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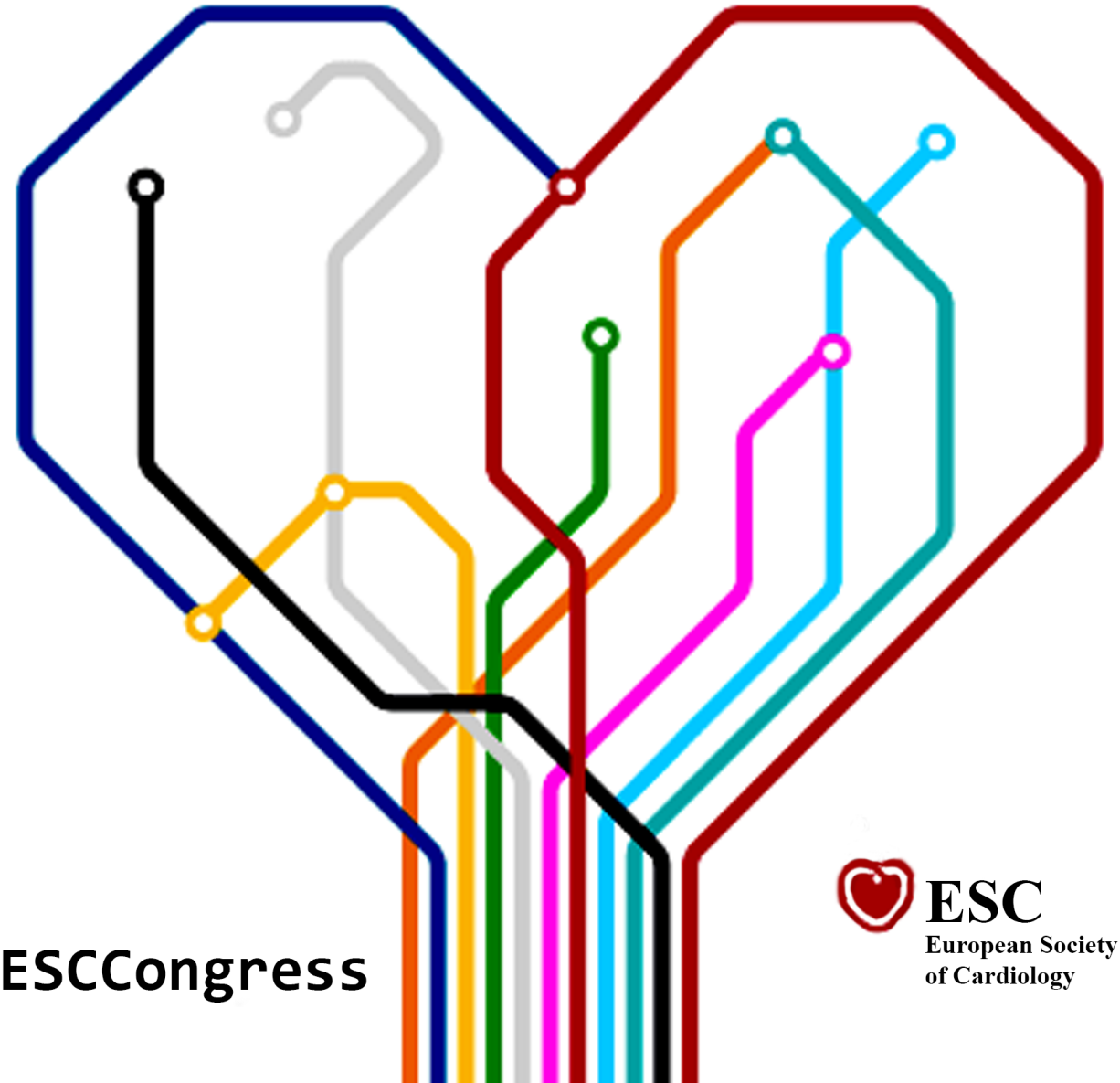

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REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Cardiovascular Diseases and Adipokines: The Role of Visfatin in Coronary Atherosclerosis

Zh. Marinova-Zlatinova, M. Negreva, Tr. Chervenkov

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Abstract

Coronary artery disease (CAD) is a leading cause of cardiovascular disease-related mortality globally. Traditional risk indicators for CAD, such as age, gender, hypertension, and cholesterol levels, may not reliably predict the existence and severity of the disease in all individuals. Furthermore, these risk variables may not provide information regarding the long-term prognosis and survival of the patients with stable CAD. The limitations of present screening approaches suggest the need to develop novel prognostic biomarkers for the detection of coronary atherosclerosis.

This review focuses on the significance of adipokines in the pathophysiology of coronary atherosclerosis. The article discusses the role of various cytokines, focusing on visfatin in developing and progressing coronary atheroma and its potential to improve risk stratification, increase diagnostic accuracy, and guide therapy decisions. Clinical evidence supporting visfatin's role as a diagnostic marker for CAD is already available, with elevated levels observed in patients with significant coronary atherosclerosis. However, the paper acknowledges certain limitations, such as the need for more extensive longitudinal studies to validate its potential use in clinical practice to improve early diagnosis and prognosis, ultimately enhancing strategies for preventing cardiovascular disease.**(International Journal of Biomedicine. 2024;14(1):9-14.)**

Keywords: visfatin • coronary artery disease • adipokines • biomarkers • screening

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Abbreviations

ACS, acute coronary syndrome; **CAD**, coronary artery disease; **CVDs**; cardiovascular diseases; **NAMPT**, nicotinamide phosphoribosyltransferase.

Introduction

Coronary artery disease (CAD), sometimes called coronary heart disease or ischemic heart disease, is one of the most diagnosed cardiovascular diseases (CVDs) among the general population. Epidemiological data for 2016 show that it remains a leading cause of mortality worldwide, affecting 154 million people and representing 32.7% of the global burden of CVDs.[1,2] Both current epidemiological data on the disease and prognostic trends for the coming years are cause for concern.[3-5]

A major clinical problem in this context is primary and secondary prevention and effective screening, especially in the subclinical stage of atherosclerosis. This has led to many studies in recent years, with a growing focus on developing and discovering new risk scores and laboratory markers for predicting the clinical course and manifestation of CAD. These markers should be more accessible and cost-effective without losing their predictive value. They should possess high specificity and sensitivity, be easily reproducible, and meet the accepted definition of a biomarker: "a characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or pharmacologic responses to a therapeutic intervention."[6,7]

To date, several biomarkers reflect disturbances in the structure, function, or various regulatory mechanisms at the cellular or tissue level.[8,9] Such laboratory indicators

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include cytokines, small signaling molecules whose primary function is intercellular communication.[10] In 1979, a family of cytokines called "interleukins" was differentiated. They are secreted and expressed primarily by leukocytes, playing a key role in immune processes. Subsequently, with the discovery of leptin, the subgroup of adipokines was formed—molecules produced by adipose tissue that participate in the evolution of metabolic syndrome and numerous CVDs. One of the representatives of the adipokine population is visfatin, a cytokine with a proven role in the pathogenesis of ischemic stroke, insulin resistance, and more.[11-13] Further research is needed to explore its potential prognostic value in CVDs, particularly CAD.[14-16]

Characteristics of Adipokines as a Subgroup of the Cytokine Family

Cytokines are small signaling molecules whose primary function is intercellular communication. The term encompasses a large and diverse family of regulatory molecules produced throughout the body by cells of different embryonic origins. Adipokines are a specific type of cytokines released by adipose cells. In the 1990s, interest in adipose tissue surged following the discovery of leptin. Over the past few decades, various molecules with hormonal activity have been isolated from adipose tissue. Adipocytes produce and release various proinflammatory and anti-inflammatory agents into circulation, including adipokines, cytokines, and chemokines.[11] The functional pleiotropy of adipose tissue is also determined by the distinction between two types, white and brown adipose tissue, which exhibit differences in the execution of various tissue functions.[17-19]

The accumulated data to this moment show that molecules with a proinflammatory nature produced by adipose tissue play an active role in the development of insulin resistance[13] and in increasing the risk of CVDs and obesity.[16] The significance of adipokines is underlined by the fact that adipose tissue is perceived as the largest endocrine "organ" in the human body. Despite the small number of molecules produced by individual adipocytes, the total volume produced by fat cells significantly influences the functions of the entire organism.[20,21] Levels of certain adipokines directly correlate with specific metabolic states and can potentially influence systemic metabolism's homeostasis directly. Dysregulation in adipokine function is implicated in the pathophysiological mechanisms of various diseases such as diabetes, obesity, atherosclerosis, and many others.[22]

The Role of Chronic Inflammation in Pathophysiology of Atherogenesis

Just a few decades ago, atherosclerosis was considered a seemingly straightforward proliferative process, according to which, extending the well-known classic Virchow's triad, endothelial damage leads to platelet aggregation and the release of platelet-derived growth factor, stimulating the proliferation of smooth muscle cells in the vascular intima, thereby forming the core of the atherosclerotic plaque. Subsequently, in addition to vascular smooth muscle cells, active immune cells and mediators were identified within atheromas, suggesting the involvement of proinflammatory mechanisms in the evolution of the disease.[6]

The immune mechanisms for combating inflammation are generally based on two types of immunity: innate and acquired (adaptive). The chronic inflammatory reaction in atherosclerosis involves elements of both types of immune responses. A significant body of evidence supports the role of the monocyte-macrophage system in atherogenesis, particularly the migration of monocytes into the vessel intima, expressing proinflammatory cytokines such as TNF-α, metalloproteinases, IL-1, and others. Activated platelets also release preformed proinflammatory cytokine mediators in response to endothelial damage.[23]

Numerous systemic anti-inflammatory strategies are applied in other diseases, such as corticosteroid therapy, non-steroidal anti-inflammatory drugs, or anti-cytokine agents. However, these treatments come with a range of unwanted side effects, which place them far from the position of ideal candidates for modulating the course of atherogenesis.[24]

Inflammation Markers as Potential Predictors of Coronary Artery Disease

Contemporary literature provides increasing evidence of the relationship between various inflammatory biomarkers and prospective CAD risk, both in asymptomatic individuals and those with already established heart failure or coronary atherosclerosis.[25-28] The clinical benefit of using such biomarkers for risk prediction in practice depends directly on their practicality, ease of measurement, cost, reproducibility of measurements, and the ability to add predictive value to existing biomarkers, such as IL-6, CRP, natriuretic peptides, myeloperoxidase, etc.[29]

Inflammatory molecules appear to have significant predictive value in individuals with known cardiovascular disease. They may be beneficial for identifying seemingly healthy persons without established CAD who cannot be diagnosed using standard risk factors. They may also effectively detect apparently healthy people without documented CAD who may be at a higher risk than established risk factors suggest.[30]

Combining such molecules may enhance CAD clinical diagnosis and prediction. More research on the discovery of new CHD-specific variables is necessary. However, no marker has firmly established itself in clinical practice at this stage. In this context, the focus in recent years has been on discovering a biomarker that meets these criteria and provides an opportunity for effective screening of individuals with an unclear coronary status and undiagnosed CAD. The goal is to initiate statin therapy earlier and prevent the development of CAD and future CAD events.

Visfatin – Adipokine with Many Faces

According to data from numerous publications over the last decade, adipokines are crucial in various aspects of systemic homeostasis.[31] However, these molecules do not share a

common denominator regarding their regulatory functions and the processes they guide. In recent years, adipokine visfatin has emerged among the diverse cytokine family. Accumulating evidence suggests that circulating levels of this molecule, also known as nicotinamide phosphoribosyltransferase (NAMPT), play a role in the pathogenesis of several conditions, including obesity, chronic inflammation, and lipid profile alterations in humans.[32,33]

Visfatin is primarily found in visceral adipose tissue and mimics the action of insulin in lowering plasma glucose levels. It is produced by various lines of immune cells (neutrophils and macrophages) and induces the expression of TNF-α and IL-6 in human monocytes, further supporting the proinflammatory qualities of this adipokine.

Clinical research data show elevated levels of visfatin in patients with type 2 diabetes, metabolic syndrome, and coronary atherosclerosis. There is also evidence that increased concentrations of this molecule raise the risk of pre-eclampsia and worsen the outcome of different types of malignancies.[34,35] Additionally, it is believed that, aside from regulating homeostasis, this cytokine is involved in disrupting atherosclerotic plaques[36] and stimulating angiogenesis.

Structure and Role of Visfatin in the Evolution of Coronary Artery Disease

Data exist regarding the participation of visfatin at various stages of atherogenesis, from endothelial dysfunction to plaque destabilization and rupture. It has been shown to be a proinflammatory mediator with a direct role in the atherosclerotic process, being involved in atherosclerotic plaque disruption, and is found in high concentrations in foam macrophages that constitute unstable plaques.[37]

Visfatin is a protein secreted by visceral adipocytes and activated lymphocytes localized in bone marrow stromal cells. It synergistically promotes B-cell proliferation in combination with IL-7 and stem cell factor (SCF). The adipokine, formerly known as Pre-B-cell colony-enhancing factor (PBEF), is also identified in an intracellular form and plays a key role in nicotinamide adenine dinucleotide (NAD) synthesis. There are two forms of visfatin/NAMPT in mammals: intracellular and extracellular (iNAMPT and eNAMPT). The intracellular form is primarily responsible for NAD+ production, while eNAMPT is associated mainly with cytokine function and an insulin-like effect in addition to its regulatory role in NAD+ biosynthesis.

To date, the importance of inflammation and the role of leukocytes at various stages of the atherogenic process has been recognized in the literature. Different types of macrophages express various effects regarding the appearance, development, and maintenance or, conversely, the remission, of inflammation. Both pro- and anti-inflammatory macrophages are present in atherosclerotic plaques, and the balance between these cellular fractions determines the fate of atheromas.[38] Macrophage polarization represents a process by which individual macrophages acquire various new functional capabilities in response to signals from their microenvironment. Polarization is regulated by multiple factors in atherosclerosis, and while numerous studies highlight its significance in atherogenesis, the regulation of the process itself remains unclear.[39,40]

In this context, visfatin has been found to be expressed in lipid-laden macrophages in the heart of the atherosclerotic plaque and may regulate lipid accumulation and the inflammatory state of these foam cells. Moreover, abnormal lipid metabolism, in combination with chronic inflammation, are key elements in atherogenesis, and this adipokine may serve as the link between these two pathological processes. Elevated concentrations of visfatin in peripheral blood are an independent factor contributing to the evolution of stable CAD into acute coronary syndrome (ACS) and increasing the risk of ischemic stroke.[41] Supporting this assertion, Zhang et al.[42] studied the levels of NAMPT in patients with confirmed ACS and a control group, demonstrating increased marker concentrations in the patient group compared to the controls.

NAMPT is a rate-limiting enzyme in NAD+ production, a substrate for the protein Sirtuin 1 (SIRT1), also known as the NAD-dependent deacetylase Sirtuin-1. This enzyme, mainly located in the cellular nucleus, is involved in the deacetylation of numerous transcription factors, thus playing a role in cellular regulation, stress response, and cellular longevity. The study results indicate an upregulation in the expression of the NAMPT/NAD+/SIRT1 signaling pathway in peripheral blood in subjects with ACS, suggesting the critical role of NAMPT in the evolution of atherosclerosis. In this context, the increased expression of eNAMPT in ACS patients likely plays a protective role precisely by regulating this signaling pathway.

Visfatin in the Diagnosis of Coronary Atherosclerosis

Several clinical studies conducted so far have reported significant results regarding the diagnostic role of visfatin in patients with CAD, from those with stable asymptomatic coronary atherosclerosis to subjects with confirmed ACS.

Kadoglou and colleagues [43] compared the serum levels of NAMPT in a cohort with established stable CAD and a control group. They found that marker concentration was significantly higher in patients with coronary atherosclerosis than in healthy individuals. However, this study was cross-sectional and not randomized, and the absence of coronary atherosclerosis in the healthy group was based on clinical data and non-invasive imaging methods, making it impossible to exclude angiographically pronounced coronary atherosclerosis.

Lu et al.[44] investigated the dynamic changes in adipokine levels in patients with ST-segment elevation myocardial infarction (STEMI), confirming the hypothesis that serum visfatin is significantly elevated in the setting of ACS, compared to patients with angina during physical exertion and healthy controls. Furthermore, the concentrations of this marker peaked approximately 24 hours after percutaneous coronary intervention (PCI) and declined to levels like those in the control group within the first week after revascularization. The authors also observed a correlation between baseline

serum levels of NAMPT and the peak levels of troponin-I, the peak levels of creatine kinase-MB fraction, total leukocyte count, and B-type natriuretic peptide. Limitations of this study were the small cohort and the short-term follow-up of the subjects, which doesn't allow dynamic tracking of the changes in visfatin levels over longer periods of time.

Other studies have also shown elevated visfatin levels in patients with in-stent restenosis following PCI[45] and those with symptomatic carotid plaques, where the biomarker is detected in the highest concentrations, particularly in lipid-laden macrophages.[36]

All this suggests that adipokines should be treated as immunomodulators, primarily localized in foam cells within unstable atherosclerotic plaques, playing a crucial role in their destabilization.

Limitations and Future Potential in the Application of Visfatin as a Biomarker for Coronary Atherosclerosis

Considering the aforementioned scientific studies, a question of particular interest arises: can circulating visfatin mark the progression of CAD and even predict its progression, potentially forecasting future ACS? Current understanding regarding this adipocytokine suggests it will shortly have potential use as part of routine laboratory indicators. However, the clinical studies discussed here contain certain drawbacks and limitations in their design or the hypotheses they investigate.

To date, no studies are tracking the marker's levels dynamically over longer periods, especially in patients for whom revascularization has not been performed for one reason or another. In most cases, subjects have been followed during their hospitalization but not in the months thereafter to determine potential fluctuations or dependencies of the adipokine on other factors. This limitation necessitates the conduct of larger, longitudinal studies.

In this context, no publications establish how visfatin concentrations react to statin or antidiabetic therapy, given the cytokine's role in the pathophysiology of both CAD and diabetes. Furthermore, the cohorts in most clinical trials are too diverse in their characteristics. The connection between insulin resistance, diabetes, obesity, and elevated levels of circulating visfatin is well known. Still, at this stage, individuals with these comorbidities have not been excluded from the study designs, compromising the "purity" of their results.

Another limiting factor in studying this adipokine is its persistently high cost of tests. It is a significant drawback from an economic standpoint and would hinder its widespread use as a potential screening method for subclinical coronary atherosclerosis, especially in regions with resource shortages for primary cardiovascular disease prevention.

Conclusion and Outlook

At the foundation of contemporary understanding of the evolution of the atherosclerotic process and plaque disruption lies a systemic inflammatory process, subject to constant regulation by numerous cytokines, one of which is the adipokine visfatin. Visfatin is found in the composition of coronary atherosclerotic plaques and has been discovered circulating in higher concentrations in the setting of acute coronary syndrome.

This molecule holds promising potential as a marker for CAD, especially in individuals with an unclear coronary status and subclinical coronary atherosclerosis. However, additional studies in this area are necessary. Such screening could significantly enhance the prevention of future cardiovascular events by initiating statin therapy and aspirin intake earlier, thus altering the long-term prognosis for these patients.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Botulinum Neurotoxin BoNT-A in the Management of Hypertrophic Scars and Keloids: A Comprehensive Review

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Abstract

Hypertrophic scars (HS) and keloids are challenging dermatological conditions that often lead to physical and psychological distress in affected individuals. Current therapeutic approaches have limitations, prompting the exploration of novel treatments. Botulinum Neurotoxin BoNT-A has emerged as a promising candidate in managing these scars. This comprehensive review delves into the pathophysiology of HS and keloids, the shortcomings of existing treatments, and the mechanisms underlying BoNT-A's potential efficacy. Through an analysis of clinical studies and evidence, the review evaluates BoNT-A's impact on scar formation and patient outcomes. Safety and side effects and the potential influence of BoNT-A on quality of life are also considered. Comparative analysis with traditional therapies underscores the advantages and challenges of BoNT-A use. The review concludes by suggesting future research directions and emphasizing the significance of Botulinum Neurotoxin BoNT-A as a promising therapeutic option. This article provides valuable insights for clinicians, researchers, and patients seeking innovative solutions for HS and keloids. **(International Journal of Biomedicine. 2024;14(1):15-19.)**

Keywords: hypertrophic scars • keloids • botulinum neurotoxin

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Introduction

Hypertrophic scars (HS) and keloids represent troublesome and often disfiguring dermatological conditions, causing both physical discomfort and profound psychological distress to those affected. Characterized by their raised, reddish appearance, these scars disrupt the seamless canvas of healthy skin, imposing significant challenges on both patients and clinicians.[1] Traditional treatment modalities, while valuable, often fall short of delivering the desired outcomes, underscoring the need for innovative therapeutic approaches.

The management of HS and keloids has conventionally relied on surgical excision, steroid injections, silicone sheeting, laser therapy, and other interventions.[2] While these methods have demonstrated varying degrees of success, they are not without limitations, including the risk of recurrence, adverse side effects, and variable patient responses. Consequently, pursuing alternative solutions has led to exploring Botulinum Neurotoxin BoNT-A as a novel and potentially transformative approach in scar treatment.[3]

BoNT-A, commonly recognized for its remarkable efficacy in aesthetic and neuromuscular applications, has shown promise in modulating the complex processes of scar formation. By targeting key molecular and cellular pathways, BoNT-A offers the prospect of not only ameliorating the physical characteristics of HS and keloids but also addressing the underlying pathophysiology.[4]

This review article aims to comprehensively evaluate the potential of BoNT-A in the context of HS and keloids, examining its mechanisms of action, clinical evidence, safety profile, and patient-reported outcomes. Through this exploration, we seek to provide a foundation for clinicians, researchers, and patients to better understand the evolving landscape of scar management and appreciate the innovative prospects that BoNT-A presents.

