 2.

Elongation data in the surgical suture knot (%)

Day	Group 1	Group 2	Group 3
1	23.63±0.14	19.42±0.115*	19.53±0.135*
3	22.33±0.114	18.5±0.1*	18.6±0.114*
7	20.6±0.14	17.6±0.1*	17.5±0.1*
14	15.5±0.28	14.7±0.16	14.34±0.09*
21	10.4±0.101	10.6±0.2	10.8±0.106

** - P≤0.05 when compared with Group 1*

One of the main requirements for a surgical suture is to maintain its strength. For absorbable threads, this indicator should correspond to the time of complete healing of tissues in the area of surgical intervention to form a postoperative scar. The elongation index in a simple knot, according to the standard applied to the suture material, should not exceed 40%. Thus, the new samples of the studied suture material, which have miramistin in their coating at a concentration of 10% and 20%, meet the regulatory requirements.

Conclusion

The polyglycolide thread coated with miramistin at a concentration of 10% and 20% did not lose its strength even on the last days of the experiment, which meets the standards for suture material and allows us to recommend it for further research.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Effective Management of Non-Surgical Periodontal Treatment in a Patient with Severe Gingival Enlargement and Periodontal Bone Loss: A Case Report

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Abstract

The immune-inflammatory response of periodontal tissue can be influenced by various systemic variables such as hormone disorders, hematologic diseases, nutrition, and medications. Inflammatory cytokines released by infected periodontal tissue are implicated in suppressing erythrocyte growth, differentiation, and erythropoiesis inhibition.

We present a case of non-surgical periodontal treatment in a 31-year-old female patient with severe gingival enlargement associated with aggressive bone destruction combined with iron deficiency anemia (IDA). The periodontal treatment plan was achieved in collaboration with the hematologist, starting with oral hygiene instruction, scaling and root planning (SRP) by sextants with systemic antibiotic therapy combined with a chemical agent and low-level-laser-therapy (LLLT), and extraction of hopeless teeth. Effective non-surgical periodontal treatment with SRP and LLLT reduces inflammation and gingival enlargements and controls the recolonization of subgingival sites by periodontal pathogens as a crucial factor to proceed with periodontal surgery. Function, aesthetics, and the patient's social communication are all improved.(**International Journal of Biomedicine. 2022;12(2):311-315.**)

Key Words: scaling and root planning • periodontal pockets • gingival enlargment • low level laser therapy

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Abbreviations

BOP, bleeding on probing; **CBCT**, cone-beam computed tomography; **GI**, gingival index; **IDA**, iron deficiency anemia; **LLLT**, low-level-laser-therapy; **MCV**, mean corpuscular volume; **MCH**, mean corpuscular hemoglobin; **MCHC**, mean corpuscular hemoglobin concentration; **PD**, periodontal disease; **PBD**, probing depth; **PI**, plaque index; **PPD**, periodontal pocket depth; **SRP**, scaling and root planning.

Introduction

The local microbiota and host immune response are the most significant etiologic and risk factors in the initiation and development of periodontitis, where uncontrolled gingival inflammation can lead to periodontal cellular injury and tooth loss.[1]

Periodontal disease (PD) with complex etiology is a dysbiotic inflammatory disease that has adverse implications for the overall health-inducing inflammatory disease at both local and distant levels.[2] It affects the host on multiple levels: at the microbial level, based on the presence of dysbiotic microbial communities with the potential for destructive inflammation; at the host level, based on genetic factors that may increase hyper-inflammatory phenotype; and at the level

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of environmental stimuli and systemic health conditions, which alter the host's protective or destructive reaction.[3,4] Periodontal microorganisms, predominantly Gram-negative bacteria, trigger an immunological response from the host and injure periodontal tissue, where inflammatory cytokines, which are released by infected periodontal tissue, are implicated in suppressing erythrocyte growth and differentiation.[5] Inflammatory cytokines, including IL-1, IL-6, and TNF, are involved in erythropoiesis inhibition. All of the facts mentioned above suggest that PD raises the danger of iron deficiency anemia (IDA). Iron is essential for the production of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation reduction, as well as the synthesis of oxygen transport proteins such as hemoglobin and myoglobin.[6] A reduction in oxygen in the tissues in the anemic condition has been hypothesized to contribute as a modifying feature in the periodontium. These diseases and conditions do not cause periodontitis, but they may contribute to accelerating its course, or exacerbating it.[7] Individuals at risk for periodontal disease can be identified by identifying risk factors associated with periodontitis, which is directly related to periodontal patient treatment.