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Pathophysiology of HS and Keloids

Scar formation is complex and dynamic due to the body's response to tissue injury. HS and keloids represent two distinct outcomes within this process, each characterized by its unique pathophysiology.

Scar Formation Mechanisms

The process of scar formation typically begins with an injury, such as a wound, incision, or burn, which triggers a series of events aimed at repairing and replacing damaged tissue to restore structural integrity and functionality in the affected area. This process consists of several critical phases. Initially, the inflammatory phase is marked by localized inflammation characterized by redness, swelling, and heat, as immune cells like neutrophils and macrophages are recruited to the wound site to remove debris and combat potential infections.[5] Subsequently, during the proliferative phase, fibroblasts, responsible for collagen production, migrate to the wound site and begin synthesizing the extracellular matrix, where collagen plays a pivotal role as the primary structural protein in the skin. Finally, in the remodeling phase, the initial collagen scaffold gradually transforms into mature collagen fibers over months or even years, resulting in a scar that becomes less prominent and more closely resembles the surrounding skin.[6]

Differentiating HS and Keloids

While HS and keloids share similarities in scar formation, they each exhibit distinctive characteristics and outcomes. HS remain confined to the boundaries of the original wound. They tend to be raised, red, or pink and may exhibit itchiness or discomfort. HS typically occur in response to injuries involving the deeper layers of the skin, such as burns, surgical incisions, or traumatic wounds. The excess collagen production and limited geographical extent distinguish them from keloids.[7]

Keloids, on the other hand, extend beyond the confines of the initial injury site and infiltrate adjacent healthy skin. They often appear more aggressive, with excessive collagen deposition, leading to their characteristic raised, nodular appearance. Keloids may grow over time, sometimes becoming significantly larger than the original injury. They are particularly common in individuals with a genetic predisposition to keloid formation and can develop in response to even minor skin trauma, such as ear piercings.[8]

Understanding the pathophysiology of HS and keloids is essential in determining the most appropriate treatment approaches. While both conditions share common mechanisms, their distinct clinical presentations and behaviors necessitate tailored therapeutic strategies.[9]

Current Treatment Options

Current therapeutic approaches for HS and keloids encompass a spectrum of interventions, ranging from non-invasive topical treatments to surgical procedures. While these treatments have offered varying degrees of success, they are not without their limitations and potential side effects.[2]

Non-Invasive and Topical Treatments

Silicone gel sheeting and creams frequently manage HS and keloids. These products work by moisturizing scar tissue and creating a protective barrier. While they can be effective in some cases, patients may find it challenging to adhere to treatment regimens.[10] Pressure garments, commonly used in burn therapy, apply even pressure to scarred areas, resulting in flattening and softening of the scar. The efficacy of these garments varies, and patient compliance can be a concern.[11] Corticosteroid injections, administered intralesionally, often with triamcinolone acetonide, are used to reduce inflammation and minimize scar thickness. However, multiple sessions may be required, and side effects like skin thinning or hypopigmentation can occur.[12] Topical imiquimod cream, an immune response modifier, has been investigated for scar management and may reduce scar volume and redness, but its effectiveness varies among patients.[13]

Laser Therapy

Pulsed dye lasers target the redness associated with HS and keloids, aiding in the reduction of redness, but may not significantly affect scar texture.[14] Fractional lasers create microthermal zones within scar tissue, promoting collagen remodeling and improving both texture and pigmentation. However, multiple sessions may be necessary, and there is a risk of post-inflammatory hyperpigmentation.[12]

Surgical Interventions

Excision and resection involve the surgical removal of HS and keloids, but there is a risk of recurrence, often necessitating postoperative therapies to mitigate this risk.[15] Radiation therapy, especially for keloids, has been used post-surgery to reduce the likelihood of recurrence. However, it is not without potential side effects and radiation-associated risks.[12]

Limitations and Side Effects

Despite the availability of various treatment options, each approach has limitations and potential side effects. Recurrence is a common issue, with HS and keloids often reappearing after treatment, necessitating repeated interventions. Adverse effects, such as skin thinning and hypopigmentation, can result from corticosteroid injections and radiation therapy. Laser therapy carries a risk of hyperpigmentation and erythema, and surgical excisions may lead to scarring. Additionally, non-invasive treatments often rely on consistent and long-term patient compliance, which can be challenging to maintain, and the efficacy of treatments varies from patient to patient, making it difficult to predict outcomes accurately.[9,12]

These limitations and side effects associated with current treatment options emphasize the need for alternative and potentially more effective therapies. Botulinum Neurotoxin BoNT-A, with its potential to address scar formation mechanisms at a cellular level, presents an intriguing opportunity for improving scar management.[12]

Botulinum Neurotoxin BoNT-A: Action Mechanisms

Mechanism of BoNT-A Action

Botulinum Neurotoxin BoNT-A, a potent neurotoxin derived from the bacterium *Clostridium botulinum*, exerts its pharmacological effects by selectively targeting and inhibiting the release of the neurotransmitter acetylcholine at the neuromuscular junction. This disruption of cholinergic

signaling results in temporary muscle paralysis, making BoNT-A a well-known and widely used agent in aesthetic medicine and neuromuscular disorders.[16]

BoNT-A is internalized by presynaptic nerve terminals, facilitated by a specific receptor-mediated endocytosis process. Within the nerve terminal, BoNT-A cleaves specific proteins known as SNARE complexes. SNARE proteins are crucial for the fusion of synaptic vesicles containing acetylcholine with the nerve cell membrane, allowing acetylcholine release into the synaptic cleft. By cleaving these proteins, BoNT-A disrupts this fusion process, preventing the release of acetylcholine. As a result, the affected neuromuscular junction fails to transmit nerve impulses, leading to muscle paralysis. This temporary paralysis, typically lasting several months, is reversible as the nerve terminals regenerate new SNARE complexes.[16,17]

BoNT-A's Potential in Mitigating Scar Formation

The application of BoNT-A in scar management is based on its ability to modulate multiple cellular and molecular pathways involved in scar formation. Skeletal muscle contraction has been implicated in the pathogenesis of HS and keloids. The continuous pulling forces created by overactive muscles in the vicinity of a healing wound can contribute to the formation of HS, especially in areas of high tension, such as the chest and shoulders. By paralyzing the underlying muscles, BoNT-A reduces mechanical stress on the wound, which, in turn, may lead to reduced scar contracture and improved cosmetic outcomes.[18]

BoNT-A's impact extends beyond muscle relaxation. It has been shown to possess anti-inflammatory properties, potentially reducing local inflammation and mitigating the inflammatory phase of scar formation. This modulation of the immune response may contribute to a less aggressive and less erythematous scar.[19]

BoNT-A may also influence fibroblast activity and collagen production. Excessive collagen synthesis is a hallmark of HS and keloids. By interfering with the fibroblast activity, BoNT-A has the potential to decrease collagen deposition, resulting in softer and less raised scars.[6,19]

Patients with HS and keloids often experience pain, discomfort, and itchiness. BoNT-A's action on muscle relaxation and its possible neuromodulatory effects may alleviate these symptoms, improving overall patient comfort and quality of life. Understanding BoNT-A's mechanism of action and its potential impact on scar formation provides a compelling rationale for its use in scar management.[20]

Clinical Studies and Evidence

Comprehensive Review of Clinical Studies

A growing body of clinical research has explored the utilization of Botulinum Neurotoxin BoNT-A in the management of HS and keloids. These studies encompass a range of patient populations and scar types, providing valuable insights into BoNT-A's efficacy and safety profile (Table 1).

Outcomes, Efficacy, and Safety of BoNT-A

Clinical studies consistently report that BoNT-A injections lead to scar softening and flattening, reducing the raised appearance characteristic of HS and keloids. Many studies indicate reduced scar erythema (redness) following BoNT-A treatment. This is particularly relevant in improving the cosmetic appearance of scars. BoNT-A demonstrates an ability to alleviate pain and itchiness associated with HS and keloids, enhancing patient comfort and quality of life. The muscle-relaxing properties of BoNT-A contribute to reduced scar contracture and mechanical tension on the wound, leading to more favorable cosmetic outcomes.[21,23,24]

Several studies report high patient satisfaction with BoNT-A treatment, highlighting the aesthetic and functional improvements. While BoNT-A has shown promise in minimizing scar recurrence, there is variability in long-term outcomes, necessitating further research to determine the optimal treatment duration and frequency.[25,26] The safety profile of BoNT-A in scar treatment appears favorable, with side effects typically mild and transient. Adverse events are infrequent and predominantly related to the injection process.[24,28]

Diversity of Patient Populations and Scar Types

Clinical studies involving BoNT-A encompass a diverse range of patient populations, including individuals of different ages, skin types, and ethnic backgrounds. This diversity underscores the applicability of BoNT-A across various demographic groups. Additionally, BoNT-A has been studied in the context of different scar types, such as scars resulting from surgical incisions, burns, trauma, and other injuries. This broad spectrum of scar etiologies highlights the versatility of BoNT-A as a potential therapeutic option.[28,29] Furthermore, the location of scars is an important consideration, as the effectiveness of BoNT-A may vary based on the anatomical site. Studies have explored the use of BoNT-A in scars located on the face, neck, chest, shoulders, and extremities. Understanding the regional nuances in scar response to BoNT-A is crucial for optimizing treatment strategies.[29,30]

Future Directions

The use of BoNT-A in scar treatment has shown promise, but there is still much to explore and innovate in this field. Table 2 represents some potential research directions and areas for further investigation.

Limitations

The study had limitations due to the high degree of patient heterogeneity. This was because the study did not impose restrictions on patient types, encompassing all research related to BoNT-A and scar formation. Consequently, there were substantial discrepancies in terms of patients' age and the types of scars across the studies included.

Conclusion

BoNT-A has emerged as a promising therapeutic option in the management of HS and keloids. Clinical studies provide substantial evidence supporting the use of BoNT-A in scar treatment. While the outcomes are generally promising, further research is needed to establish standardized treatment protocols and determine the long-term effects of BoNT-A in scar management.

Table 1.

Botulinum toxin as a primary management agent.

Study	Scar types	Outcome	Efficacy
Shaarawy et al. [21]	Keloids	Lesion volume reduction	82.7% reduction in the group receiving Intralesional (IL) steroid repeated every 4 weeks for six sessions, 79.2% reduction in the group receiving IL BTA 5 IU/cm^3 repeated every 8 weeks for three sessions
Rasaii et al. [22]	Keloids	Lesion parameters improvement	Decreased lesion height, vascularity, and pliability in both groups (intralesional triamcinolone alone vs. intralesional triamcinolone in combination with BTA).
Li et al. [23]	Keloids	Symptom relief and lesion appearance	There was no significant difference in lesion volume and appearance in the three groups: Group A - intralesional compound betamethasone injection+BTA, Group B - compound betamethasone injection+fluorouracil and Group C - compound betamethasone injection alone. Group A reported better pain and itching scores.
Zhou et al. [24]	Keloids	Symptom relief and lesion thickness	Greater reduction in Visual Analogue Scale (VAS) and keloid thickness in the joint treatment group
Zhibo et al. [25]	Keloids	Symptom relief and lesion parameters	BTA was an effective and safe treatment for keloids of all sizes and any duration. In addition to flattening in all cases, peripheral regression of lesions was noted, and there was no evidence of recurrence after 1 year.
Gauglitz et al. [26]	Keloids	Macroscopic and morphological appearance	No significant changes in scar volume, height, and appearance in intralesional BTA treatment.
Pruksapong et al. [27]	Keloids after surgery	Vancouver Scar Scale (VSS) improvement	Favorable outcome in the BTS group at 1 and 3 months, while the control group (corticosteroid therapy) performed better at 6 months

BTA *- botulinum toxin type A*

Table 2.

Research directions and areas for further investigation.

Future Directions	Description
Standardized Treatment	To develop standardized treatment protocols for BoNT-A in scar management, including optimal dosages, injection intervals, and treatment duration to enhance efficacy and ensure safety.
Combination Therapies	To explore combining BoNT-A with other scar management methods (e.g., laser therapy, silicone sheeting) to achieve synergistic effects and improve scar outcomes.
Scar Types and Locations	To investigate the effects of BoNT-A on various scar types and locations to tailor treatment plans based on regional and etiological differences.
Long-Term Effects	Conduct longitudinal studies to assess the durability of BoNT-A treatment and factors influencing scar recurrence for patient guidance.
Mechanistic Insights	To investigate the underlying mechanisms of BoNT-A in scar management, focusing on collagen production, fibroblast activity, and inflammation for potential targeted therapies.
Scar Prevention	To explore BoNT-A's potential in preventing excessive scar formation, particularly in high-risk cases like post-surgical incisions and trauma.
Safety and Adverse Events	To conduct large-scale safety studies to assess the incidence of adverse events related to BoNT-A treatment and optimal strategies for their management.

Competing Interests

The authors declare that they have no competing interests.

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Imaging Characteristics and Hormonal Receptor Correlations in Varieties of Breast Cancer

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Abstract

Background: While there is acknowledgment of the vital role of factors such as the status of histological grades and hormone receptor (HR) in shaping targeted treatment strategies for breast cancer (BC), comprehensive research that unifies the study of imaging features with the evaluation of HR characteristics and histopathological data is notably absent in Albania, creating a critical research gap that this study endeavors to fill. This study aimed to investigate the imaging characteristics observed in ultrasound and the possible correlations between expression levels of HRs in ductal and lobular types of BC to elucidate potential prognostic and therapeutic implications.

Methods and Results: This descriptive study, conceived as a series of cases, leveraged a prospective approach to scrutinize the dynamics of the study population over four years (2019-2023) in the Mother Teresa University Hospital Center and a private oncology clinic in Tirana. The convenience sampling strategy enlisted 238 female patients (mean age 60.5±12.5 years) diagnosed with BC who had been tested for HRs and consented to participate. Diagnostic imaging was facilitated using a Chison US equipped with a 10 MH linear probe. The results were adjudged based on the BI-RADS tumor classification. HR markers were discerned through rigorous immunohistochemical analyses. Utilizing SPSS version 21.0, statistical analyses incorporated a variety of tests, including Spearman's rho to assess correlations between hormonal receptors and imaging morphological characteristics and ordinal logistic regression to evaluate the relationships between hormonal receptors and cancer grades.

Analyzing the localization of the tumor revealed that a slightly higher proportion had it on the left side, accounting for 52.9% compared to 47.1% on the right side. Regarding the BI-RADS classification observed through echographic examination, a vast majority were classified as BI-RADS 5 (92.8%), followed by a smaller percentage distributed amongst BI-RADS 4 (5.9%), BI-RADS 3 (0.84%), and BIRADS 6 (0.42%). Examining the cancer grades determined that 68.3% were at Grade 2, whereas Grades 1 and 3 were noticeably less common, standing at 1.7% and 30.2%, respectively. Estrogen receptor (ER) and progesterone receptor (PgR) sensitivity were high in most patients, exhibiting 77.7% and 70.6% positivity, respectively, alongside a notable presence of high Ki67 levels in 75.2% of the individuals. The investigation into HER2 status demonstrated that a significant number were negative (76.1%), as opposed to 17.6% being positive and 6.3% equivocal. Remarkably, 5.5% of the patients had a triple-negative status upon biopsy evaluation. The Spearman's rho correlations displayed a moderate positive correlation between ER and PgR (rho=0.563) and a weak negative correlation between ER and Ki67 (rho=-0.343) (P<0.05 in both cases). PgR and Ki67 show a weak negative correlation (rho=-0.353, P<0.05), suggesting a tendency for higher PgR values to correspond with lower Ki67 values. The ordinal logistic regression analysis identified a statistically significant negative relationship between the ER variable and the outcome variable, denoted by a coefficient of -2.137, P<0.05. Additionally, Ki67 showcased a positive relationship with the outcome, as indicated by a coefficient of 5.150, P<0.05.

Conclusion: This study delineates the nuanced relationships between biomarkers such as ER, PgR, and Ki67 in different types of infiltrative cancers, pointing to a complex interplay that necessitates further exploration while also noting the independence of BI-RADS imagery in these correlations.**(International Journal of Biomedicine. 2024;14(1):20-25.)**

Keywords: breast cancer • hormonal receptor • BI-RADS imagery • Albania

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Abbreviations

BI-RADS, Breast Imaging Reporting and Data System; **BC**, breast cancer; **ER**, estrogen receptor; **HER2**, human epidermal growth factor receptor 2; **HR**, hormone receptor; **PgR**, progesterone receptor.

Introduction

Breast cancer (BC), silently developing and predominantly detected through routine screenings, is the most frequently diagnosed cancer in women globally, accounting for over a tenth of new annual diagnoses and standing as the second leading cause of cancer-related death among women.[1] In the nuanced pathway to predicting BC prognosis, the role of ultrasound emerges as critical, working in tandem with assessing histological grade and other characteristics, such as hormone receptor (HR) (estrogen receptor [ER] and progesterone receptor [PgR]) status and human epidermal growth factor receptor 2 [HER2] condition, to furnish a comprehensive picture that can guide targeted treatment strategies.[2] A study conducted in Albania indicated a promising Area Under the Curve value of 0.81 in the ROC curve analysis, underlining that utilizing ultrasound with BI-RADS categorization stands as a reliable instrument for identifying malignant breast tumors across all age groups, offering satisfactory precision for tertiary diagnostic services.[3] In Albania, there is a notable lack of studies that unify the examination of imaging features and HR characteristics, along with their potential intrinsic correlations or associations with histopathological data. Numerous differences by histological type—including lobular, ductal/lobular, tubular, and medullary histologies—are also observed by race/ethnicity. However, there was no clear evidence of substantial differences in a 21-gene recurrence score or ER1, PgR, or HER2 RNA expression.[4,5] Despite ethnicity or population-specific characteristics, it is critically important to meticulously analyze every potential correlation between these diagnostic-prognostic factors to forecast the prognosis of BC and inform treatment choices accurately. Evidence shows that ultrasound echo patterns in BC significantly correlate with ER, PR, and HER2/neu expression, potentially guiding prognosis and hormonal therapy responses.[6] Furthermore, a study analysis delineated significant associations between various molecular cancer subtypes, the presence of ER, PgR, and HER2, and elevated a proliferation marker Ki67 levels, revealing direct and inverse relationships.[7] Immunohistochemistry, essential in identifying biomarker expressions pivotal in breast pathology and predicting therapy responses using prevalent immunomarkers such as ER, PgR, HER2, and Ki67, offers a practical and cost-effective alternative to the more cumbersome and expensive gene profile analysis for determining BC prognoses and therapeutic strategies.[8]

This study aimed to investigate the imaging characteristics observed in ultrasound and the possible correlations between expression levels of HRs in ductal and lobular types of BC to elucidate potential prognostic and therapeutic implications.

Materials and Methods

The study was conceived as a series of cases, rigorously fitting the inclusion criteria. The guiding approach of the study was prospective, tracking the dynamics of the study population. However, the retained type of the study was descriptive, different from comparative cohort studies that still have a compelling analytical approach with two comparison groups. The study was conducted in the Oncology Service at the Mother Teresa University Hospital Center and Tirana's private oncological diagnostic clinic.

This study spanned from 2019 to 2023, according to the proper protocol, and employed a convenience sampling strategy, selecting cases until sufficient sample size was achieved to test various hypotheses and yield statistically significant results adequately. The sample size reached 238 female patients. The inclusion criteria were patients diagnosed with BC who had been tested for HRs and consented to participate.

Data Collection Instruments

The data accrued in the study hailed from a diversity of categories and utilized an array of tools. Diagnostic imaging was facilitated using a Chison US equipped with a 10 MH linear probe, administered by a skilled radiologist who later adjudged the results based on the BI-RADS tumor classification. A single anatomical pathologist affirmed the histopathological aspects. Moreover, HR markers were discerned through rigorous immunohistochemical analyses.

Variable Definitions and Measurements

In our study, we analyzed various patient variables and their clinical characteristics. The age of the patients is treated as a continuous variable, with statistics such as mean, median, standard deviation, and interquartile range elucidating the distribution. Tumor location and Ki67 level are binary variables differentiated as left versus right and high versus normal, respectively. Furthermore, we worked with categorical variables including BI-RADS classification (with categories BI-RADS 5, 4, 3, 6), cancer grade (categorized into Grades 1, 2, 3), estrogen and progesterone receptor sensitivity (each divided into positive, slightly positive, and negative), and HER2 status (divided into positive, equivocal, and negative). Triple-negative BC was characterized by the absence of three types of receptors (ER, PgR, and HER2).

Statistical Analysis

Data from the cases were initially processed in Microsoft Excel and subsequently in SPSS version 21.0. Absolute frequencies and corresponding percentages were calculated for all categorical variables. Central tendency measures (mean, median) and respective dispersion measures (standard deviation [SD], standard error [SE], percentile) were calculated for all numeric variables. Kolmogorov-Smirnov and Shapiro-Wilk tests assessed normal distribution for numeric variables like age, tumor size, and hormonal receptor values. The Student's t-test and ANOVA assessed the differences in hormone receptor values among cancer types and grades. The Chi-squared or Fisher's exact tests evaluated differences among cancer types, BI-RADS classification, and cancer localization. Spearman's correlation coefficient assessed connections between hormonal

receptors and imaging morphological characteristics and between the hormonal receptors themselves. Ordinal logistic regression assessed the relationship between independent variables (hormonal receptors) and cancer grades. Odds ratios (OR), 95% confidence intervals (95%CI), and statistical significance values were calculated in bivariate models of ordinal logistic regression. In all cases, values of $P<0.05$ were considered statistically significant.

Ethical Considerations

Data collection was conducted in accordance with the ethical principles set forth by the Helsinki Declaration for scientific research involving human subjects. Study participants were ensured confidentiality and privacy.

Results

A total of 238 patients were meticulously examined to gather pertinent data on their demographic details and health metrics. The average age among this population was 60.5 years, with a standard deviation of 12.5 years, indicating a moderate variability in the age of the patients. The median age stood at 61 years, offering a central tendency that is slightly higher than the mean age. When considering the interquartile range, the middle 50% of the data clustered between the ages of 53 and 68 years, highlighting the predominant age group in the study. It is noteworthy that the age of patients spanned a substantial range, with the youngest being 28 years and the oldest reaching 91 years, showcasing a wide generational gap in the study demographic. Furthermore, in assessing the normal distribution testing of the age variable using the Kolmogorov-Smirnov method, a *P*-value of 0.2 was derived, which suggests that the age distribution did not significantly deviate from a normal distribution at the conventional 0.05 threshold for statistical significance (Table 1).

Table 1.
Patient demographics and age (years) distribution normality test.

Parameter	Value	Normal Distribution Testing of the "Age" Variable (Kolmogorov-Smirnov), P Value
Patient Demographics		0.2
Number of patients studied	238	
Average age	60.5	
Standard deviation of age	12.5	
Median age	61	
Interquartile range of age	53-68	
Minimum age	28	
Maximum age	91	

A detailed examination of various critical aspects of the disease portrays a substantial variation in the cancer characteristics. Analyzing the localization of the tumor revealed that a slightly higher proportion had it on the left side, accounting for 52.9% compared to 47.1% on the right side. Regarding the BI-RADS classification observed through echographic examination, a vast majority were classified as BI-RADS 5 (92.8%), followed by a smaller percentage distributed amongst BI-RADS4 (5.9%), BI-RADS 3 (0.84%), and BIRADS 6 (0.42%). Examining the cancer grades determined that 68.3% were at Grade 2, whereas Grades 1 and 3 were noticeably less common, standing at 1.7% and 30.2%, respectively. Estrogen and progesterone receptor sensitivity were high in most patients, exhibiting 77.7% and 70.6% positivity, respectively, alongside a notable presence of high Ki67 levels in 75.2% of the individuals. The investigation into HER2 status demonstrated that a significant number were negative (76.1%), as opposed to 17.6% being positive and 6.3% equivocal. Remarkably, 5.5% of the patients had a triple-negative status upon biopsy evaluation, underscoring a critical area of focus in BC research and treatment (Table 2).

Table 2.
Clinical characteristics of BC patients.

Characteristics	Number of Patients	Percentage (%)
Tumor Location		
Left side	126	52.9
Right side	112	47.1
BI-RADS Classification		
BI-RADS 5	221	92.8
BI-RADS 4	14	5.9
BI-RADS 3	2	0.84
BI-RADS 6	1	0.42
Cancer Grade		
Grade 2	162	68.1
Grade 1	4	1.7
Grade 3	72	30.2
ER Sensitivity		
Positive	185	77.7
Slightly positive	10	4.2
Negative	43	18.1
PR Sensitivity		
Positive	168	70.6
Slightly positive	11	4.6
Negative	59	24.8
Ki67 Level		
High	179	75.2
Normal	59	24.8
HER2 Status		
Positive	42	17.6
Equivocal	15	6.3
Negative	181	76.1
Biopsy Results		
Triple negative (ER, PgR, HER2)	13	5.5

The presented Spearman's rho correlations between various variables, including ER, PgR, Ki67, and BI-RADS, provide insight into the strength and direction of the relationships between each pair of variables (Table 3). A noteworthy observation is the moderate positive correlation between ER and PgR (rho=0.563) and a weak negative correlation between ER and Ki67 (rho=-0.343) ($P<0.05$ in both cases), indicating that higher ER values are generally accompanied by higher PgR and lower Ki67 values,

respectively. Meanwhile, the ER and BI-RADS relationship manifests as a very weak correlation (rho=0.045), which isn't statistically significant, showcasing a negligible association between the two variables. Similarly, PgR and Ki67 show a weak negative correlation (rho=-0.353, P<0.05), suggesting a tendency for higher PgR values to correspond with lower Ki67 values. Regarding PgR and BI-RADS, a very weak correlation is observed (rho=0.038), representing a slight, non-significant association. Lastly, the Ki67 and BIRADS association is characterized by a very weak correlation (rho=0.018), further underscoring a non-significant relationship (Table 3).

Table 3.

Correlations among different variables (Spearman's Rho).

	ER	PgR	Ki67	BI-RADS
ER	-	0.563*	-0.343*	0.045
PgR	0.563*	-	-0.353*	0 .038
Ki67	-0.343*	-0.353*	-	0 .018
BI-RADS	0.045	0.038	0.018	-

*- P-value <0.05

Starting with the ER marker, ductal infiltrative cancer, with a substantial sample size of 103, shows a higher mean (0.7413) than the lobular infiltrative category, which had a mean of 0.6222 based on 9 observations. Though the ductal infiltrative type exhibits a higher mean, it is essential to consider the relatively wider confidence interval in the lobular category, reflecting a greater uncertainty around the mean estimate. Similarly, for the PgR marker, the ductal infiltrative type, analyzed across 98 cases, had a higher mean value (0.5791) than the lobular infiltrative category, which had a mean value of 0.3375 derived from 8 cases. The lobular infiltrative group here indicates a negative lower bound in the confidence interval, indicating a more substantial dispersion in the data and a potential for more extreme lower values.

Examination of the Ki67 marker found that the trend continues with the ductal infiltrative type having a higher mean (0.3230 from 80 cases) than the lobular infiltrative group, which stands at a mean of 0.3000, calculated from a much smaller sample size of 5 (Table 4).

Table 4.

Biomarker distributions across different histological types of breast cancer.

Variables	N	M	SD	SE	Lower	Upper
ER						
Ductal infiltrative	103	0.7413	0.39652	0.03907	0.6638	0.8188
Lobular infiltrative	9	0.6222	0.47376	0.15792	0.2581	0.9864
Mixed	3	0.4667	0.41633	0.24037	-0.5676	1.5009
Other	6	0.7667	0.38816	0.15846	0.3593	1.1740
Total	121	0.7269	0.40068	0.03643	0.6547	0.7990
PgR						
Ductal infiltrative	98	0.5791	0.40778	0.04119	0.4973	0.6608
Lobular infiltrative	8	0.3375	0.43732	0.15462	-0.0281	0.7031
Mixed	3	0.7333	0.30551	0.17638	-0.0256	1.4922
Other	5	0.5600	0.51284	0.22935	-0.0768	1.1968
Total	114	0.5654	0.41248	0.03863	0.4888	0.6419
Ki67						
Ductal infiltrative	80	0.3230	0.13689	0.01530	0.2925	0.3535
Lobular infiltrative	5	0.3000	0.07071	0.03162	0.2122	0.3878
Mixed	2	0.2250	0.10607	0.07500	-0.7280	1.1780
Other	4	0.3500	0.17321	0.08660	0.0744	0.6256
Total	91	0.3208	0.13441	0.01409	0.2928	0.3488

Turning our attention to the independent variables of Table 4, several biomarkers labeled HER2Plus with Grades from 0 to 3, ER, PGR, and Ki67 are presented. The HER2Plus Grade 3 category is indicated as a reference group. For other grades of HER2Plus, the coefficients represent the change in the logged odds of the outcome variable per unit increase in the predictor; however, none of these reach statistical significance (P>0.05).

The variable ER has a coefficient of -2.137 and a statistically significant P-value of 0.028, implying a negative relationship with the outcome variable, within a 95% CI of -4.045 to -0.230. The PGR variable, despite having a positive coefficient of 1.144, does not reach a conventional level of statistical significance with a P-value of 0.164. Conversely, the Ki67 variable displays a strongly positive relationship with the outcome variable, having a coefficient of 5.150 and a significant P-value of 0.025, showcasing a strong positive influence within a 95% CI of 0.655 to 9.645 (Table 5).

Table 5.

Ordinal Logistic Regression Analysis of Breast Cancer Biomarker Grades and Predictors.

		Coef.	Std. Error	Wald	df	P-value	Lower	Upper
Dependent variable	[Grade = 1]	-3.165	1.470	4.632	1	0.031	-6.047	-0.283
	[Grade = 2] (Border)	2.636	1.142	5.330	1	0.021	0.398	4.873
Independent variables	[HER2Plus=0]	1.420	0.962	2.178	1	0.140	-0.466	3.306
	[HER2Plus=1]	0.824	0.968	0.725	1	0.394	-1.073	2.722
	[HER2Plus=2]	0.946	1.239	0.584	1	0.445	-1.482	3.375
	[HER2Plus=3]	0[a]			0			
	ER	-2.137	0.973	4.822	1	0.028	-4.045	-0.230
	PgR	1.144	0.823	1.934	1	0.164	-0.468	2.756
	Ki67	5.150	2.294	5.042	1	0.025	0.655	9.645

Discussion

In the multifaceted landscape of BC research, understanding the interrelationships between various biomarkers, demographic details, and imaging characteristics stands pivotal in forging paths toward more personalized and effective treatments. The analysis presented encompassed a meticulous exploration of a cohort of 238 patients, diving deep into demographic patterns, pathological characteristics, and the intricate web of relationships between key biomarkers. The realm of focus spanned from age distributions to a fine-grained analysis of variables such as tumor localization, BI-RADS classification, and receptor sensitivities, navigating through correlations and dissecting the distribution patterns across different cancer types.

The data surrounding age distributions hinted at a moderately varied age range, with a substantive span between the youngest and the oldest individuals, painting a rich tapestry of generational diversity. The average and median ages of our study population are comparable to those in BC studies generally, as older age is a well-established risk factor for BC. Several studies have found the median age of diagnosis to be around the 60s, which aligns well with our results. A slightly higher prevalence of tumor localization on the left side raises questions on whether biological, genetic, or environmental factors play a role in this disparity. In fact, another study emphasized that left-sided BC is more common and is associated with more aggressive biology and poorer outcomes than right-sided BC.[11] The overwhelming presence of BI-RADS-5 (92.8%) indicates a cohort with largely high percentages of malignancy due to the inclusion criteria and the type of study population. A dominant percentage of patients with cancer Grade 2 (68.3%) show a moderate differentiation in the tumors. The stark contrast with Grade 1 patients could suggest a late diagnosis in many cases. High positivity rates for both ER (77.7%) and PgR (70.6%) sensitivities indicate that hormone therapy might be a viable treatment route for a substantial fraction of the cohort. Recent studies on current populations indicate a rise in the occurrence of BC that test positive for ER and PgR, with the overall rates now ranging between 79% and 84%.[12,13]

While representing a smaller fraction (5.5%), the triple-negative patients underline a crucial subgroup that generally faces limited treatment options and a poorer prognosis. In general, this cancer is responsible for more than 15%–20% of all BCs.[14]

In the same line, the prominent negativity in HER2 status in 76% of patients signals a predominant type of BC that traditionally responds well to certain therapies, albeit with a less aggressive disease course than HER2-positive types.

There is a moderate positive correlation between the ER and PgR variables (rho=0.563, $P<0.05$), indicating a generally concurrent increase in the values of ER and PgR. This might occur due to the modulation by the PgR of the ERα activity in BC; the PR is a target gene of ER that is upregulated, and its expression is dependent on estrogen.[15]

Furthermore, a weak negative correlation was observed between ER and Ki67 (rho=-0.343, $P<0.05$) and between PgR and Ki67 (rho=-0.353, $P<0.05$), suggesting a tendency that higher levels of ER and PgR are generally associated with lower Ki67 levels. Tumors that are ER-positive or PgR-positive tend to grow more slowly than ER-negative or PgR-negative tumors.[16] Therefore, ER-positive and PgR-positive tumors might exhibit a lower Ki67 index than ER-negative and PgR-negative tumors, indicating a slower proliferation rate. However, this is a general trend, and individual cases may vary significantly.

Our observations of elevated ER expression in ductal infiltrative cancer, as opposed to lobular infiltrative cancer, diverge from the established consensus in current literature, which generally affirms a higher propensity for ER expression in lobular types.[17,18]

This difference may be attributed to a variety of factors, including a potential selection bias due to the small sample size for lobular cancers, distinct geographic and demographic variables influencing receptor expression, unique histopathological characteristics potentially not represented evenly in the sample, advancements in technology altering the sensitivity and specificity of receptor detection over time, and inherent intra-tumor heterogeneity causing a diverse range of expressions even within the same cancer subtype.

However, in our study, and according to existing literature, there is no significant difference in ER and PgR expression levels between lobular and ductal carcinoma.[19] Ki67, a marker of proliferation, was found to be slightly higher in ductal infiltrative cancers in our study, consistent with the literature.[20] The other results establish that higher Ki67 levels are associated with increased logged odds of the outcome variable, underlining the crucial role of Ki67 as a marker for cancer proliferation and potentially indicating aggressive tumor characteristics, a finding that is in line with the extensive body of literature emphasizing Ki67 as a vital prognostic marker in cancer studies.[21] High-grade lesions, in general, are more likely to be ER and PR negative;[22] this explains why an increase in the ER level was associated with a decrease in the logged odds of the outcome variable.

The study analyzed a diverse patient cohort, revealing critical insights into age distributions, tumor localizations, and the prevalence of different cancer grades, thus laying a foundation for more personalized and effective treatments in BC. Furthermore, it challenged existing literature on ER expression in different cancer subtypes, opening avenues for fresh debates and encouraging deeper exploration in this sphere to offer nuanced perspectives in BC research.

Conclusion

Our study offers a profound glimpse into the intricate relationships between various biomarkers and their associations with different types of infiltrative cancers. By carefully analyzing patient demographics and tumor localizations, we elucidated potential trends and patterns that might guide future research. Our analysis revealed a discernable pattern of associations between ER levels with PgR and Ki67 markers, showcasing a complex interplay that warrants further exploration. BI-RADS, a radiological assessment, doesn't exhibit a significant correlation with any of the three markers, indicating it might be independent of the hormonal status and the proliferative index

of the tumor. Furthermore, when we delineated the trends across different cancer types, a discernible pattern emerged, with the ductal infiltrative category generally portraying higher mean values across all markers than the lobular infiltrative category. The negative coefficient for ER suggests a declining trend with increasing grades. At the same time, Ki67 demonstrates a strong positive relationship with higher cancer grades, underlining its role as a potent marker for cancer proliferation and potentially higher grades of cancer. This study lays substantial groundwork for future research, promising to foster more targeted and effective therapeutic avenues, nurturing hope for better, individualized cancer treatment strategies.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Onco-Hematology

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Pediatric Hodgkin Lymphoma Overview in Albania during the Last Decade

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Abstract

Background: Hodgkin lymphoma (HL) is a rare malignancy characterized by a malignant proliferation of cells in the reticuloendothelial system, mainly lymph node, and the presence of Reed-Sternberg cells with a relatively good prognosis compared to other pediatric malignancies. This study aimed to produce epidemiologic and clinical data on HL for Albania, aiming for a better understanding of the disease presentation to diagnose it at an earlier stage with the result of a better outcome.

Methods and Results: This single-center, retrospective study performed in the Mother Theresa University Hospital Center (Tirana, Albania) analyzed epidemiological and clinical data of pediatric patients under 14 years of age diagnosed with HL during a 10-year study period from 2012 to 2022. During the last 10 years, 25 children (ages 0-14) were diagnosed with HL at UHC "Mother Theresa," Tirana. From the demographic data of our study, most patients (68%) were in the age group of 10-14. The male-to-female ratio was 2.12:1. The first clinical presentation was mainly because of lymphadenopathy in 92% of patients, with the presence of B symptoms in 68%. In most cases, the CBC was not affected, yet 24% of patients had high platelets, and 12% had low RBCs, while WBCs increased in 16% of patients and decreased in 12%. Lymphopenia and monocytosis were found in more than half of cases. Around 68% of patients had high levels of LDH and CRP. ESR and ALP were high in 64% of patients, Ferritin was high in 32%, and fibrinogen level was high in 28%. According to the Ann Arbor system, most of our patients were at stage II (32%), followed closely by stage I (28%) and stage III (24%), and only 16% were at stage IV upon presentation. The most common histopathologic type was nodular sclerosis classical HL, presented in 44% of cases.

Conclusion: HL is a relatively frequent pediatric malignancy in young adults, affecting mainly males, and is diagnosed at a relatively early stage in our country.**(International Journal of Biomedicine. 2024;14(1):26-29.)**

Keywords: pediatric Hodgkin lymphoma • lymph node • complete blood count • diagnosis

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Abbreviations

CBC, complete blood count; **HL**, Hodgkin lymphoma; **LN**, lymph node; **LRCHL**, lymphocyte-rich classic HL; **LDCHL**, lymphocyte-depleted classical HL; **MCCHL**, mixed cellularity classical HL; **NSCHL**, nodular sclerosis classical HL; **NLPHL**, nodular lymphocyte-predominant HL.

Introduction

Hodgkin lymphoma (HL) is a rare malignancy characterized by a malignant proliferation of cells in the reticuloendothelial system, mainly lymph node (LNs), and the presence of Reed-Sternberg cells with a relatively good prognosis compared to other pediatric malignancies.[1] There are several risk factors, but the strongest until now are the family history of previous lymphoma or adenopathy and previous Epstein-Barr virus infection.[2]

According to histological features, two main variants of Hodgkin's Lymphoma are classic HL in 95% of cases and nodular lymphocyte-predominant HL (NLPHL) in 5%. Classic HL is divided into four subtypes: nodular sclerosis classical

HL (NSCHL), mixed cellularity classical HL (MCCHL), lymphocyte-rich classic HL (LRCHL), and lymphocyte-depleted classical HL (LDCHL).[1,3] It is mainly presented with superficial lymphadenopathy with or without B symptoms, which, if diagnosed in an early stage, has a very good survival rate of over 90% in developed countries.[1,2]

Unfavorable prognostic factors of pediatric HL include age between 5 and 10, male gender, stage IV disease (the Ann Arbor staging system), presence of bulky disease and B symptoms at presentation, a hemoglobin level <10.5 g/dL, WBC>15×10³/μL, lymphocyte count <600×10³/μL, a serum albumin level <3.5g/dL.[1,4]

Materials and Methods

This study aimed to produce epidemiologic and clinical data on HL for Albania, aiming for a better understanding of the disease presentation to diagnose it at an earlier stage with the result of a better outcome. This single-center, retrospective study performed in the Mother Theresa University Hospital Center (Tirana, Albania) analyzed epidemiological and clinical data of pediatric patients under 14 years of age diagnosed with HL during a 10-year study period from 2012 to 2022. Data were extracted from charts. A positive diagnosis of HL was considered only if confirmed by a biopsy sample histopathologic examination. This is a retrospective descriptive study where patient identity and sensitive information are not revealed, even though the parents have signed the hospital-informed form on scientific data usage. Since this is not an interventional study, ethics approval was not recommended.

Results

During the last 10 years, 25 children (ages 0-14) were diagnosed with HL at UHC "Mother Theresa," Tirana. From the demographic data of our study, most patients (68%) were in the age group of 10-14, followed by the age group of 5-9 (28%). HL is rare in children under 5; in our study, only one patient was diagnosed under 5. Age distribution is presented graphically. The male-to-female ratio was 2.125:1, and all males were in the age group of 1-9. Regarding geographic distribution, most of the patients lived in central Albania (28% from Tirana County, 16% from Elbasan County, 16% form Dibra County), as more than half of the population is concentrated in this region.

From clinical data, we found that the first clinical presentation was mainly because of lymphadenopathy in 92% of patients, with the presence of B symptoms in 68%. Table 1 presents the frequency of encountering each symptom and other common clinical findings on physical examination. Hepatosplenomegaly was present in 40% of patients, and cough and dyspnea in 44% and 32%.

The most common site of LN involvement was the cervical region, found in up to 91% of patients, followed by the supraclavicular region in 69%. Meanwhile, the axillar and inguinal regions were each affected in 44% of all cases. Affected nodes were mostly not painful, often found bilaterally in 83%, mobile locally in 78% of cases, and with a firm consistency in 78% of cases. Most patients had a package of LNs affected, rather than just one (74%).

Table 1.

The frequency of encountering each symptom and other common clinical findings on physical examination.

Clinical Finding	Number of cases	%
B symptoms	17	68%
Peripheral lymphadenopathy	23	92%
Hepatosplenomegaly	10	40%
Cough	11	44%
Dyspnea	8	32%
Malaise	12	48%
Abnormal breath sounds	16	64%
History of antibiotic intake	21	84%

Important diagnostic information was also extracted from the laboratory investigations. In most cases, the CBC was not affected, yet 24% of patients had high platelets, and 12% had low RBCs, while WBCs increased in 16% of patients and decreased in 12%. A leukocyte count >15×10³/μL is an unfavorable prognostic factor,[4] and in our study, two patients had leukocytosis, with 80% of all having left formula shift with increased bands. Lymphopenia and monocytosis were found in more than half of cases. Hemoglobin <10.5 g/dL is another unfavorable prognostic factor,[4] but most of the patients had a hemoglobin level >11.5 g/dL, and only 32% had a hemoglobin level <10.5g/dL. The median neutrophil count of our patients was 5.4×10³/μL while the median lymphocyte count was 1.7×10³/μL, giving the value of the neutrophil/lymphocyte ratio of 3.17.