Periodontitis treatment attempts to prevent the infection from progressing further in order to lower the possibility of tooth and bone loss, as well as the symptoms and complications of the disease. The key approaches in periodontal treatment are oral hygiene instructions, a smoking cessation program, diets, plaque and calculus removal, scaling and root planning (SRP), local and systemic medication, low-level-laser-therapy (LLLT), and various forms of periodontal surgery.[8,9]

Case Presentation

A 31-year-old female patient was referred to our clinic, the Department of Periodontology at the University Dentistry Clinical Center in Kosovo. The patient had several complaints of progressive swelling and bleeding gums associated with difficulty in swallowing and speaking, time after time, with pain in gums and luxation of front teeth. The complaints continued for several months, and she did not receive any periodontal treatment except some mouthwashes.

The patient's anamnestic data stated that she has fatigue and weak immunity, and tends to get cold very often. The extraoral clinical examination showed no significant change, while the intraoral examination showed changes in the gingiva: reddish-purple color, enlarged, covering the crown on all existing teeth, soft, shiny, with a tendency to bleed at the slightest touch. In gingival compression in some teeth, there was purulent secretion, and some of the front teeth were dislocated. There was a considerable amount of supra and subgingival deposits, and dental plaque on the teeth and gums. Due to the existing deep periodontal pockets, some of the teeth have migrated, forming two rows of teeth in the lower jaw, or changed position and migrated in different directions (Figures 1 and 2). The patient's dental cone-beam computed tomography (CBCT) revealed significant bone resorption, as well as teeth in the advanced stages of periodontitis that should be removed and periodontal infrabony pockets in the majority of the remaining teeth (Figure 3). The diagnosis was aggressive, severe periodontitis associated with severe gingival enlargement based on the symptoms, clinical examinations, and periodontal parameters: periodontal pocket depth (PPD), clinical attachment level, gingival index (GI), sites with bleeding on probing (BOP), and plaque index (PI) described above.

Fig. 1. *The first visit to our clinic: Gingival inflammation with reddish-purple color, enlarged covering the crown on existing teeth, soft, shiny, with a tendency to bleed at the slightest touch.*

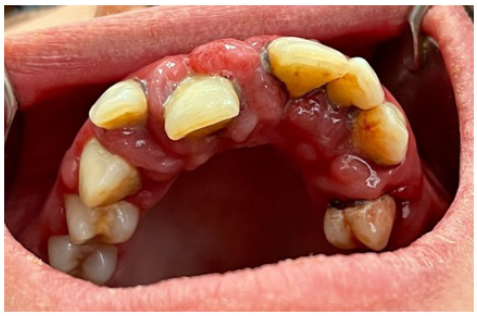

Fig. 2. *The first visit to our clinic: Several gingival enlargements in palatinal side associated with tooth migration.*

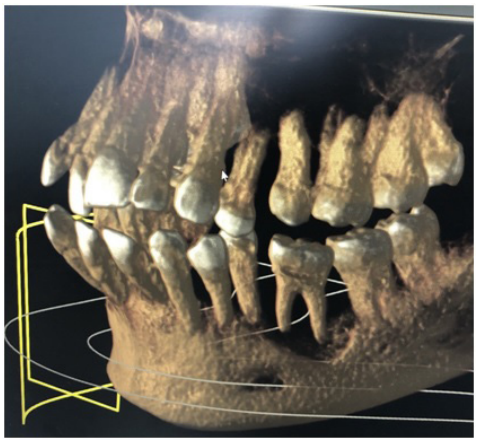

Fig. 3. *Bone loss: vertical resorption*

Laboratory examination revealed decreased levels of red blood cell (RBC) count, hemoglobin (HGB), and hematocrit (HCT), low levels of ferritin and iron in serum, MCH, MCHC and MCV, and slight elevation of leukocytes (Table 1). Other blood parameters were within the normal range.