Other inflammatory markers like ESR, CRP, ferritin, fibrinogen, LDH, ALP, and albumin were also studied (Chart 1). Around 68% of patients had high levels of LDH and CRP from the initial laboratory tests. ESR and ALP were high in 64% of patients, Ferritin was high in 32%, and fibrinogen level was high in 28%. Albumin is another parameter with a predictive value considered unfavorable if lower than 3.5g/dL,[4] and such levels were encountered in only 20% of our patients.

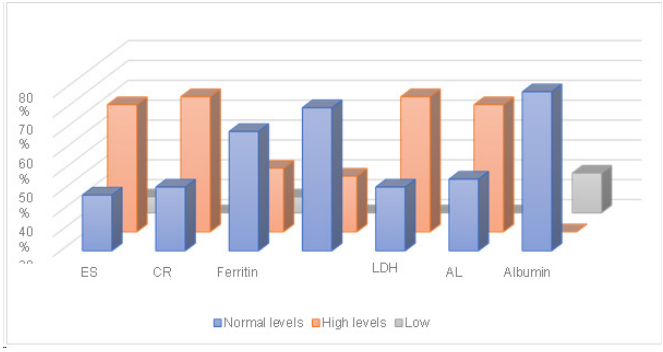

Chart 1. *Inflammatory markers levels on initial tests on patients diagnosed with HL*

We analyzed the presence of bulky disease that was also considered a bad prognostic factor[4] in the following cases: a mediastinal mass with a maximum width equal to or greater than one-third of the internal transverse diameter of the thorax at any level on a chest X-ray or a nodal mass of more than 6 cm diameter.[1,5] In our study, bulky disease was observed in 32% of cases, and 5 out of 8 patients had a mediastinal mass.

CT scan revealed even more affected LN sites than the physical examination. However, the most affected sites were still the cervical regions (76%), and 64% of patients had affected mediastinal nodes. Submandibular and occipital regions were rarely affected.

The liver and spleen are two other frequently affected organs, and they were found enlarged in 40% of patients through physical examination and in 88% through abdominal ultrasound.

Evaluation of metastasis is very important in staging HL. In our study, the metastatic sites were as follows: 41% of patients had splenic metastasis, 23% had lung metastasis, followed by liver metastasis in 12% of patients, and very rarely (in 4 patients) metastasis was observed in the peritoneum, mesentery, bone (right pelvis) and adrenal gland.

After all the investigations, patients were staged at presentation based on the Ann Arbor system. Most of our patients were at stage II (32%), followed closely by stage I (28%) and stage III (24%), and only 16% were at stage IV upon presentation.

In this cohort, we also studied histopathology characteristics. The most common histopathologic type was NSCHL, presented in 44% of cases. The second most frequent was MCCHL in 32% of patients. The LRCHL type was present in 12% of patients, while the LDCHL was present in only 4%. NLPHL was observed in 8% of patients (Chart 2).

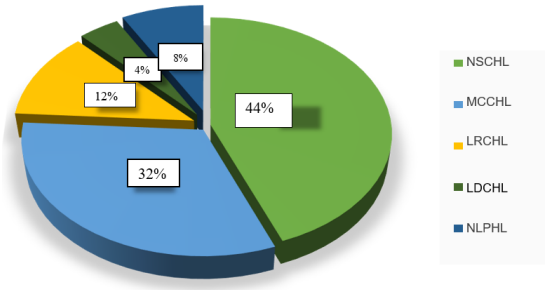

Chart 2. *Distribution of patients according to the histological HL subtypes*

Discussion

This study has the advantage of being realized in a single country center, giving us a good national HL overview. From the data, we calculated an incidence of 6-7 cases/year/million children up to 14 years of age, even though we are not very accurate since several cases are lost from the system because patients left to continue treatment abroad before coming to our center. The age and gender distributions are like those of other worldwide studies, where males under 15 have a higher incidence of HL than females.[1,6-8]

The most common clinical presenting symptom in pediatric patients is peripheral lymphadenopathy, but most superficial enlarged LNs in children are benign; therefore, physicians should search for other indicators that would lead the diagnosis towards malignity, like persistence of lymphadenopathy more than 6 weeks, the presence of constitutional B symptoms, the imagining characteristic of these LNs and unresponsiveness to a course of antibiotics. In our study, B symptoms were also observed frequently, in around 68% of cases, which is higher than what is expected from international studies. Probably being a retrospective study, this is overestimated and considered positive for any case of mild fever, weight loss, or sweating.[8]

As for the lab test, no specific investigations besides histopathology can differentiate a benign from malignant LN, but several persistent blood changes and inflammatory markers indicate an underlying malignant process or a bed prognosis. Present in about one-third of our cohort were a high leukocyte count with neutrophil/lymphocyte ratio >3, hemoglobin <10.5g/dL, a high CRP, ESR, ferritin, or fibrinogen, and a low albumin level. These are predictors of harmful diseases.[4,9]

Imaging increases the chances of finding affected LNs and distant metastasis, especially to the spleen and lung that are the commonest, since it is much more sensitive and, for instance, scaled up the Ann Arbor staging.

In our study, around 92% of patients had classic HL, and only 8% were nodular lymphocyte-predominant HL, like the international statistics. We want to mention two atypical presentations of HL. First, a 13-year-old female patient referred only to hip pain, localized in her right pelvis. She had high levels of inflammatory markers and normal CBC. The CT scan revealed multiple bone lesions in her pelvis as well as on her inguinal LNs. Biopsy revealed HL on her LNs and osteoma on her bone. At this point, it was difficult to evaluate which malignant process had started first. After multiple discussions and other studies, such as immunophenotyping, it was concluded that the patient had primary HL of the bone. We were able to find other similar cases published.[10] Luckily, this is considered the first stage, according to the Ann Arbor staging system, and has generally a good prognosis. It is important to note that a differential diagnosis with osteomyelitis, Ewing sarcoma, and osteosarcoma should be made in such cases.

Our second, a 4-year-old male patient, presented with idiopathic thrombocytopenic purpura and superficial adenopathy. A biopsy of the enlarged LN revealed HL. Our main dilemma was whether idiopathic thrombocytopenic purpura and HL coexisted independent of each other, or one brings the other. After researching this topic,[11,12] we concluded that these conditions were related. Our leading theory is that HL can cause a great deal of inflammation, triggering different autoimmune processes that lead to idiopathic thrombocytopenic purpura.

Conclusion

HL is a relatively frequent pediatric malignancy in young adults, affecting mainly males, and is diagnosed at a relatively early stage in our country. Most pediatric HL patients present with persistent enlarged LNs that do not resolve with an antibiotic course. Other organs affected or the presence of B symptoms, such as hepatosplenomegaly and persistent inflammatory changes in laboratory tests, should not be dismissed. Paying attention to each of the findings that indicate a malignancy will enable early detection and treatment with the result of a better prognosis.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Pediatrics

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Association of Overweight and Obesity with Monosymptomatic Nocturnal Enuresis in 5-15 Years Old Children

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Abstract

***Background*:** Monosymptomatic nocturnal enuresis (MNE) has increasingly been reported as a challenging issue for families and children due to its impact on the psychological aspects of children and on reducing their concentration at school the next day. Obesity might serve as a risk factor for voiding dysfunction in children. Our study aimed to evaluate the relationship between excess body mass index (BMI) in children and MNE.

Methods and Results: This case-control study included 60 children diagnosed with MNE (the main group [MG]) and 60 children without MNE (the control group [CG]) aged 5-15 years. Proper matching between the two groups concerning age and sex was adopted. Age, weight, family history, and complete medical history were recorded for each participant. In the MG, 18(30%) children had excess BMI, and 42(70%) had normal BMI. In contrast, 9(15%) children in the CG had excess BMI, and 51(85%) children had normal BMI, indicating a statistically significant association between increased BMI and MNE (P=0.049). The frequency of positive family history was significantly higher among the MG than the CG (P=0.0001). The findings of this study showed no significant relationship between gender and a family history of enuresis with excess BMI in children with MNE (P=0.679 and P=0.234, respectively).

Conclusion: Obesity and overweight in children have an influence on the development of MNE. **(International Journal of Biomedicine. 2024;14(1):30-35.)**

Keywords: monosymptomatic nocturnal enuresis • bedwetting • children • obesity • overweight

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Abbreviations

BMI, body mass index; **MNE**, monosymptomatic nocturnal enuresis; **UTI,** urinary tract infection

Introduction

Nocturnal enuresis is a common disorder affecting children and adolescents worldwide. It is described as the involuntary leakage of urine while sleeping in children at least five years old.[1] Enuresis can be classified as monosymptomatic enuresis (MNE) and non-monosymptomatic enuresis. Monosymptomatic nocturnal enuresis is referred to as enuresis in children with no other symptoms of the lower urinary tract or history of bladder dysfunction. Non-monosymptomatic enuresis is enuresis in children with additional symptoms of the lower urinary tract, such as urgency, hesitancy, and postmicturition non-monosymptomatic.[2] In addition, monosymptomatic nocturnal enuresis can be classified as primary enuresis - when the child has never achieved a satisfactory period of night dryness; or secondary enuresis - when the child has had at least six months of dry nights and has begun to experience nighttime enuresis again.[3]

Monosymptomatic nocturnal enuresis has increasingly been reported as a challenging issue for families and children due to its impact on the psychological aspects of children and on reducing their concentration at school the next day. Spontaneous resolution of primary monosymptomatic nocturnal enuresis is expected as prevalence decreases from about 15% at age 5 to 5% at age 10 and finally to 1%-2%

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at age ≥15 years.[2] Numerous factors have been proposed to contribute to the etiology of monosymptomatic nocturnal enuresis, including genetic factors, sleep arousal disorder, nocturnal polyuria, maturational delay, psychological factors, detrusor overactivity, and small bladder capacity.[4]

Nocturnal enuresis hurts the child and the entire family. Children experience guilt and low self-esteem, which might lower their confidence or prevent them from participating in social activities like camping and sleepovers. Additionally, enuresis might worsen their sleep quality and cause stress and conflict within the family.[5-8]

Furthermore, there have been some indications in the research that monosymptomatic nocturnal enuresis might be related to obesity and overweight, which are defined as an increase in body fat content to the extent that it negatively impacts a child's health and which are other significant pediatric problems that have increased over the past few decades.[9] Obesity in children and adolescents can contribute to a variety of comorbid conditions, including cardiovascular diseases such as hypertension and dyslipidemia; psychological disorders such as anxiety, depression, and social isolation; gastrointestinal conditions such as fatty liver and cholelithiasis; respiratory diseases such as asthma and obstructive sleep apnea; and hormonal disorders such as metabolic syndrome and diabetes mellitus. Furthermore, it can predispose the child to obesity in later adult life.[10-12]

Studies on the relationship between childhood obesity and voiding disorders, particularly enuresis, have been conducted by many researchers.[13-16] Some of these studies have posited that obesity might serve as a risk factor for voiding dysfunction in children;[17-21] however, other studies have failed to find evidence of a direct correlation between excess body mass and lower urinary tract symptoms.[22-27]

Considering the inconclusive and contradictory findings of these studies, the current study was conducted aiming to evaluate further the relationship between excess BMI in children and monosymptomatic nocturnal enuresis.

Materials and Methods

This case-control study was conducted at Al-Khansaa maternity and pediatric hospital. Over 6 months, 60 children aged 5-15 years diagnosed with MNE were enrolled in the main group (MG) according to the International Children's Continence Society criteria.[28] The control group (CG) included 60 children without MNE who were present for routine check-ups or treatment for minor illnesses. To ensure unbiased sampling, every second child who presented to the center was selected for the main or the CG. Furthermore, proper matching between the two groups concerning age and sex was adopted.

Exclusion criteria were neurodevelopmental disorders, including autism spectrum disorder, intellectual disability, and global developmental delay; urological conditions such as UTI, history of recurrent UTI, chronic kidney disease and posterior urethral valve; diurnal enuresis; polyuria-related diseases such as diabetes insipidus and diabetes mellitus; seizure disorders; sickle cell disease and spinal dysraphism.

A semi-structured interview was conducted with both the children and their caregivers. Additionally, a structured questionnaire was employed, which requested the demographic data of the participants, neurological and endocrinological disorders, developmental milestones of the child, frequency of nocturnal enuresis within the past three months, recurrent UTI history, past medical history, and the attainment of night dryness for a minimum of six months. Subsequently, the children were given a thorough examination, encompassing a neurological assessment, anthropometric measurements, and an evaluation of developmental milestones. Afterward, the researchers requested a urine specimen for urine culture and instructed the family on the appropriate technique for obtaining a clean catch, midstream urine sample.

The weight of the children in the MG and CG was measured to the nearest 0.1kg, while they were wearing lightweight clothes, by using the Seca 700 weight scale (SECA, Hamburg, Germany). The weight scale also contains a measuring rod to measure the standing height of the children to the nearest 0.1 cm. The children were barefoot during the examination and looked straight ahead with their hands at their sides.

Thereafter, we calculated the BMI by dividing the children's weight in kilograms by the square of their height in meters, and to assign the children to weight categories, their parameters were entered into a web-based BMI percentile calculator designed by the Centers for Disease Control and Prevention. Children were categorized as overweight if their BMI was between the 85th and the 95th percentile and obese if their BMI was equal to or above the 95th percentile.[29]

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean (M) ± standard deviation (SD) for continuous variables. For data with normal distribution, inter-group comparisons were performed using Student's t-test. The frequencies of categorical variables were compared using a chi-square test, and a compare proportions test was applied. 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 conducted in accordance with the ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee at the Ninevah University/College of Medicine. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Results

We found non-significant differences between the MG and the CG regarding age, sex distribution, or BMI; at the same time, we found a predominance of boys in both groups (Table 1). The primary MNE accounted for the most significant percentage among the MG (93.3%).

In the MG, 18(30%) children had excess BMI, and 42(70%) had normal BMI. In contrast, 9(15%) children in

the CG had excess BMI, and 51(85%) children had normal BMI, indicating a statistically significant association between increased BMI and MNE (P=0.049) (Table 2). However, the boxplot showed no significant difference between the mean BMI between groups (Figure1). The frequency of positive family history was significantly higher among the MG than the CG (P=0.0001) (Table 3).

Table 1.

Demographic characteristics of control versus MNE patients.

	CG (n=60)	MG (n=60)
Age (years)	7.58±2.6	7.25±2.3
Sex (M/F)	39/21	39/21
BMI (kg/m^2)	16.8±2.1	16.9±2.6

Table 2.

The frequency of high BMI in the study groups.

Group	BMI		*P*-value
	Excess BMI n (%)	Normal BMI n (%)	
MG	18 (30)	42 (70)	0.049
CG	9 (15)	51 (85)	
Total	27	93	

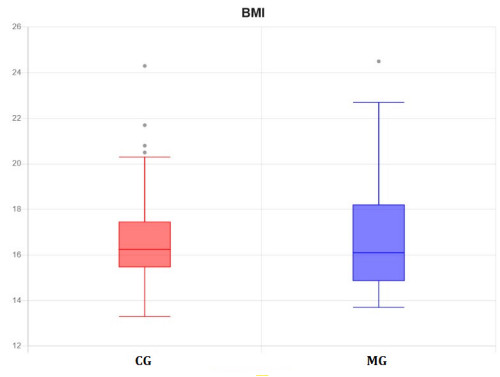

Fig. 1. *BMI in the control versus MNE patients.*

Table 3.

The frequency of family history in the study groups.

Group	-Family History	+Family History	Odds ratio	*P*-value
CG (n=60)	57 (95%)	3 (5%)	23.2	<0.0001
MG (n=60)	27 (45%)	33 (55%)		

The findings of this study showed no significant relationship between gender and a family history of enuresis with excess BMI in children with MNE (P=0.679 and P=0.234, respectively) (Table 4). A Pearson correlation coefficient was computed to assess the relationship between BMI/age percentile and weekly enuresis frequency in the MG. There was a non-significant weak correlation between the two variables (r =-0.1032, P=0.434). The coefficient of determination (R^2) was 0.011 (Figure 2).

Table 4.

Gender and family history as risk factors for excess BMI in children with MNE.

Factors		Excess BMI n (%)	Normal BMI n (%)	*P*-value
Gender	Male	11(61)	28 (67)	0.679
	Female	7 (39)	14 (33)	
	Total	18 (100)	42 (100)	
Family history of enuresis	Positive	12 (67)	21 (50)	0.234
	Negative	6 (33)	21 (50)	
	Total	18 (100)	42 (100)	

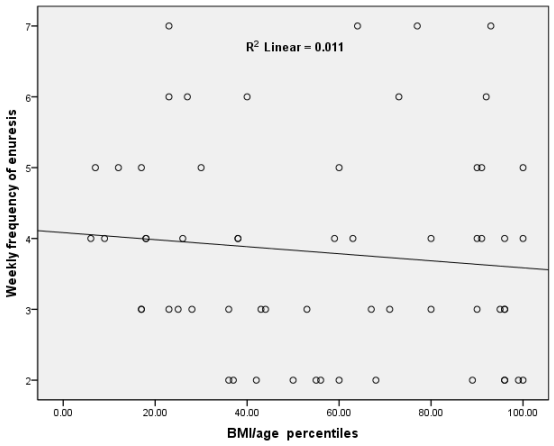

Fig. 2. *Correlation of BMI with the weekly frequency of enuresis.*

Discussion

Our study demonstrated that children with MNE had a higher prevalence of obesity and overweight than controls had, which is consistent with previous studies.[18,24,30] However, other studies reported no differences in the prevalence of MNE between obese or normal-weight children.[22,26]

The underlying pathogenesis of the increased prevalence of MNE in obese children is obscure; however, there have been reports about suggested explanations, including the impact of obesity on bladder pressure and reducing the bladder capacity to hold urine, causing bedwetting.[17,31]

Obesity is also known to alter the sleep pattern, resulting in fragmented sleep with association with frequent waking periods, resulting in bedwetting.[19,27] Moreover, mood alteration and psychological disturbances associated with obesity resulted in increased stress, which overburdened the child and resulted in bullying by peers and reduced self-esteem with subsequent increased chance of bedwetting.[32] Obese children are more susceptible to snoring, which is associated with difficulties in breathing during sleep, with subsequent frequent waking, resulting in sleep disturbances.[27] Sleep difficulties are usually related to next-day fatigue and reduced concentration with reduced mood; all of these increase the chance of nocturnal enuresis.[17] However, in contrast to our study, other studies did not find a significant association between overweight/obesity and monosymptomatic nocturnal enuresis.

A possible explanation for these contradictory findings is the study design and sample size variations. In particular, Zahra et al.[22] studied 180 children with monosymptomatic nocturnal enuresis in a retrospective review of patient records; a study by Uzan et al.[26] was an observational study with a cross-sectional design; Monkhouse et al.[27] conducted a retrospective review of the records of 1000 children; and Ibrahim et al.[34] conducted a community-based cross-sectional study which included 866 children.

The present study enrolled MNE children from 5-15 years old (with a mean age of 7.25±2.3 years), the frequently reported age for bedwetting. The evidence confirmed that MNE is typically highly prevalent in children under 10 years old,[35-37] declining in children older than 10 years.[37] The results demonstrated that MNE is distributed unequally in both sexes and is more prevalent in boys than girls, which disagreed with previous reports by Hamed et al.[37] and Karaci et al.,[17] who found equal distribution. Nonetheless, non-significant differences existed between boys and girls regarding the presence or absence of obesity and overweight among the 60 children with MNE. Conversely, Yeung et al.[38] confirmed a higher prevalence of MNE in girls than boys; however, Schum et al.[39] and Schum et al.[40] reported that MNE is more prevalent in boys than girls due to earlier development of successful toilet training in girls than boys.

Despite the impact of obesity on developing bedwetting, however, family history might also partially contribute to MNE, especially after the determination of several certain genetic links that might predispose to the hereditary development of MNE.[23] Obesity by itself has a genetic association with parents, which means that parents who have a family history of obesity will develop obesity in their childhood with subsequent development of MNE.

Nonetheless, in our study, non-significant differences (P=0.23) existed between participants' positive and negative family histories of enuresis regarding the presence or absence of obesity and overweight in the MNE children. Moreover, a non-significant correlation existed between BMI increments and weekly frequency of enuresis, indicating that obesity is only partially a risk factor for enuresis and that the etiology of MNE is multifactorial, involving family history,[23] sex,[40] age,[35-37] psychological situation,[19,23] and socioeconomic factors.[27,34]

Despite these explanations of the role of obesity and family history in developing MNE, social and family history shouldn't be ruled out due to their impact on children's psychology, considering that children react more sensitively toward their environment than adults.[17,19,23,27,34]

Conclusion

The findings of this study provide evidence that obesity and overweight in children have an influence on the development of MNE. The children under 10 years of age are more active in developing MNE with no impact of gender differences on increasing BMI among girls and boys with MNE. The present study found that a positive family history of enuresis has a significant impact on the development of MNE but no additional impact on the prevalence of overweight and obesity in those children suffering from MNE.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Nephrology

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The Influence of the Time Factor and Marital Status on the Quality of Life of Patients After Kidney Transplantation in Uzbekistan

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Abstract

***Background*:** Currently, compared to program hemodialysis and peritoneal dialysis, kidney transplantation is considered to be the preferred method of renal replacement therapy in patients with end-stage chronic kidney disease (CKD) in terms of patient survival and improvement in their quality of life (QOL). This study aimed to investigate the influence of the post-transplantation period and marital status on the health-related QOL of patients with CKD who underwent kidney transplantation.

Methods and Results: A cross-sectional study was conducted among 78 patients who had received a kidney transplant from living related donors in the Republican Specialized Scientific and Practical Medical Center for Nephrology and Kidney Transplantation between January and April 2022. Kidney transplant recipients (KTRs) were divided into four groups depending on the time after kidney transplantation: 3, 6, 12 months, and 2 years or more after surgery. The study used 2 questionnaires. The first included questions about the sociodemographic data of patients; the second was the standardized health-related QOL questionnaire SF-36.

This study of the QOL of KTRs, residents of the Republic of Uzbekistan, showed an improvement in most scales of physical and mental health components 12 months and 2 years or more after kidney transplantation. On the scales of physical and mental health components, unmarried KTRs had a higher self-assessment of QOL than married KTRs, and among married KTRs, those with children had higher QOL indicators.

Conclusion: Clarifying how the time after kidney transplantation and individual sociodemographic and medical factors influence QOL indicators requires further research (including longitudinal studies) in a wider KTR population.**(International Journal of Biomedicine. 2024;14(1):36-40.)**

Keywords: chronic kidney disease • kidney transplantation • quality of life • Uzbekistan

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Abbreviations

CKD, chronic kidney disease; **KTRs**, kidney transplant recipients; **PF**, physical functioning; **PCS**, physical component summary; **QOL**, the quality of life; **RP**, role-physical functioning; **MCS**, mental component summary; **SF-36**, the 36-Item Short-Form Health Survey; **SF**, social functioning; **VT**, vitality.

Introduction

Currently, compared to program hemodialysis and peritoneal dialysis, kidney transplantation is considered to be the preferred method of renal replacement therapy in patients with end-stage chronic kidney disease (CKD) in terms of patient survival and improvement in their quality of life (QOL). In recent years, various researchers and international working groups have noted that the study of health-related QOL is an important tool for indirectly assessing the functioning of the transplant, as well as the effectiveness and quality of patient-centered care provided to kidney recipients.[1] In most international studies of QOL in kidney transplant recipients (KTRs), the universal health-related QOL questionnaire SF-36 was used.[2,3]

Several retrospective studies have reported an improvement in QOL over time on most scales in KTRs 1-5 years after kidney transplantation.[4] At the same time, a longitudinal study using data from the Korean Kidney Transplant Outcome Cohort Study reported a significant improvement in QOL measured by SF-36 in KTRs 2 years after kidney transplantation. However, a follow-up study in a larger cohort of KTRs demonstrated that although QOL showed improvement 2 years after kidney transplantation, it began to decline after 4 years of follow-up in the same cohort.[5] Thus, researchers have not come to a unified conclusion about QOL in patients with a transplanted kidney at various time periods after transplantation.

A number of researchers attempted to assess the QOL of the KTRs from the point of view of demographic and socio-economic aspects. The influence of gender, age, education, marital status, employment, and income level was studied.[6-8]

When studying the influence of marital status on self-assessment of QOL, Ryu et al.[5] concluded that married KTRs have better QOL scores than unmarried ones. At the same time, Junchotikul et al.[9] and Chisholm et al.[10] obtained the opposite result – family KTRs showed lower rates on QOL scales than did single patients.

Thus, the question of the influence of marital status on the QOL in people with a kidney transplant is still open since the findings of earlier studies contradict one another.

To date, it has been shown that the study of the QOL of KTRs using the SF-36 universal questionnaire is an important criterion for assessing the state of functioning of the kidney graft and the effectiveness of this type of renal replacement therapy.[1] At the same time, the ambiguity of researchers' opinions about the influence of certain sociodemographic factors, as well as the duration of the graft functioning on the assessment of the QOL of kidney recipients, indicates the need for further research in this direction.

This study aimed to investigate the influence of the post-transplantation period and marital status on the health-related QOL of patients with CKD who underwent kidney transplantation.

Materials and Methods

A cross-sectional study was conducted among 78 patients who had received a kidney transplant from living related donors in the Republican Specialized Scientific and Practical Medical Center for Nephrology and Kidney Transplantation between January and April 2022. The age of patients ranged from 18 to 60. KTRs were divided into four groups depending on the time after kidney transplantation: 3, 6, 12 months, and 2 years or more after surgery.

The study used 2 questionnaires. The first included questions about the sociodemographic data of patients; the second was the standardized health-related QOL questionnaire SF-36.

The following sociodemographic information and anthropometric data were obtained from the respondents: gender, age, height, weight, marital status, level of education, employment status, and residential area.

The QOL was evaluated using the 36-item Short Form Survey (SF-36) questionnaire.[11] The SF-36 contains eight domains: Physical functioning [PF (10 items)], Role physical [RP (4 items)], Bodily Pain [BP (2 items)], General Health [GH (5 items)], Vitality [VT (4 items)], Social functioning [SF (2 items)], Role emotional [RE (3 items)], and Mental Health [MH (5 items)]. These eight scales can be aggregated into two summary measures: the Physical Component Summary (PCS)and Mental Component Summary (MCS). The higher scores suggest a better assessment of one's health status, and the maximum score (100) indicates the predominance of positive statements and a very favorable assessment of one's health.

The survey was conducted online through Google Forms in Uzbek and Russian. QOL indicators were calculated and evaluated with the use of a specially developed computer program.

Results were statistically processed using Microsoft Excel 2019. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results

The sociodemographic characteristics of participants in the study are presented in Table 1: 95% of KTRs were young patients, 77% were men, and most of the KTRs were married and had children. The number of people with secondary education and who were unemployed among KTRs was higher than that of the highly educated and employed.

Table 1.

Patient characteristics (n=78).

Patients' characteristics	n (%)
Gender	
Male	60 (77.0)
Female	18 (23.1)
Age (years)	
Range	18-55
Mean ± SD	32.7 ± 7.3
Education	
Higher education	24 (30.7)
Secondary education	54 (69.3)
Employment status	
Employed	20 (25.6)
Not employed	58 (74.4)
Marital status	
Married	64 (82.1)
Not married	14 (17.9)
Children	
KTRs with children	54 (69.2)
KTRs without children	24 (30.8)

In our study, we found a clear trend toward an increase in QOL indicators over time after kidney transplantation (Figures 1 and 2): after 6 months, there was a slight increase in 2 scales (PF, RP) of the physical component of health, as well as an integrated indicator, PCS; after 12 months, there was an increase in QOL indicators on all 8 scales of physical and mental health components, of which a significant increase was noted on the PF, SF, RE, and PCS (P<0.05).

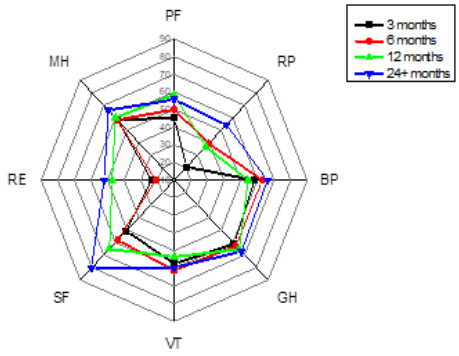

Fig. 1. *Items of SF-36 scales in the postoperative period.*

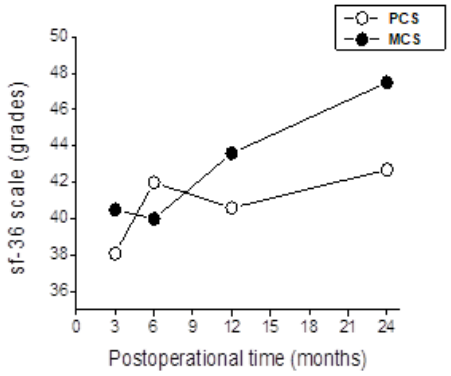

Fig. 2. *The Physical Component Summary (PCS) and Mental Component Summary (MCS) of the SF-36 in the postoperative period*

In the KTR group with a period of more than 2 years after transplantation, higher QOL indicators were found, while significant increases were noted on the RP (P<0.001), SF (P<*0.0001*), RE (P<0.05), PCS, and MCS (P<0.05).

KTRs were divided into groups based on their marital status: single, married, and with children. The study of the influence of marital status on QOL indicators showed a higher self-assessment of QOL on physical and mental health components scales in unmarried KTRs compared to married KTRs, with statistically significant improvements in 3 out of 8 scales (RP, GH, VT [P<0.05]), (Figure 3). Among married KTRs, QOL indicators were slightly higher in patients with children, than in patients who were married but did not have children (PF [P<0.05], BP [P<0.001], SF [P<0.0001]), (Figure 4).

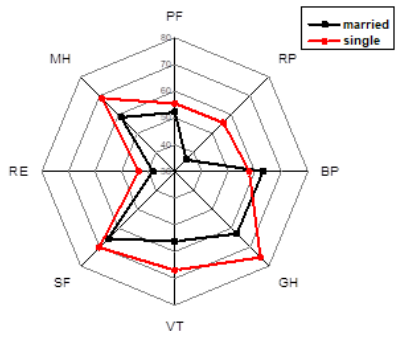

Fig. 3. *Indicators of QOL depending on marital status of KTRs.*

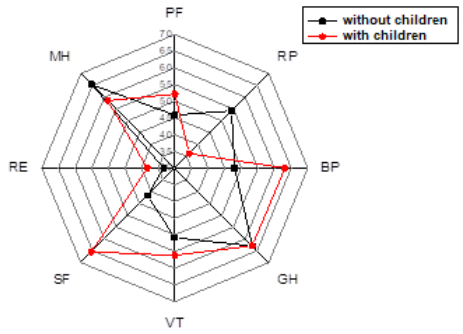

Fig. 4. *Indicators of QOL among married KTRs with and without children.*

Discussion

In general, our results align with the findings of earlier studies, although we found some differences that deserve discussion, mainly having to do with the period of assessment following surgery. Vu et al.[12] point to higher QOL in KTRs with extended postoperative periods. It should be noted that, according to most researchers, the most optimal assessment of QOL is observed in KTRs with a period after transplantation from 1 to 5 years. At the same time, some researchers indicate that a significant improvement in QOL in KTRs is observed 5, 10, or more years after kidney transplantation.[4]

To study the influence of the time factor on the formation of a subjective assessment of QOL indicators according to the SF-36 questionnaire, the KTRs who took part in the study were divided into four groups depending on the timing of kidney transplantation - with a period of 3, 6, 12 months and 2 years or more after the operation. Our analysis of this study showed a clear trend toward an increase in QOL indicators over time after kidney transplantation: after 6 months, there was a slight increase in 5 scales (PF, RP, BP, VT, SF) of physical and mental components of health, as well as PCS; after 12 months, there was an increase in QOL indicators on 6 scales of physical and mental components of health (a statistically significant increase on the SF and RE scales), and a statistically insignificant increase in the integrated indicators - PCS and MCS.

In the KTR group with a period of more than 2 years after transplantation, higher QOL indicators were found on all

scales, while significant increases were noted on 3 scales of the physical and mental components of health (RP, SF, RE) and integrated PCS and MCS. Our results are consistent with findings reported in the literature.

These results can be explained by the fact that patients need physical activity restrictions in the early stages after surgery, more frequent visits to medical institutions, and intensive immunosuppressive therapy. In addition, during this period, there is a risk of developing postoperative complications, as well as complications, including infectious ones, associated with taking immunosuppressive drugs. Within 1 to 5 years, the patient adapts and stabilizes, physical activity increases, and social contacts expand, which is obviously reflected in a higher subjective assessment of QOL.

When studying the influence of some sociodemographic factors on the QOL parameters of KTRs, our study found that the age and gender did not significantly affect the QOL. Regarding the influence of the level of education and employment on the self-assessment of QOL, patients with higher education and working people had higher scores on the SF-36 scale than did patients with secondary education and non-working people. The findings regarding the impact of education and employment on the QOL after kidney transplantation have been previously published.[13] Given the limited sample size, there is a need for further research on these issues.

We also studied the influence of marital status on the indicators of QOL for KTRs. Higher rates of QOL were found in young unmarried KTRs on the scales of GH, VT, and SF of the physical and mental health components, as well as PCS, and a slightly higher rate on other scales, compared with married kidney recipients. The results obtained are consistent with the research findings of Junchotikul et al.[9] and Chisholm et al.,[10] which indicated lower QOL rates in married KTRs. At the same time, our findings contradict the results of Ryu et al.,[5] who concluded that married KTRs have better indicators on the QOL scales than unmarried ones.

Among married KTRs, in our study, the QOL indicators are slightly higher for patients with children than for married KTRs without children. A higher self-assessment of the QOL in KTRs with children could be because they live in a complete family and have optimistic hopes and expectations associated with raising a child. This circumstance should be considered when organizing comprehensive medical and social assistance to families of KTRs wishing to have a child. Further research is needed to obtain more reliable results.

Thus, a number of sociodemographic, psychological, and medical factors take part in forming indicators of the QOL of KTRs. At the same time, according to the results of our study, with an increase in the period after kidney transplantation of more than 12 months, there is a noticeable improvement in the QOL parameters of physical and mental health components on most scales. Depending on marital status, the QOL indicators were somewhat higher in unmarried KTRs, and among married KTRs with children.

The legal framework for transplantology in the Republic of Uzbekistan was created in 2017, and the regulatory framework on this issue continues to improve. From 2017 to 2022, more than 700 kidney transplants from a living related donor have been performed in the country.

This study has some limitations. This is a cross-sectional study, which was conducted in one Republican Center for Nephrology and Kidney Transplantation and included a relatively small cohort of CKD patients who underwent donor kidney transplantation in the period 2018-2022. In this regard, the results of this study cannot be extended to the entire population of KTRs in Uzbekistan.

At the same time, a study of health-related QOL indicators among KTRs living in Uzbekistan has not been previously conducted. In this regard, we believe it is advisable to continue and expand research on the study of the QOL of KTRs for further clarification of the role and nature of the influence of sociodemographic and medical factors on its formation. The organization and conduct of longitudinal studies are of interest to assess the QOL in the population of KTRs at various periods (including long-term) after kidney transplantation.

Conclusion

This study of the QOL of KTRs, residents of the Republic of Uzbekistan, showed an improvement in most scales of physical and mental health components 12 months and 2 years or more after kidney transplantation. On the scales of physical and mental health components, unmarried KTRs had a higher self-assessment of QOL than married KTRs, and among married KTRs, those with children had higher QOL indicators. Clarifying how the time after kidney transplantation and individual sociodemographic and medical factors influence QOL indicators requires further research (including longitudinal studies) in a wider KTR population.

Ethical Considerations

The protocol of this study was approved by the ethical committee of the Ministry of Health of the Republic of Uzbekistan (No. 6/13-1696.2022) and carried out following the Helsinki Declaration of 1964. Informed consent was obtained from all KTRs who took part in the study.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Nephrology

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Determination of Platelet Count and Platelet Indices among Sudanese Patients with Chronic Kidney Disease

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Abstract

Background: Patients with chronic kidney disease (CKD) are at a considerably higher risk of thrombotic and hemorrhagic challenges. Platelet function studies in CKD are contradictory, ranging from decreased platelet function to normal or even increased platelet reaction. Our study aims to evaluate the change in platelet count (PC) and platelet indices (platelet distribution width [PDW], platelet large cell ratio [PLCR], and mean platelet volume [MPV]) among Sudanese patients with CKD.

Methods and Results: This case-control study was conducted from February to August 2014 at East Nile Hospital, Khartoum, Sudan. The study involved 75 patients diagnosed with CKD (mean age 52.43±18.4 years) and 75 healthy individuals (mean age 50.3±14 years) as a control group.

PC, PDW, PLCR, and MPV tests were conducted using a Sysmex KX-21N Automated Hematology Analyzer (Sysmex Corporation, Japan), and creatinine level was measured by Roche/Hitachi Cobas C311 analyzer (Basel Switzerland). The creatinine level was significantly higher in CKD patients than in the control group: 7.91±4.8 mg/dL and 1.4±1.3 mg/dL, respectively (P=0.000). We found an insignificant difference between CKD patients and the control group in terms of PC, MPV, PDW, and PLCR; an insignificant difference in PC and all PI between CKD patients with creatinine levels <6 mg/dL and >6 mg/dL; and an insignificant difference in PC and all PI between the group with CKD duration <2 years and >2 years and between CKD patients on hemodialysis and without hemodialysis.

Conclusion: This study found no difference in PC and studied platelet indices in CKD patients, compared to the control group and no difference in PC and PI (MPV, PDW, and PLCR) in patients on hemodialysis versus patients not on hemodialysis. **(International Journal of Biomedicine. 2024;14(1):41-44.)**

Keywords: chronic kidney disease • platelet count • platelet indices

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Abbreviations

CKD, chronic kidney disease; **GFR**, glomerular filtration rate; **MPV**, mean platelet volume; **PC**, platelet count; **PI**, platelet indices; **PDW**, platelet distribution width; **PLCR**, platelet large cell ratio.

Introduction

Chronic kidney disease (CKD), defined as kidney damage or an estimated glomerular filtration rate (eGFR) <60 ml/min per 1.73 m^2, persisting for 3 months or more, increases the risk of other health problems.[1-4] A reduction in kidney function and structure for more than 3 months distinguishes CKD from acute renal disease. According to Kidney Disease: Improving Global Outcomes (KDIGO), CKD is defined by markers of kidney damage or decreased GFR persisting for >3 months and is classified according to cause, GFR, and albuminuria criteria (CGA classification).

Disease manifestation varies according to etiology, severity, and degree of progression.[5-7] Early CKD treatment is critical for eliminating disease progression.[8,9] Kidney

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disease has been estimated to kill 5-10 million individuals annually.[10] The prevalence of CKD varies between 7% and 12% globally.[11] Adult prevalence was reported as 1.7% in China,[12] 3.1% in Canada,[13] 5.8% in Australia,[14] and 6.7% in the United States.[15] In Europe, the frequency ranges from 2.3% in Germany[16] to 2.4% in Finland,[17] and in England, the rate ranges from 9.2% to 5.2%.[18] CKD is a public health problem throughout Africa, with a prevalence range from 2% to 41%.[19] In Sudan, the overall prevalence of CKD was estimated to be from 8% to 11%.[20]

CKD patients are at a considerably higher risk of thrombotic challenges.[21-24] However, they also have an increased risk of hemorrhagic consequences. Along with thrombocytopenia, CKD has been associated with platelet abnormalities. Platelet function studies in CKD are contradictory, ranging from decreased platelet function to normal or even increased platelet reaction.[25-27] Our study aims to evaluate the change in platelet count (PC) and platelet indices (platelet distribution width [PDW], platelet large cell ratio [PLCR], and mean platelet volume [MPV]) among Sudanese patients with CKD.

Materials and Methods

This case-control study was conducted from February to August 2014 at East Nile Hospital, Khartoum, Sudan. The study involved 75 patients (main group) diagnosed with CKD (mean age 52.43±18.4 years) and 75 healthy individuals (mean age 50.3±14 years) as a control group.

Exclusion criteria included patients who had recent blood loss or transfusion, patients who had previous or current thrombosis, and patients with an infection that might affect the investigation parameters.

For study purposes, 5 ml of blood samples were collected: 2.5 ml into the EDTA blood containers for analysis of PC, PDW, PLCR, and MPV tests conducted using a Sysmex KX-21N Automated Hematology Analyzer (Sysmex Corporation, Japan) and 2.5 ml into the heparin containers for measurement of creatinine level by Roche/Hitachi Cobas C311 analyzer (Basel Switzerland).

Statistical analysis was performed using statistical software package SPSS version 16.0 (Chicago: SPSS Inc.). For descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

The study was conducted by the ethical principles stated in the Declaration of Helsinki (1964, ed. 2013) and was approved by the Scientific Ethics Committee of Sudan University Science and Technology, Khartoum, Sudan. All participants provided written informed consent.

Results

The creatinine level was significantly higher in CKD patients than in the control group: 7.91±4.8 mg/dL and 1.4±1.3 mg/dL, respectively ($P=0.000$). We found an insignificant difference between CKD patients and the control group in terms of PC, MPV, PDW, and PLCR (Table 1); an insignificant difference in PC and all PI between CKD patients with creatinine levels <6 mg/dL and >6 mg/dL (Table 2); and an insignificant difference in PC and all PI between the group with CKD duration <2 years and >2 years (Table 3) and between CKD patients on hemodialysis and without hemodialysis (Table 4).

Table 1.

Mean levels of PC, PI, and blood creatinine in the main and control groups.

Parameter	Main group (n = 75)	Control group (n = 75)	*P*-value
PC, $\times10^3/\mu L$	274.01±102.5	286.60±76.5	0.395
MPV, fL	9.27±1.1	9.57±1.6	0.183
PDW, fL	11.58±2.3	11.85±1.8	0.425
PLCR, %	21.07±8.0	22.50±4.7	0.184
Creatinine, mg/dl	7.91 ± 4.8	1.4±1.3	0.000

Table 2.

PC and PI in CKD patients with creatinine levels <6 mg/dL and >6 mg/dL.

Parameter	Creatinine level		*P*-value
	< 6 mg/dL (n = 32)	> 6 mg/dL (n = 43)	
PC, $\times10^3/\mu L$	290.06 ± 112.1	262.07 ± 94.5	0.245
MPV, fL	9.37 ± 1.1	9.20 ± 1.2	0.532
PDW, fL	11.60 ± 2.2	11.57 ± 2.5	0.957
PLCR %	21.52± 7.7	20.76 ± 8.4	0.689

Table 3.