Table 1.

Blood parameters prior to periodontal treatment

Parameter	Measured values	Normal range
Leukocytes (10^3/μL)	11.5	4-11×10^3/μL
Neutrophils (%)	55.3	40-60%
Lymphocites (%)	31.0	25-40%
Monocites (%)	8.0	2-8%
Bazophiles (%)	0.4	0-1%
Eosinophils (%)	2.3	1-4%
Red blood cell (10^6/μL)	3.4	4.1-5.4 ×10^6/μL
Hemoglobin (g/L)	9.1	12-17.5 g/L
Haematocrit (%)	32	35-50%
Erythrocyte sedimentation rate (mm/h)	15	3-15 mm/h
MCH (pg)	25	27-32 pg
MCHC (g/dL)	28.2	32-36 g/dL
MCV(fL)	85.7	80-95 fL
Serum iron (μmol/L)	25	60-150 μmol/L
Ferritin (μg/L)	6.7	15-200 μg/L

IDA was diagnosed after referring the patient to a hematologist, and additional laboratory testing and iron-based treatment were followed. Basic periodontal treatment is started after the anemia has improved. Periodontal treatment for the patient included oral hygiene instruction based on the Bass technique and flossing, full mouth root scaling and planning with an ultrasonic scaler and Gracey curettes by sextants, and systemic antibiotic therapy (Amoxiclaf 625 mg tablets + metronidazole 250 mg tablets twice daily for 7 days). The patient was recommended to rinse her mouth twice daily for 14 days using 15 ml of 0.2% chlorhexidine gluconate mouthwash and the teeth in the late stage of periodontitis were extracted. After a month, periodontal pockets were irrigated with 0.2% chlorhexidine gluconate (Figure 4).

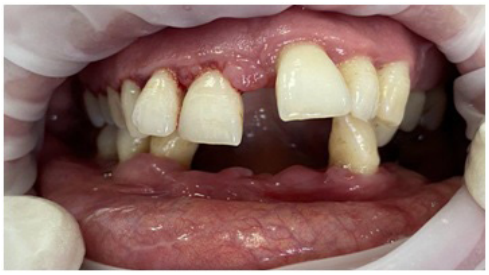

Fig. 4. *Four weeks after a visit to our clinic: After the full mouth SRP combined with systemic antibiotic therapy and mouth wash.*

After two months, the periodontal pockets were treated with LLLT. The patient was treated with a diode laser (Laser HF, Hager-Werken, Germany), with each tooth inside the periodontal pocket exposed for one minute using a light of wavelength 980 nm and a power of 10 mW. The laser fiber was placed in the periodontal pocket during the exposure (Figure 5).

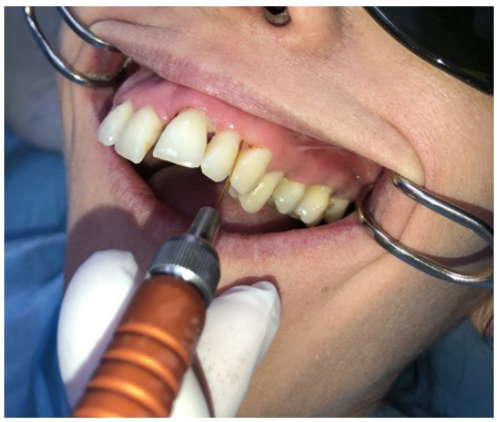

Fig. 5. *Two months after visit to our clinic: LLLT of periodontal pockets.*

After non-surgical periodontal treatment, we had an improvement in the periodontal parameters PPD, GI, BOP, and PI. Complete remission of gingival inflammation resolved 11-12 weeks after initial admission to our department. Gingiva was a pink color, with normal form and size, did not bleed after probing, and the patient had no bad breath. In terms of functionality, she could eat more comfortably and communicate more easily, and her social life improved (Figures 6 and 7).