PC and PI in patients with CKD duration <2 years and >2 years.

Parameter	CKD duration		*P*-value
	<2 years (n = 62)	>2 years (n = 13)	
PC, $\times10^3/\mu L$	275.73 ± 102.7	265.85 ± 105.6	0.754
MPV, fL	9.32 ± 1.2	9.07 ± 0.8	0.476
PDW, fL	11.58 ± 2.4	11.77 ± 2.5	0.797
PLCR, %	21.32 ± 8.4	19.96 ± 8.5	0.598

Table 4.

PC and PI in CKD patients on hemodialysis and without hemodialysis.

Parameter	Patients on hemodialysis (n = 45)	Patients without hemodialysis (n = 30)	*P*-value
PC, $\times10^3/\mu L$	266.80 ± 100.6	284.83 ± 106.2	0.460
MPV, fL	9.25 ± 1.2	9.30 ± 1.1	0.856
PDW, fL	11.67 ± 2.5	11.47 ± 2.2	0.723
PLCR, %	21.0 ± 8.2	21.20 ± 7.9	0.917

Discussion

Our study revealed an insignificant difference in PC among CKD patients compared to the control group. There was no relation between PC and serum creatinine level. This study also showed no significant difference in PC according to the duration of CKD. The present result showed an insignificant difference in PC among patients on hemodialysis and those who were not; this result agrees with a study by Arogundade et al.[28] in India and Mohamed et al.[29] in Sudan. On the other hand, our findings disagree with the study conducted by Shittu et al.[30] in Nigeria. They found in their study a significant reduction in PC in CKD patients, and this may be due to the use of erythropoietin therapy, which potentiates the effect of megakaryocyte colony-stimulating factors, platelet-activating factor acetylhydrolase, and paraoxon.

Our study revealed insignificant differences in all PI (MPV, PDW, and PLCR) among CKD patients, compared to the control group. There was no relation between MPV and the serum creatinine level and the duration of CKD. The present result revealed an insignificant difference in MPV among patients on hemodialysis and those who were not, which agrees with a study by Bilen et al.[31] There was no relation between PDW, PLCR and the serum creatinine level, as well as the duration of CKD. The present result revealed insignificant differences in PDW and PLCR among patients on hemodialysis and those who were not, which agrees with a study by Schoorl et al.[32]

Further research with a high sample size is required, particularly on platelets in CKD patients, such as platelet function, because there is evidence of an effect of uremia on platelet function.

In conclusion, this study found no difference in PC and studied PI in CKD patients, compared to the control group and no difference in PC and PI (MPV, PDW, and PLCR) in patients on hemodialysis versus patients not on hemodialysis.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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The Role of Hepcidin, sTfR, and sTfR/Log Ferritin Index for the Differential Diagnosis of Iron Deficiency Anemia and Anemia of Chronic Disease

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Abstract

Background: In chronic diseases characterized by persistent inflammation, anemia of chronic disease (ACD) and iron deficiency anemia (IDA) are commonly encountered forms of anemia and can often co-occur. In such situations, the conventional iron status tests used for differential diagnosis are influenced by inflammation, reducing their diagnostic accuracy. The primary objective of this study was to assess the significance of hepcidin as a crucial diagnostic marker for ACD and to investigate the correlation between hepcidin and inflammation-related indicators. Furthermore, a significant secondary aim of this research was to ascertain the diagnostic role of novel biochemical markers, specifically soluble transferrin receptor (sTfR) and the derived sTfR/log ferritin index (sTfR-F index).

Methods and Results: This study was conducted at the Laboratory Service of the University Hospital Center «Mother Teresa» and included a cohort of 187 subjects, comprising 156 patients (83 females and 73 males) who were admitted to and received treatment at the Rheumatology and Cardiology Departments of the «Mother Teresa» University Hospital Center in Tirana. Additionally, 31 individuals without anemia and inflammatory conditions were included as a control group. All subjects incorporated into the study were classified into five distinct groups based on a comprehensive analysis of their complete blood profiles, iron status, and inflammation-related biomarkers: IDA, ACD, ACD+IDA, patients without anemia, and the control group without anemia.

The comparison between groups showed that the mean values of pro-hepcidin, TNFα, IL6, Hs-PCR, and ferritin are significantly decreased in IDA vs. ACD, while sTFR and sTfR-F index are significantly increased. In comparing ACD vs. ACD+IDA groups, ferritin increased significantly in the ACD group, while sTFR and sTfR-F index decreased significantly in ACD, compared to the ACD+IDA group. The ROC curves analysis of the biochemical parameters selected for the comparison of ACD vs IDA showed that the pro-hepcidin test is a perfect test for the differential diagnosis of ACD vs. IDA. The suggested cut-off value for pro-hepcidin was ≥153 ng/ml, yielding a sensitivity of 100% and a specificity of 100% for the diagnosis of ACD. As regards sTFR, the suggested cut-off value for the diagnosis of IDA vs. ACD was ≥ 4.9 µg/ml resulting in 84% sensitivity and 100% specificity. The sTfR-F index was also very useful for the diagnosis of IDA vs. ACD: for a cut-off value of ≥2.06, the sensitivity was 90% and the specificity was 96%. sTfR resulted in a good test for the diagnosis of ACD+IDA vs. ACD: the cut-off value was ≥ 3.7 µ/ml, the sensitivity was 92.3% and the specificity was 93.2%. Similarly, the parameter sTfR-F index resulted in a very good test for the diagnosis of ACD+IDA vs. ACD: for the suggested cut-off value of ≥2.3, the sensitivity was 92.3% and the specificity was 100%.

Conclusion: As a result of the study, it was found that the pro-hepcidin test was a highly accurate test for distinguishing between ACD and IDA. Meanwhile, sTfR and the sTfR-F index proved to be excellent indicators for the differential diagnosis of IDA in chronic inflammatory conditions.**(International Journal of Biomedicine. 2024;14(1):45-51.)**

Keywords: anemia of chronic disease • iron deficiency anemia • pro-hepcidin • transferrin receptor

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Abbreviations

ACD, anemia of chronic disease; **IDA**, iron deficiency anemia; **CHF**, chronic heart failure; **RA**, rheumatoid arthritis; **TfR**, transferrin receptor; **sTfR**, soluble TfR; **TfS**, transferrin saturation; **sTfR-F index**, sTfR/log ferritin index.

Introduction

Chronic infection, inflammation, and neoplastic pathology are commonly associated with anemia called "anemia of inflammation" or anemia of chronic disease (ACD). This form of anemia and iron deficiency anemia (IDA) are the most frequent forms of anemia encountered.[1,2] Various studies have reported the prevalence of ACD in chronic disease as 30%-70% in patients with rheumatoid arthritis (RA),[3,4] 28%-55% in patients with HIV infection (depending on the extent of the disease),[5] 30%-70% in patients with chronic liver disease[6] and 57% in patients with congestive heart failure.[7] In patients with malignant pathologies, the prevalence of anemia varies greatly depending on the stage of the disease and the therapy used. In most cases, the anemia of these pathologies has the characteristics of ACD.[8]

The pathophysiology of ACD during inflammation is intricately linked to the action of cytokines and reticuloendothelial system cells. These mechanisms result in various alterations, including the regulation of iron homeostasis, inhibition of erythroid precursor proliferation, attenuation of the erythropoietin response to anemia, and a reduction in the lifespan of erythrocytes. These modifications are predominantly mediated by hepcidin, recognized as the principal hormone governing iron balance.[9,10]

Inflammatory cytokines, particularly interleukin-6 (IL-6), are pivotal in stimulating hepatocyte hepcidin production.[11] Hepcidin functions by obstructing the functional activity of ferroportin, which serves as the primary conduit for iron export, leading to diminished absorption of dietary iron in enterocytes and reduced iron release into the bloodstream from splenic red pulp macrophages and hepatocytes. Consequently, this sequesters iron within the splenic red pulp macrophages, thereby decreasing iron availability for supporting erythropoiesis, even when iron stores are sufficient. This state is frequently referred to as «functional iron anemia.»[12]

As a distinctive feature of ACD, the serum typically displays normal or elevated ferritin levels, reflecting the accumulation and storage of iron within the reticuloendothelial system. Further elevations in ferritin levels occur due to immune activation.[13] The combination of reduced serum iron concentration and normal or elevated serum ferritin concentration is paramount in distinguishing ACD from IDA.[14]

In the context of chronic diseases, it is not uncommon to encounter not only ACD and IDA but also a mixed form of anemia known as ACD+IDA, particularly in cases of chronic inflammatory diseases that involve bleeding and/or malnutrition.[15,16]

The laboratory diagnosis of iron deficiency anemia in the presence of chronic inflammation poses significant challenges. This is because the acute phase of inflammation impacts transferrin saturation (TfS) and ferritin levels, making identifying an absolute iron deficiency quite challenging. Conversely, markers such as soluble transferrin receptor (sTfR), fragments generated through the proteolytic cleavage of the extracellular domain of transferrin receptor (TfR), directly reflect the functional iron status. Notably, sTfR is not influenced by acute inflammation, unlike ferritin, an acute-phase protein. Moreover, the sTfR/log ferritin index (sTfR-F index), calculated as the ratio of sTfR to the logarithm of ferritin, offers an even more accurate representation of the body's iron status.[17-19]

The primary objective of this study was to assess the significance of hepcidin as a crucial diagnostic marker for ACD and to investigate the correlation between hepcidin and inflammation-related indicators. Furthermore, a significant secondary aim of this research was to ascertain the diagnostic role of novel biochemical markers, specifically sTfR and the derived sTfR-F index. These markers were particularly evaluated for their diagnostic utility in distinguishing IDA and, more notably, in the differential diagnosis of IDA in chronic inflammatory conditions.

Materials and Methods

This study included a cohort of 187 subjects, comprising 156 patients (83 females and 73 males) who were admitted to and received treatment at the Rheumatology and Cardiology Departments of the «Mother Teresa» University Hospital Center in Tirana. Additionally, 31 individuals without anemia and inflammatory conditions were included as a control group. All patients were meticulously selected following a protocol that had obtained approval from the Biochemical-Clinical Laboratory Service in collaboration with the respective clinical departments.

Conforming to the guidelines set forth by the WHO, individuals were categorized as anemic if their hemoglobin levels fell below 12 g/dl for women and 13 g/dl for men. The subjects incorporated into the study underwent classification into five distinct groups based on a comprehensive analysis of their complete blood profiles, iron status, and inflammation-related biomarkers:

1. Patients with IDA: These individuals exhibited diminished sideremia levels and ferritin concentrations below 30 ng/ml.

2. Patients with ACD: This group presented reduced sideremia levels and manifested either elevated ferritin levels exceeding 100 ng/ml or ferritin levels ranging from 30 to 100 ng/ml, coupled with an sTfR-F index <1.

3. Patients with ACD+IDA: These patients exhibited diminished sideremia, alongside ferritin levels ranging from 30 ng/ml to 100 ng/ml, and notably, a sTfR-F index >2.

4. Patients suffering from RA or CHF without anemia: These subjects had been diagnosed with either RA or CHF, yet they did not present symptoms of anemia.

5. Control group without anemia: Comprising individuals who had undergone outpatient assessments, this group was characterized by the absence of anemia, as well as the presence of indicators denoting normal iron status and inflammation.

The prevalence of different forms of anemia in the patients with these chronic diseases included in the study was evaluated.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Mean, standard deviation (SD), Median (Me), and 95% confidence interval (95% CI) were calculated. For comparisons between 2 independent groups, Student's t-test was applied. One-way ANOVA with the Tamhane post hoc test was used for multiple groups. Spearman's rank correlation coefficient 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.

Receiver Operating Characteristic (ROC) curves were employed to assess the sensitivity and specificity of selected laboratory parameters, namely hepcidin, sTfR, and the sTfR-F index, in the context of distinguishing between ACD and the IDA, as well as between ACD and ACD+IDA. Hepcidin was evaluated through the DRG Elisa prohormone hepcidin kit for the dosage of the prohormone hepcidin in serum, which correlates with the levels of hepcidin.

The sensitivity and specificity levels of each chosen laboratory parameter were meticulously documented. The Area Under the Curve (AUC) was calculated, serving as a metric to gauge the capacity of the respective laboratory parameter to accurately categorize patients with distinct forms of anemia. Various threshold values, or cut-offs, were identified for these laboratory parameters, and corresponding sensitivity and specificity values were reported for each cut-off. For the purposes of differential diagnosis, the most suitable cut-off values were determined based on clinical judgment.

Results

The study's findings revealed the presence of anemia in 45.7% of patients with RA, with 45.4% attributed to ACD, 29.5% to IDA, 20.4% to the mixed form ACD+IDA, and 4.5% to other forms of anemia. In patients with CHF, anemia was detected in 50.7% of cases, with 64.9% of those cases corresponding to ACD, 16.2% to IDA, 10.8% to ACD+IDA, and 8.1% to other forms of anemia.

Table 1 presents the data on the ages, genders, and laboratory test results of 156 patients and 31 healthy individuals. Table 2 compares laboratory parameters between different groups in the study. The Tamhane t-test was used according to the ANOVA post hoc procedure to compare continuous parameters between groups with unequal variances. The comparison between groups showed that the mean values of pro-hepcidin, TNFα, IL6, Hs-PCR, and ferritin are significantly decreased in IDA vs. ACD, while sTfR and sTfR-F index are significantly increased ($P<0.001$ in all cases). In comparing ACD vs. ACD+IDA groups, ferritin increased significantly in the ACD group, while sTfR and sTfR-F index decreased significantly in ACD, compared to the ACD+IDA group ($P<0.001$ in all cases).

The above data for the parameters of blood iron, ferritin, transferrin, TfS, sTfR, sTfR-F index, pro-hepcidin, and IL-6 are presented visually below by Error Bars graphics presenting the average values of the corresponding laboratory parameter in a 95% confidence interval (CI) for different groups of the study.

Table 1.

Gender, age and laboratories tests of 156 patients and 31 healthy individuals

Variable	The study groups				
	IDA (n=19)	ACD (n=44)	ACD+IDA (n=13)	PWA (n=75)	CG (n=31)
Male/ Female*	6/13	25/19	5/8	35/40	22/9
Age (years)^	54.7±12.0	58.6±13.2	59.8±14.0	56.6±11.7	58.6±11.9
RBC (×10⁶/μL)	4.3±0.3	4.0±0.6	4.1±0.4	4.6±0.5	4.9±0.4
Hb (g/dL)	10.2±1.4	11.2±1.4	11.3±1.3	13.8±1.3	14.4±1.3
HCT (%)	31.4±4.4	32.8±4.7	34.2±3.7	39.1±4.6	41.0±8.1
MCV (fL)	73.0±5.9	81.6±7.2	80.5±6.8	85.2±5.3	85.9±5.4
MCH (pg)	23.5±3.2	28.6±3.4	27.9±3.0	29.8±1.8	29.6±2.2
RET (/1000)	6.1±2.8	4.5±2.9	5.3±3.0	6.5±3.1	7.5±3.0
RDW (%)	16.2±1.6	14.0±1.1	15.2±1.4	13.9±1.7	14.1±1.3
Fe (μg/dL)	21.4±10.7	36.5±13.3	31.3±12.2	97.8±35.9	106.4±44.9
Ferritin (ng/mL)	17.6±12.4	270.7±211.2	55.5±19.4	132.4±82.7	140.9±83.4
Tf (mg/dL)	344.2±54.6	241.0±51.8	225.6±48.9	266.8±46.9	285.5±51.8
TFs (%)	3.5±2.8	8.2±5.7	7.0±5.0	21.3±14.2	26.0±9.6
sTfR (μg/mL)	7.9±4.0	2.2±1.1	7.0±2.8	2.1±1.0	2.1±0.9
sTfR-F index	7.8±4.9	0.9±0.5	4.2±1.9	1.1±0.6	1.1±0.5
TNFα (pg/dL)	5.9±1.6	13.9±4.7	12.9±6.7	6.1±1.7	5.6±1.9
Pro-hepcidin (ng/mL)	105.0±27.4	542.2±437.8	266.1±190.9	103.3±33.8	102.1±33.5
IL-6 (pg/mL)	3.8±1.0	16.8±10.0	11.2±6.1	3.9±1.4	3.4±1.2
Hs-PCR (mg/dL)	0.2±0.1	5.3±8.3	2.8±2.5	0.2±0.1	0.2±0.1
ESR (mm/hr)	20.3±15.8	43.1±12.0	38.1±5.2	17.5±14.8	10.2±5.0

**Gender presented as n/%; ^ Age and laboratories parameters presented as M ± SD; PWA- patients without anemia; CG -control group.*

Table 2.

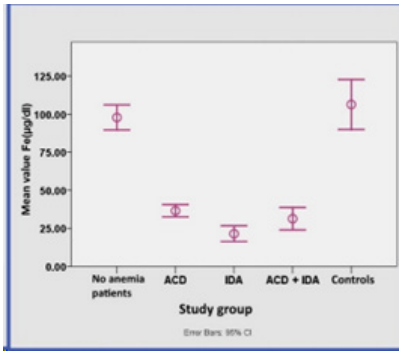
Laboratory parameters between different groups in the study.

Factor	Compared groups in the study					
	IDA vs. ACD	ACD vs. ACD+IDA	IDA vs. PWA	IDA vs. CG	ACD vs. PWA	ACD vs. CG
RBC (×10⁶/μL)	↑ 0.038	NS	↓ <0.031	↓ <0.001	↓ <0.001	↓ <0.001
MCV (fL)	↓ <0.001	NS	↓ <0.001	↓ <0.001	NS	↓ 0.047
MCH (pg)	↓ <0.001	NS	↓ <0.001	↓ <0.001	NS	NS
RDW (%)	↑ <0.001	NS	↑ <0.001	↑ <0.001	NS	NS
Fe (μg/dL)	↓ <0.001	NS	↓ <0.001	↓ <0.001	↓ <0.001	↓ <0.001
Ferr (ng/mL)	↓ <0.001	↑<0.001	↓ <0.001	↓ <0.001	↑ 0.001	↑ 0.005
Tf (mg/dL)	↑ 0.001	NS	↑ <0.001	↑ 0.006	NS	↓ 0.005
Tfs (%)	↓ 0.001	NS	↓ <0.001	↓ <0.001	↓ <0.001	↓ <0.001
sTfR (μg/mL)	↑ <0.001	↓ <0.001	↑ <0.001	↑ <0.001	NS	NS
sTfR-F index	↑ <0.001	↓ 0.001	↑ <0.001	↑ <0.001	NS	NS
TNF-α (pg/mL)	↓ <0.001	NS	NS	NS	↑ <0.001	↑ <0.001
Pro-Hep (ng/mL)	↓ <0.001	↑0.059	NS	NS	↑ <0.001	↑ <0.001
IL-6 (pg/mL	↓ <0.001	NS	NS	NS	↑ <0.001	↑ <0.001
HsCRP (mg/dL)	↓ 0.002	NS	NS	NS	↑ 0.002	↑ 0.002

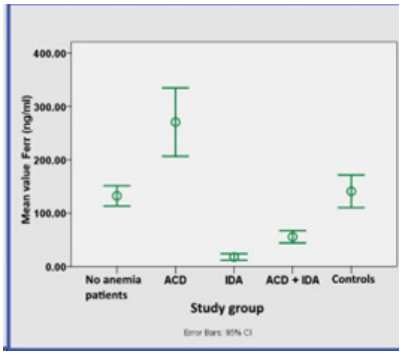
↓-Significantly lower compared to the group being compared

↑-Significantly higher compared to the group being compared;

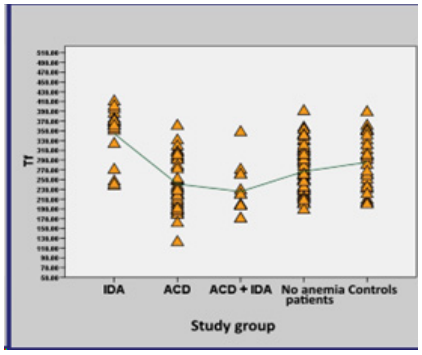
PWA- patients without anemia; CG -control group.