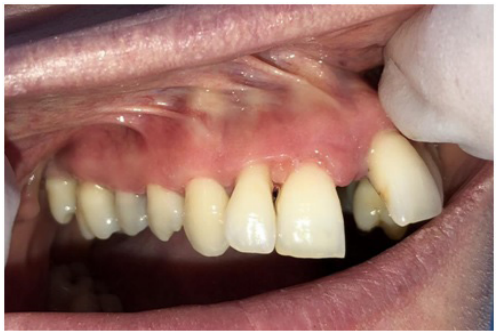

Fig. 6. *Three months after the first visit to our clinic: Gingiva on right buccal maxillary side is healthy with no signs of inflammation*

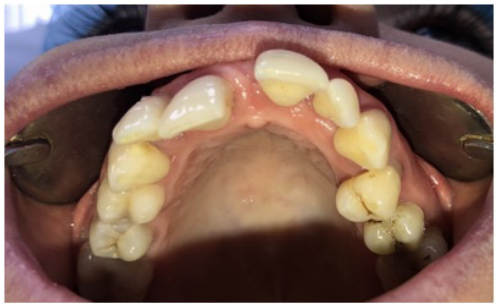

Fig. 7. *Three months after the first visit to our clinic: Gingiva on palatinal side is healthy with no signs of inflammation*

Following a definitive SRP, sites with continuous active probing depths and symptoms of inflammation, and levels of attachment loss associated with intra-bony defects, are considered for periodontal regenerative surgery.

Discussion

The treatment of periodontal disease in its different forms has progressed over the last century, with studies demonstrating that the elimination of biofilms, both through SRP periodontal therapy and rigorous home oral hygiene, combined with systemic or local administration of chemotherapeutic drugs, showed impressive clinical, microbiological, and inflammatory outcomes.[10] According to studies, SRP reduces the pocket depth and eliminates clinical symptoms of inflammation, such as edema and bleeding on probing (BOP), by aiming at the primary objective: reduce the bacterial load and keep infection under control.[11]

According to current research, inflammation caused by periodontal pathogens impacts both the oral cavity and the patient's overall health, and the presence of calculus and biofilm is definitely linked to periodontal inflammation,[12] and it is also evident in our case study, where local irritational factors dental plaque, calculus, debris and staining associated with poor oral hygiene were evident in all existent teeth.

It is essential to identify systemic diseases or conditions that are directly related to periodontitis progression but also have an impact on the periodontal treatment approach. As the periodontal tissues produce an immunological inflammatory response to pathogens and their byproducts, systemic exposure to these substances triggers a significant vascular response. Certain inflammatory cytokines released during periodontal inflammation can inhibit erythropoietin production, resulting in anemia;[13] findings of a periodontal examination and blood sample in over 800 subjects revealed that periodontitis is characterized not only by leukocytosis but also by a predisposition to "anemia of inflammation."[14] The recent data show that periodontitis reduces Hb concentration and alters iron metabolism balance, confirming the severity of the link between severe periodontitis and the tendency of "anemia of inflammation" to develop.[15]

SRP with LLLT has been suggested for its photochemical significance in anti-inflammation, biostimulation, and analgesia and has a biomodulatory effect on periodontal tissue. The results of a low-level laser are attributable to its non-heating effects, which stimulate fibroblast reproduction; LLLT can accelerate the repair process, reduce post-operative pain, influence inflammation, improve wound healing after gingivectomy, and other procedures.[16-18]

SRP helps periodontal tissues heal by significantly reducing PBD, GI, BOP, and PI due to recollagenation of supracrestal fibers, reducing the inflammatory infiltrate, and changing the supra- and subgingival bacterial flora, with a decrease in Gram-negative and an increase in Gram-positive bacteria.[19-22] LLLT can only be used in combination with non-periodontal treatment and cannot replace regular SRP procedures. Crispino et al.[23] reported that combining mechanical treatment (SRP) with diode laser therapy gives more significant effects than laser treatment alone, in this regard.

In conclusion, mechanical, nonsurgical periodontal therapy, also referred to as scaling and root planing or subgingival debridement, is significantly efficacious in not only treating periodontitis but is also integrated as a part of overall health management.

Competing Interests

All authors declare that they have no competing interests.

Ethics Approval and Consent to Participate

This case report was reviewed and approved by the Joint Ethics Committee of the University Dentistry Clinical Center of Kosovo. Written informed consent was obtained from our patient.

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