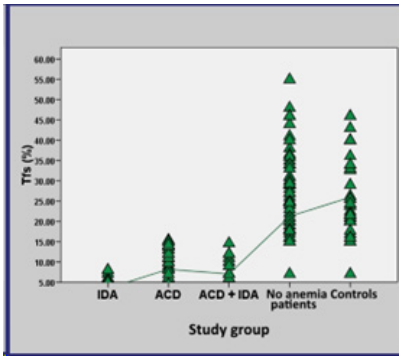
Graph 1. *Me±95% CI values of blood iron.*



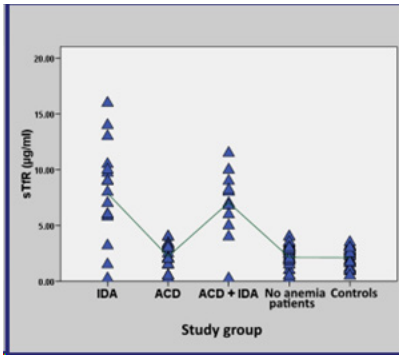
Graph 2. *Me±95% CI values of ferritin.*



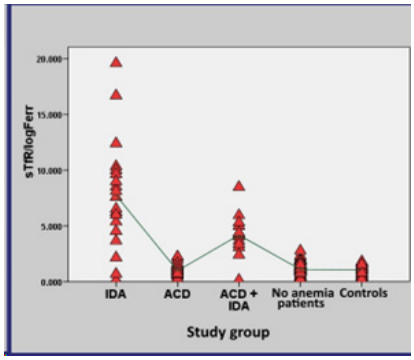
Graph 3. *Me±95% CI values of transferrin.*



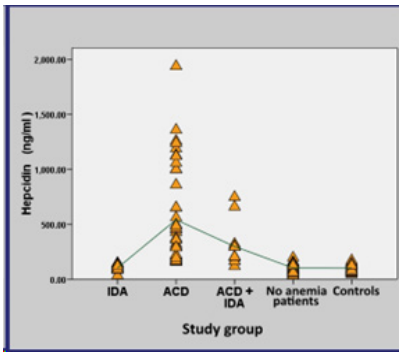
Graph 4. *Me±95% CI values of TfS.*



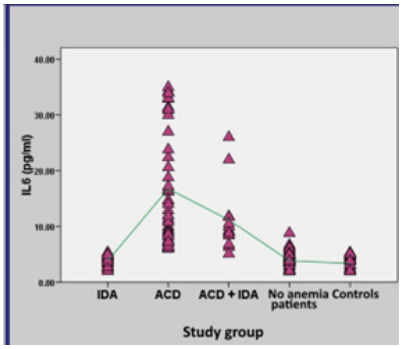
Graph 6. *Me±95% CI values of sTfR.*



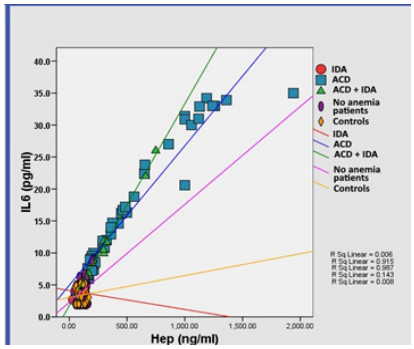
Graph 6. *Me±95% CI values of sTfR-F index.*



Graph 8. *Me±95% CI of pro-hepcidin.*



Graph 8. *Me±95% CI of IL-6.*



Graph 9. *The relationship between pro-hepcidin and IL-6.*

It can be clearly seen that for the ACD and ACD+IDA groups, the relationship between hepcidin and IL-6 is linear, while for the remaining groups it is not. The results of the linear regression confirm this: for patients with ACD, the increase in IL6 predicts the increase in pro-hepcidin for 91% of the cases. For patients with ACD+IDA, this reaches 98% (P<0.001).

The ROC curves analysis of the biochemical parameters selected for the comparison of ACD vs IDA showed that the pro-hepcidin test is a perfect test for the differential diagnosis of ACD vs. IDA. The suggested cut-off value for pro-hepcidin was ≥153 ng/ml, yielding a sensitivity of 100% (100% of those with ACD are correctly diagnosed and differentiated from those with IDA) and a specificity of 100% (all without ACD are correctly classified as without ACD). As regards sTFR, the suggested cut-off value for the diagnosis of IDA vs. ACD was ≥ 4.9 µg/ml resulting in 84% sensitivity and 100% specificity. The sTfR-F index was also very useful for the diagnosis of IDA vs. ACD: for a cut-off value of ≥2.06, the sensitivity was 90% and the specificity was 96%. sTfR resulted in a good test for the diagnosis of ACD+IDA vs. ACD: the cut-off value was ≥ 3.7 µ/ml, the sensitivity was 92.3% and the specificity was 93.2%. Similarly, the parameter sTfR-F index resulted in a very good test for the diagnosis of ACD+IDA vs. ACD: for the suggested cut-off value of ≥2.3, the sensitivity was 92.3% and the specificity was 100%.

Discussion

Anemia is a prominent symptom among hospitalized patients, and as discussed earlier, ACD and IDA are the most

prevalent forms of anemia in this context, which is further validated by the findings of our study.

Primarily, the evaluation of chronic disease-related anemia involves an assessment of iron status to exclude the possibility of IDA. In our study, we observed a decrease in both iron and saturated transferrin levels in the serum in both ACD and IDA when compared to the control group. (Graphs 1 and 2) This decline can be attributed to the complete absence of iron in IDA and iron sequestration within the splenic red pulp macrophages in ACD.[2,15,16,20]

Additionally, our study revealed that the levels of TfS in IDA patients are significantly lower than in ACD patients (P<0.001). This can be explained by the fact that concentrations of plasma iron transporter transferrin increase in IDA. However, other studies have also demonstrated that in ACD, the levels of transferrin, which acts as a negative antagonist of the acute phase, either remain normal or decrease due to the influence of inflammatory cytokines in this particular form of anemia.(1) When comparing TfS levels in ACD vs. ACD+IDA, the ANOVA test indicates that these differences are not statistically significant (P>0.05).

Retrospective studies[21] have previously shown that the transferrin concentration in IDA tends to be higher than in ACD, a finding that our study confirmed. However, it is worth noting that although the group of patients with ACD+IDA displayed variations in their transferrin concentrations, in comparison to the ACD group, most of these values still fell within the range of normal values when compared to those of healthy subjects (Table 1, Graph 3). Consequently, our results, consistent with the existing literature, lead us to conclude that the serum transferrin level does not provide a definitive indicator for detecting IDA in the presence of inflammation.

Ferritin, being a crucial iron status indicator that signifies iron storage, presents a subject of debate when it comes to determining a cut-off value for defining depleted iron reserves.[22,23] In various studies, this cut-off threshold varies, with some researchers adopting values as low as ≤12 ng/ml while others propose a higher threshold, such as ≤15 ng/ml. Additionally, due to ferritin's responsiveness as an acute-phase protein, some experts recommend even higher values like 22 ng/ml or 30 ng/ml.

Must et al.[24] found that a ferritin level below 12 ng/ml exhibits an excellent specificity of 98% but a low sensitivity of 20% in diagnosing IDA. In contrast, a cut-off value of 30ng/ml essentially transforms it into an almost flawless test for IDA diagnosis, boasting 100% sensitivity and 98% specificity. Other studies confirm this finding.

Patients who struggle with ACD while concurrently experiencing depleted iron reserves pose a particular challenge in the differential diagnosis of these two types of anemia. When we compared IDA to ACD and the control group, we observed that the serum ferritin concentration was significantly lower in IDA (P<0.001). Likewise, when comparing ACD to ACD+IDA and the control group, we noticed that the serum ferritin concentration was significantly higher in ACD than in ACD+IDA and the control group (P<0.001). In the case of patients with ACD, ferritin levels remain within the normal range or become elevated due to the influence of inflammation and the actions of inflammatory cytokines, which play a central role in the pathogenesis of ACD. Consequently, iron deposition and storage within the splenic red pulp macrophages are increased.[20,22] In the differential diagnosis between ACD and ACD+IDA, the cut-off value of ferritin for the diagnosis of IDA is calculated to be approximately 40-60 ng/dL.[23,25] Therefore, for ferritin values 30-100ng/dl, iron deficiency can neither be detected nor excluded based on the concentration of ferritin in the serum.

Based on the limitations of the above tests, which are influenced by the acute phase of inflammation, as well as the fact that sTfR concentrations are not influenced by acute-phase reactions, we decided to study the role of this biomarker, compared to other biomarkers of iron status, both in diagnosing pure IDA and in detecting IDA in conditions of inflammation.[22,26]

The mean and standard deviation of sTfR were evaluated in all groups included in the study, such as IDA, ACD, ACD+IDA, and patients without anemia, and the control group (Graph 5). Our data show that the serum concentration of sTFR in IDA presents high values when we compare this group with ACD and the control group (P<0.001); and it presents values within the normal limits in ACD when we compare this group with the control group. The value of sTfR 4.9 µg/ml serves as a cut-off to make the differential diagnosis between IDA and ACD (sensitivity 84% and specificity 100%). Also, when we compare ACD with the mixed form of anemia, we notice that the concentrations of sTfR in the serum are significantly lower in ACD, and the sensitivity and specificity of this indicator go toward a perfect test. Thus, the sTfR value >3.7 µg/ml serves as a cut-off (sensitivity 92.3% and specificity 93.2%) to make the differential diagnosis between ACD and ACD+IDA. The superiority of this test over other parameters influenced by the acute phase of inflammation is also confirmed by other authors.[23,27,28]

Since sTfR evaluates only functional iron and ferritin stores iron, i.e., static iron, studies have recommended a new index that evaluates the entire iron kinetics and improves the diagnostic efficiency of ferritin. This index was evaluated in our study, and calculations were made to find its value. This parameter did not lose its significance in all possible study groups (IDA vs. ACD, IDA vs. the control group, ACD vs. ACD+IDA). Therefore, this index is a very important indicator not only in diagnosing IDA but also in discovering its existence in the conditions of inflammation. As such, it is also useful to determine the patients expected to benefit from supplemental iron. The value of sTfR-F index >2.06 is that it has a sensitivity of 90% and a specificity of 96% when IDA is compared with ACD. While comparing ACD+IDA vs. ACD for values of sTfR-F index >2.3, the sensitivity and specificity of this test go toward a perfect test (92.3% and 100% respectively).

The scientific basis for the combined use of ferritin and sTfR is their different behavior toward reduced iron reserves. Serum ferritin concentrations are linearly related to reduced iron stores, but there is no cut-off concentration to indicate when a given patient's iron stores are so depleted that iron availability has become a limiting factor for erythropoiesis.

The opposite is the case with sTfR, which at this point corresponds to the increased concentration in the serum. These studies have shown that the sTfR-F index is the most efficient parameter for diagnosing ACD+IDA vs. ACD.[23,28]

The role of hepcidin in iron metabolism has now been made very clear by many prestigious papers and is widely accepted.[29,30] The results of our study are in support of the great role of hepcidin in the control of iron metabolism by removing it from the circulation toward the RE block, a fact which we verify with the high levels of ferritin in the serum of patients with ACD, compared to the control group (P<0.001). The data of our study also confirm the strong relationship between hepcidin and mediators of inflammation, such as IL-6 (Graph 9). Our data confirm the value of the pro-hepcidin test for the differential diagnosis of ACD vs IDA. The suggested cut-off from the respective ROC curve is pro-hepcidin ≥153ng/ml. For this given level, the sensitivity is 100% (100% of those with ACD are correctly diagnosed and differentiated from those with IDA), and the specificity is 100% (all without ACD are correctly classified as without ACD).

In conclusion, in patients with ACD, the serum concentration of pro-hepcidin shows a notable and significant increase, compared to patients with IDA, individuals without anemia, and healthy controls. Hepcidin emerges as a highly accurate diagnostic tool for differentiating ACD and IDA. Moreover, a robust positive correlation exists between the serum concentration of pro-hepcidin and IL-6. Additionally, the parameters sTfR and sTfR-F index demonstrate their effectiveness as excellent diagnostic tests for IDA in chronic inflammation.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Prevalence and Correlation between High-Risk HPV Genotypes and Pap Smear Findings in Bahrain: A Retrospective Approach

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Abstract

Background: Human papillomavirus (HPV) is currently the most common pathogen responsible for cervical cancer (CC), a major cause of cancer-related deaths in women. This study aimed to estimate the current HPV prevalence in Bahrain and to determine the association of high-risk HPV (HR-HPV) genotypes with cytological findings, age, and clinical history of the patients.

Methods and Results: Our study used a retrospective approach. Convenience sampling was used to gather 100 cases of HPV-positive women with abnormal and normal Pap smear findings from January 2017 to April 2023 in the Cytology Department at King Hamad University Hospital. Out of 100 HR-HPV positive cases, non-16/18/45 HR-HPV was found in 62%, and 73% had abnormal Pap smear findings. Among Pap smear findings, ASC-US/ASC-H (P=0.038) and LSIL/HSIL (P=0.017) were significantly associated with HR-HPV genotypes. ASC-US was found to be more frequently associated with HPV16+non-16/18/45 HR-HRV and LSIL with HPV18/45+non-16/18/45 HR-HRV. Most HR-HPV cases (59%) were aged ≤40 years, 25% - from 41 to 50 years old, while only 16% were >50. The age group ≤40 had the highest peak with non-16/18/45 HR-HPV genotype (35%) and HPV16 (19%). The predominant genotypes for age groups 41-50 and >50 were non-16/18/45 HR-HPV types (19% and 8%, respectively).

Conclusion: In Bahrain, non-16/18/45 HR-HPV infection is becoming more prevalent, with ASC-US being the most common abnormal Pap finding and the highest HPV infection in women aged 40 years or younger. Based on our findings, we recommend effective screening and vaccine programs for women aged 40 years and younger, as early detection can lower infection rates and improve recovery.**(International Journal of Biomedicine. 2024;14(1):52-58.)**

Keywords: HR-HPV • cervical cancer • Pap smear • ASC-US

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Abbreviations

ASC-US, atypical squamous cells of undetermined significance; **ASC-H**, atypical squamous cells, HSIL cannot be excluded; **CC**, cervical cancer; **DI**, double infection; **HPV**, human papillomavirus; **HR-HPV**, high-risk HPV; **HSIL**, high-grade SIL; **LR-HPV**, low-risk HPV; **LSIL**, low-grade SIL; **NILM**, negative for intraepithelial lesion or malignancy; **SIL**, squamous intraepithelial lesion; **SQC**, squamous cell carcinoma.

Introduction

Human papillomavirus (HPV) is currently the most common pathogen responsible for cervical cancer (CC), a major cause of cancer-related deaths in women. Globally, CC is the fourth most common type of cancer among women worldwide, with over 600,000 new cases and over 300,000 deaths.[1] The rate at which CC has affected young women has increased dramatically from 10% to 40% over the past 30 years.[2] In the Kingdom of Bahrain, the estimated incidence rate of CC is 4.3 per 100,000 people per year, making it the fourth most prevalent cancer in the country and the second-highest incidence rate of

CC among the Cooperation Council for the Arab States of the Gulf countries (GCC).[3] Unfortunately, CC is difficult to detect early because it typically has no symptoms.[4]

The introduction of the Papanicolaou (Pap) test led to a notable decrease in CC mortality in developed countries during the 20th century. Pap test results are classified as negative for intraepithelial lesion or malignancy (NILM) or positive (ASC-US, LSIL, ASC-H, HSIL or SQC) based on altered squamous cells, severity of abnormalities, preservation of specimens, and clinical setting (Table 1).[5]

Human papillomavirus (HPV) is considered the most common cause of CC in women. HPV is a small, non-enveloped, circular, double-stranded DNA virus belonging to the Papillomaviridae family. Many HPV infections that result in warts or subclinical infections are spread through personal, non-sexual contact. However, there are more than 40 types that can spread through sexual contact and are classified into two categories according to their risk of causing CC.[6] These categories include LR-HPVs and HR-HPVs. LR-HPV is responsible for anogenital and cutaneous warts, while HR-HPV is responsible for oropharyngeal cancers and anogenital cancers, including cervical, anal, vulvar, vaginal, and penile cancers.[7] There are over 150 different HPV genotypes, out of which 14 HR-HPV genotypes are known to be carcinogenic. The most frequently detected are HPV types 16, 18, 52, 31, and 58.[8] Several studies suggested that the prevalence of HPV genotypes varies geographically based on the incidence and genotypic distribution of HPV infections across nations (Table 2). Moreover, there is a relationship between HR-HPV genotypes and cytology results.[8-10]

This research will be the first study in Bahrain to correlate the HR-HPV genotypes with patients' cytological findings, demographics, and clinical profiles. This study aimed to estimate the current HPV prevalence in Bahrain and to determine the association of HR-HPV genotypes with cytological findings, age, and clinical history of the patients.

Table 2.

Global findings on HR-HPV genotypes based on recent articles.

Location	HPV Genotype Prevalence
Saudi Arabia (Kussaibi et al., 2021) [8]	HPV16 (5%), HPV16+18/45 (1%), Other HR-HPV (9%)
Oman (Al-Lawati et al., 2020) [21]	HPV82 (10.77%), HPV68 (7.69%)
Latin America (Correa et al., 2022)[13]	HPV16/18 (71.1%)
Africa (Seyoum et al., 2022) [14]	HPV16 (42.1%), HPV52 (30.3%), HPV18 (27.7%)
China (Yu et al., 2022) [15]	HPV16 was the most common in all regions except southern and eastern China, where HPV52 was predominant.

Materials and Methods

Our study used a retrospective approach. Convenience sampling was used to gather 100 cases of HPV-positive women with abnormal and normal Pap smear findings from January 2017 to April 2023 in the Cytology Department at King Hamad University Hospital.

Cytology Procedures

Thin-Prep

Thin-Prep collects cervical specimens using a cervix brush, which is rinsed in a vial with preservative fluid based on methanol. By pushing the brush to the bottom, forcing the bristles apart, and swirling it in the fluid, cells are released. The brush is then discarded.

Sure-Path

Sure-Path collects cervical specimens with a broom-like device with a detachable head. The practitioner must snip off the tip (detachable head) of the collection device and place it in a sample vial that contains an ethanol-based preservative fluid.

Table 1.

Squamous abnormalities in Pap Smears.[5, 25]

Squamous Abnormality	Definition	Cytomorphology
Atypical Squamous Cells (ASC)	ASC is cytologic changes indicating SIL and is divided into ASC-US and ASC-H. ASC-US is atypical mature squamous cells with SIL features, accounting for 4.3% of all pap smear diagnoses. ASC-H is immature squamous cells with HSIL-like cytologic changes, accounting for 0.3% of all pap smear diagnoses.	ASC-US has nuclei 2.5-3 times larger than normal intermediate squamous cells or twice the size of squamous metaplastic cells. It has a slightly increased nuclear-to-cytoplasmic ratio (N:C) and minimal nuclear hyperchromasia. ASC-H has a few immature squamous cells with high N:C and mild-to-moderate nuclear atypia. Clustered squamous cells show atypical nuclear features, with nuclei 1.5-2 times larger than normal and significant nuclear membrane irregularity. The N:C is markedly increased, similar to HSIL.
Squamous Intraepithelial Lesion (SIL)	Squamous intraepithelial lesion (SIL) refers to precursors to invasive squamous cell carcinoma, previously known as dysplasia, carcinoma in situ, borderline lesion, and CIN. SIL can be categorized into mature cell lesion (LSIL) and immature cell lesion (HSIL) types, with both highly associated with HR-HPV 97%. Both types account for 3% of pap smear findings.	LSIL cells are intermediate-sized squamous cells with abundant cytoplasm and a low but slightly increased N:C. They are hyperchromatic with variable size and uniformly distributed chromatin. HSIL are smaller and show less cytoplasmic maturity than LSIL cells. They occur singly, in sheets, or in syncytial-like aggregates. The N:C is higher compared to LSIL, and the degree of nuclear enlargement is more variable. Hyperchromatic nuclei with coarse granular or fine chromatin are common.
Squamous Cell Carcinoma (SQC)	SQCs are invasive epithelial tumors with varying degrees of differentiation. They can be well-differentiated, keratinizing or poorly differentiated, non-keratinizing. SQC accounts for 75% of cervical cancer cases, with HPV-16 in 50-60% and HPV-18 in 10-15%.	Keratinizing SQC cells are isolated, single cells with marked pleomorphic cells, dense orangeophilic cytoplasm, and variable nuclei, irregular nuclear membranes, and coarsely granular chromatin. Non-keratinizing SQC cells are smaller, occur singly or in syncytial aggregates, have poorly defined cell borders, and have variable nuclei size, irregular nuclear membranes, and coarsely clumped chromatin with chromatin clearing. Tumor diathesis is often present.

HR-HPV Detection

All cervical samples with ASC-US features must comply with CAP (College of American Pathologists) standards and be sent for HR-HPV testing. Patients at risk must undergo HR-HPV testing and Pap smears. The Cepheid Xpert HPV Assay is a fully automated, qualitative in vitro test for detecting the E6/E7 region of the viral DNA genome from HR-HPV in patient specimens. The test performs multiplexed amplification of target DNA by RT-PCR of 14 HR-HPV types in a single analysis. Xpert HPV specifically identifies HPV16 and HPV18/45 types in two distinct detection channels. It reports 11 non-16/18/45 HR-HPV types (31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68) in a pooled result. Specimens are limited to cervical cells collected in PreservCyt® Solution (Hologic Corp.). Cervical samples collected in PreservCyt Solution pretreated with glacial acetic acid to lyse excess red blood cells for cytology review have also been validated with the Xpert HPV Assay.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Group comparisons concerning categorical variables were performed using Fisher's exact test. A probability value of $P<0.05$ was considered statistically significant.

Ethical Statements

The Institutional Review Board of King Hamad University Hospital (KHUH) approved this research study in March 2023 (Ref. #: 23-590).

Results

Over the specified period, 100 cases were positive for the HR-HPV test; HPV16 was detected in 24%, HPV18/45 in 6%, and non-16/18/45 HR-HPV in 62%. The remaining 8% revealed double HR-HPV genotypes, which was distributed among the cases (5% with HPV16 & non-16/18/45 HR-HRV, 2% with HPV18/45 and non-16/18/45 HR-HRV and 1% with HPV16 & HPV18/45) (Figure 1).

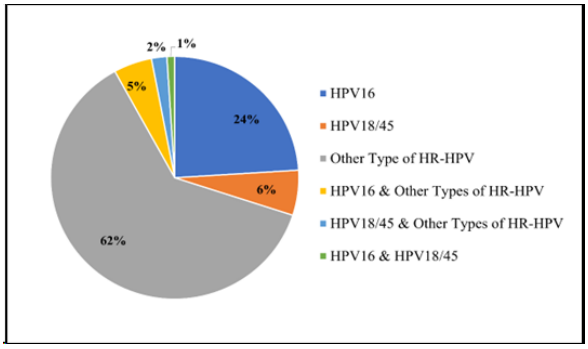

Fig. 1. *Frequency of positive HR-HPV cases between Jan 2017 and April 2023 (n=100).*

Among 100 positive HR-HPV cases, 73% had abnormal Pap smear findings (50% ASC-US - 50%, 1% ASC-H - 1%, 13% LSIL - 13%, 7% HSIL - 7%, and SQC - 2%), while 27% had negative Pap smear. Figure 2 shows the abnormal Pap smear findings retrieved from the cases (Figure 2)

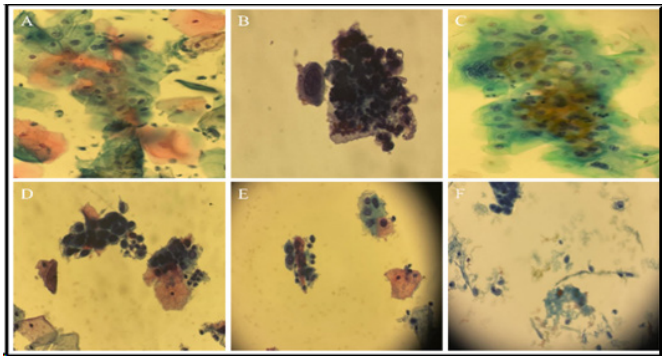

Fig. 2. *Abnormal Pap smear findings in patients. A: ASC-US, B: ASC-H, C: LSIL, D: HSIL, E: Keratinizing SQC, F: Non-keratinizing SQC.*

Correlation between HR-HPV and Pap smear findings

This study has found a significant correlation between HR-HPV and Pap smear findings (P=0.003). Among Pap smear findings, ASC-US/ASC-H (P=0.038) and LSIL/HSIL (P=0.017) were significantly associated with HR-HPV genotypes. However, no significant relationship was determined regarding SQC and negative Pap smear results with HR-HPV genotypes.

Out of the 24% of the total HPV16-positive cases, 8% showed negative Pap smear while 16% had an abnormal Pap smear result (ASCUS - 9%, ASC-H - 1%, LSIL - 3%, HSIL - 2%, SQC-1%). Six percent of the total cases were positive for HPV18/45; 2% had negative Pap smear, and 4% had abnormal Pap smear (ASC-US - 1%, LSIL - 3%). Among 62% of non-16/18/45 HR-HRV genotypes, 16% of the cases had negative Pap smear results, while 46% had abnormal Pap smear findings (ASC-US - 38%, LSIL - 6%, HSIL - 2%). Among the 8% of total DI-HR-HPV, 1% had negative Pap smear (HPV16 & non-16/18/45 HR-HRV), and 7% had abnormal Pap smear (ASC-US - 2%, LSIL - 1%, HSIL-3%, and SQC - 1%). In other words, ASC-US was found to be more frequently associated with HPV16+non-16/18/45 HR-HRV and LSIL with HPV18/45+non-16/18/45 HR-HRV. HSIL was detected in 2% of the cases with HPV16+non-16/18/45 HR-HPV genotype and in 1% of the cases with HPV18/45+non-16/18/45 HR-HRV, whereas only one case of SQC was found with DI (HPV16 + HPV18/45) (Table 3).

Table 3.
Correlation between HR-HPV and Pap smear findings.

	HPV16	HPV18/45	Non-16/18/45 HR-HRV	DI-HR-HPV	Total	*P*-value
Negative	8%	2%	16%	1%	27%	0.680
ASC-US	9%	1%	38%	2%	50%	0.038*
ASC-H	1%	0%	0%	0%	1%	
LSIL	3%	3%	6%	1%	13%	0.017*
HSIL	2%	0%	2%	3%	7%	
SQC	1%	0%	0%	1%	2%	0.086
Total	24%	6%	62%	8%	100%	0.003*

** A significant correlation between HPV types and Pap smear findings.*

Correlation between HR-HPV and age

Patients' ages ranged from 23 to 67 years. All patients were grouped into three categories (≤40, 41-50, and >50). Most cases (59%) were aged ≤40 years, 25% - from 41 to 50 years old, while only 16% were >50 years old (Figure 3).

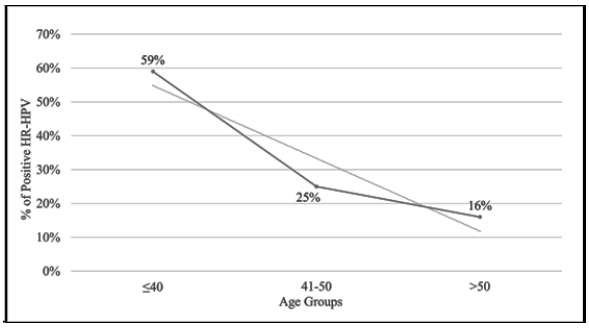

Fig. 3. *Overall age-related percentage for positive HR-HPV cases between Jan 2017 and April 2023 (n=100).*

Considering the relationship between HR-HPV genotype and age, this study has found a significant correlation between HPV16 and HPV18/45 with all age categories (P=0.022 and P=0.035, respectively). On the other hand, DI-HR-HPV and non-16/18/45 HR-HPV genotypes showed no significant correlation with the age groups (Table 4).

Table 4.

Correlation between HR-HPV and age.

HR-HPV genotypes	40	41-50	50	*P*-value
HPV16	19%	1%	4%	0.022*
HPV18/45	1%	2%	3%	0.035*
DI-HR-HPV	4%	3%	1%	0.694
Non-16/18/45 HR-HRV	35%	19%	8%	0.198
Total	59%	25%	16%	0.012*

* *A significant correlation between HR-HPV type and age.*

The relation between the age and HR-HPV genotype was further analyzed, and it was found that the age group ≤40 had the highest peak with non-16/18/45 HR-HPV genotype (35%) and HPV16 (19%). The predominant genotypes for age groups 41-50 and >50 were non-16/18/45 HR-HPV types (19% and 8%, respectively) (Table 4).

Patient history in relation to HR-HPV

Based on the available patients' history, data was collected only from 39 out of 100 cases. The genital wart was the most common diagnosis, with 23.1% infected with non-16/18/45 HR-HPV, 5.1% (2/39) with HPV16, and 2.6% (1/39) with DI (HPV16 and non-16/18/45 HR-HRV). The frequency of the patients with other symptoms was shown in Table 5.

Discussion

When discussing the results obtained, it should be noted that 62% of the patients with a single infection were positive with non-16/18/45 HR-HPV. This coincides with the results reported by Kussaibi et al.,[8] revealing that non-16/18/45 HR-HPV caused most HR-HPV-positive cases. The last study, conducted in Bahrain in 2014, found HPV52 to be the most frequent genotype (1.4%), which agrees with the current findings.[11] A recent study reflecting the HPV prevalence in the GCC region also exhibited similar results, whereas non-HPV16/18 were mainly responsible for HR-HPV-positive cases.[12] On the contrary, a study conducted by Correa et al.[18] found that HPV16 is the most common HR-HPV genotype, while non-16/18/45 HR-HPV was found to be the least common. Similar results were found in other studies. Among the DI cases, the majority were positive with HPV16+non-16/18/45 HR-HPV. This was, in accordance with Ali et al.[12] and Liao et al.,[16] which reported the highest number of DI cases involving HPV16+non-16/18/45 HR-HPV infection. Opposing the current study and most other

Table 5.

Review of patient history with HR-HPV genotypes.

HPV Genotype	Number of cases (n=39)
HPV16	
Abnormal vaginal bleeding	1
Cervicitis	1
Fungal infection	1
Genital warts	2
Nulligravida & Nullipara	1
Postmenopause	1
Postcoital bleeding	1
Vaginal discharge	1
HPV16 & HPV18-45	
Perimenopausal Bleeding	1
HPV16 & non-16/18/45 HR-HRV	
Vaginal discharge	1
Genital warts	1
HPV18-45	
History of chronic cervicitis	1
HPV18-45 & non-16/18/45 HR-HRV	
History of irregular periods	1
Vaginal discharge	1
History of HPV-18	1
non-16/18/45 HR-HRV	
Abdominal pain	2
Bacterial vaginosis	1
Genital warts	9
Irregular periods	1
Nulligravida & Nullipara	3
Pelvic inflammatory disease	1
Postmenopause	2
Postcoital Bleeding	2
History of HPV	2

studies where the single infection is more prevalent than DI, Gallegos-Bolaños et al.[17] observed DI as more frequent with HPV51+HPV52. The observation that infection with more than one HPV genotype increases the likelihood of developing CC remains controversial. In comparison to SI, double and concurrent HPV infections may be linked to HPV persistence and a higher risk of CC. A biological mechanism by which one HPV genotype leads to the development of another genotype can be considered as one of the reasons for DI.[12] The substantial variations in the prevalence of HR-HPV genotypes in different areas worldwide can be attributed to geographical location, sociocultural norms, socioeconomic status, age, and ethnicity of the population.

For the relationship between Pap smear findings and HR-HPV genotypes, the current study found an overall significant correlation (P=0.003) with ASC-US, ASC-H, LSIL, and HSIL being significantly associated with HR-HPV genotypes. Of 100 HR-HPV-positive cases, 73% had abnormal Pap smear findings, while 27% had negative smear. Non-16/18/45 HR-HPV (46%) was the most prevalent genotype among the abnormal Pap smear findings, with ASC-US in 38%, LSIL in 6%, and HSIL in 2%. Over-diagnosis of patients with inflammation is one reason ASC-US is the most recurrent abnormal Pap finding. The results of the current study for Pap smear findings agree with other research, which reported the highest number of cases diagnosed with ASC-US and the lowest with SQC.[8,18,19] In contrast with our findings, a study by Umakanthan et al. found the HR-HPV detection rate remarkably higher in LSIL, followed by HSIL, ASC-US, and SQC.[20]

Concerning the relationship of HR-HPV infection with normal Pap smear results, the current study found that non-16/18/45 HR-HPV was most frequently associated with normal Pap smears, thus complying with one of the research's objectives. The presence of HR-HPV infection in women with normal Pap smear findings could be attributed to the patient's age during the infectious stage, immunity status, as well as the degree of progression due to pathological changes. A similar association was found in a study conducted in Oman, where researchers reported the HPV prevalence in women with normal Pap results to be 17%, which was higher than previous studies conducted in other GCC countries, such as Kuwait and Bahrain, where the prevalence was 2.4% and 9.8% respectively. According to their findings, the higher prevalence could be due to the methodological assays being more sensitive than the techniques used in the prior studies, thus allowing the detection of HPV in samples with a low viral load that would otherwise be considered negative.[21]

Besides the HPV-positive cases where one squamous abnormality "progressed" to the other, the current study also observed a "regression" phenomenon in some patients. Four cases in our study with a history of ASC-US (1/4), LSIL (2/4), and HSIL (1/4) showed normal Pap results in the follow-up Pap test, with three of the cases infected with non-16/18/45 HR-HPV genotype. This regression of Pap result could be explained by the patient's age at the time, aiding in self-resolution of the infection and/or early diagnosis and leading to effective treatment. Additionally, one of the patients who was diagnosed with HSIL was found to have developed DI (HPV16 and non-16/18/45 HR-HRV) one year later, with her Pap result being negative, exhibiting regression even in the presence of HR-HPV infection. This case emphasizes the importance of understanding the potential risk factors for HPV infection persistence in patients with HSIL to optimize the postoperative monitoring program and clinical treatment. Zang and Hu's study reported several pathological and physiological factors such as high viral load, presence of HPV16 infection, age over 50 years, and positive surgical margins were associated with persistent HPV infection in patients with HSIL, but this finding remains controversial.[22]

Concerning age, the current study concluded that there is a significant correlation between HR-HPV genotypes and patients' age. A similar correlation was found in a study done in Nigeria.[23] On the other hand, other studies, for instance, a study done in Saudi Arabia[8] and a study done in China,[24] found no significant correlation between HR-HPV genotypes and patients' age. Although new HPV infections may occur at any age, it is difficult to determine at what age a woman acquires the HPV infection that leads to CC. Nonetheless, women's age is one of the significant risk factors for HPV infection. The data from this study revealed that most HR-HPV-positive cases fell in the category of ≤40 years, with the second most frequently infected age group being 41-50, and >50 years ranked last. Thus, in Bahrain, HPV rates decrease with age. In all age groups, non-16/18/45 HR-HPV prevailed as the most common cause of HPV infection, followed by HPV16/18/45. This coincides with the findings of the Kussaibi et al.[8] study, which reported that 66% of the HR-HPV-positive patients were younger than 40 years, with non-16/18/45 HR-HRV being the most frequent genotype. Similar results were seen in a study conducted by Clarke et al.[26] On the contrary, a study conducted by Yan et al. found that young women under the age of 20 had the highest infection rates, probably because of their newness to intercourse and the sensitivity of their immune systems to HPV infections. They also found that the age group 61-70 showed a decline in HPV infection rate.[27] The cause may be related to the virus's persistence or the potential reactivation of HPV triggered by physiologic and immunological problems during the menopausal transition period. In addition to the results from our data, other recent studies also suggest most of the HR-HPV infections are diagnosed in women between the ages of 30 and 40.[28] Henceforth, the WHO recommends screening programs in developed countries beginning at 30.

When comparing the patients' HPV status and Pap findings with their clinical manifestations, genital warts were found to be the most common symptom among HR-HPV-positive women in the current study. Among the women exhibiting genital warts, 23.1% of them were infected with non-16/18/45 HR-HPV, thus revealing the main cause of the symptom. Besides genital warts, other symptoms observed in HR-HPV-infected women include abdominal pain, history of irregular periods, abnormal vaginal discharge, abnormal uterine bleeding, previous HPV infection, and microbial infection. Conversely, a study by Ozaydin-Yavuz et al.[29] reported that most genital warts cases were caused by LR-

HPV. In a general sense, most cases of genital warts are caused by LR-HPV, but in recent years, it has been discovered that 20%–50% of patients with genital warts develop HR-HPV infection. There is no sufficient research on the relationship of HPV genotypes with clinical signs and symptoms. Therefore, a definite conclusion regarding the patient's history cannot be formed based on the current studies.

Conclusion

This study is the first in Bahrain to establish the prevalence of HR-HPV genotypes and analyze the correlation between HR-HPV genotypes and Pap smear findings, age groups, and clinical profiles among women. Results showed that non-16/18/45 HR-HPV infection is becoming more prevalent, with ASC-US being the most common abnormal Pap finding and the highest HPV infection in women aged 40 years or younger. Further investigation is needed to distinguish specific genotypes in the non-16/18/45 HR-HPV pool. Based on our findings, we recommend effective screening and vaccine programs for women aged 40 years and younger, as early detection can lower infection rates and improve recovery. Understanding the patient's clinical history can also help determine the appropriate testing and treatment approach. Future research should focus on the relationship between the Pap smear results, patient progression, regression status, and influential factors.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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Factor V Leiden G1691A, *Prothrombin* G20210A, and *MTHFR* C677T Mutations among Sudanese Women with Recurrent Pregnancy Loss

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Abstract

Background: Various factors, such as genetic causes, anatomic abnormalities of the uterus, infectious diseases, coagulative disorders, and endocrinological and immunological diseases, might influence recurrent pregnancy loss (RLP). This study aimed to evaluate the prevalence and frequency of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms in Sudanese women with RPL.

Methods and Results: This descriptive cross-sectional study involved 100 women with a history of 3 or more RPLs (the case group) and 94 healthy multiparous women without pregnancy complications (the control group). DNA was extracted from peripheral blood samples. The study of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms was performed by PCR and RFLP analysis. For the *FII* G20210A, the genotype distribution in the case group and control group was as follows: GG=97.0%, GA=3.0%, AA=0% and GG=94.0%, GA=0%, AA=0%, respectively. In the case group, the allelic distribution was as follows: G=98.5%, A=1.5%. In the control group, the A allele was absent, and the frequency of the G allele was 100%. For the *MTHFR* C677T, the genotypic and allelic frequencies in the case group were 97%, 3%, and 0%, respectively, for the CC, CT, and TT genotypes, and 98.5% and 1.5%, respectively, for the C and T alleles. In the control group, the genotype distribution was as follows: CC-100% CT-0%, TT-0%; the T allele was absent, and the frequency of the C allele was 100%. For the *FVL* G1691A, the genotype distribution in the case group and control group was as follows: GG=92.0%, GA=8.0%, AA=0% and GG=93.6%, GA=6.4%, AA=0%, respectively. For G and A alleles, the frequencies were 96.0% and 4.0%, respectively, for the case group, and 96.8% and 3.2%, respectively, for the control group. Our analysis did not reveal a significant positive association between the *MTHFR* C677T, *FII* G20210A, and *FVL* G1691A polymorphisms and the risk of RPL across the dominant model, multiplicative model, and a comparison of the frequencies of the heterozygous and homozygous dominant genotypes.

Conclusion: The research findings suggest that the *MTHFR* C677T, *FVL* G1691A, and *FII* G20210A variants do not significantly contribute to the increased susceptibility to RPL in this specific population of Sudanese women. **(International Journal of Biomedicine. 2024;14(1):59-65.)**

Keywords: recurrent pregnancy loss • Factor V Leiden • methylenetetrahydrofolate reductase • prothrombin • Sudanese women

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Abbreviations

APC, activated protein C; **FVL**, Factor V Leiden; **MTHFR**, methylenetetrahydrofolate reductase; **RPL**, recurrent pregnancy loss; **RFLP**, restriction fragment length polymorphism.

Introduction

Recurrent pregnancy loss (RPL), also referred to as recurrent miscarriage or habitual abortion, is historically defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period.[1] The Practice Committee of the American Society for Reproductive Medicine has defined RPL as 2 or more failed pregnancies before the 20th week of pregnancy.[2,3] Various factors, such as genetic causes, anatomic abnormalities of the uterus, infectious diseases, coagulative disorders, and endocrinological and immunological diseases, might influence RLP.[4-9] The association between thrombophilia and RPL has become an undisputed fact. The *FVL* G1619A mutation, prothrombin or factor II (*FII*) G20210A, and *MTHFR* gene polymorphisms are believed to play a key role in the pathogenesis of RPL. These genetic conditions have been linked to a range of obstetric complexities, including venous thromboembolism, recurrent miscarriage, abruption of placentae, preeclampsia, and the delivery of a fetus that is small for its gestational age.[10]

The *FVL* G1619A mutation occurs by substituting guanine with adenine at the nucleotide 1691 in exon 10. As a result of this missense mutation, arginine (Arg) at amino acid 506 is substituted with glutamine (Gln), leading to the generation of FVL resistant to the APC. APC is a natural anticoagulant that, in normal situations, cleaves activated factor V at amino acid 506 and makes it inactive.[11-17] This results in a hypercoagulable state with a 5- to 10-fold risk of thrombosis in heterozygotes and an 80-fold risk in homozygotes.[18] Studies investigating the relationship between *FVL* mutation and RPL found an association, with odds ratios ranging from 0.5 to 18.[19-22]

A single missense mutation on the *FII* gene, leading to the substitution of guanine by adenine at nucleotide position 20210, was recently identified as a genetic risk factor for thrombosis. The *FII* G20210A polymorphism is associated with increased plasma prothrombin levels, and its carriers present a 2 to 3-fold increased risk for developing venous thromboembolism.[23] The *FII* gene mutation was found in 4%–9% of women with RPL, compared with 1%–2% of those with uncomplicated pregnancies, with odds ratios ranging from 2 to 9.[24,25]

Mutations in the *MTHFR* gene lead to decreased enzyme activity and hyperhomocysteinemia, which induces platelet aggregation.[26] The *MTHFR* C677T is a missense mutation in exon 4 of this gene, which converts an alanine to a valine residue in the N-terminal catalytic domain of the protein, resulting in decreased enzymatic activity and hyperhomocysteinemia, which induces platelet aggregation.[27,28] Homozygous C677T mutations and the *MTHFR* 677T allele have been associated with elevated levels of homocysteine and are identified as risk factors for thrombosis.[29,30]

Earlier research has demonstrated variations in the presence of the *FVL*, *FII*, and *MTHFR* mutations across different geographical regions and racial and ethnic backgrounds.[31,32]

This study aimed to evaluate the prevalence and frequency of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms in Sudanese women with RPL.

Material and Methods

This descriptive cross-sectional study involved 100 women (mean age of 25±4.0 years) with a history of 3 or more RPLs (the case group) and 94 healthy multiparous women (mean age of 30±4.0 years) without pregnancy complications (the control group).

DNA was extracted from peripheral blood samples using a Master Pure DNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) according to the manufacturer's standard protocol. The study of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms was performed by PCR and RFLP analysis. For *FII* G20210A polymorphism, a 345-bp genomic DNA segment, including the mutation site, was amplified using forward and reverse primers.[33] Digestion of the PCR products containing the wild-type heterozygous and homozygous allele with the restriction enzyme Hind III results in 345 bp, 322 bp, 23 bp, and 322 bp, 23 bp fragments, respectively. For *FVL* G1691A polymorphism, a 267-bp genomic DNA segment was amplified as described by Bertina et al.[34] The 267 bp amplification product was digested with Mnl I for 60 minutes at 37°C, and the resulting fragments were separated by electrophoresis in a 3% agarose gel. The presence of the mutant allele was indicated by a 200-bp product and the normal allele by a 163-bp product, the heterozygotes having both. The *MTHFR* C677T polymorphism was detected according to the method described by Frosst et al.[35] A length of 198bp in exon 4 of the *MTHFR* gene was amplified using the special primers 5′ TGAAGGAGAAGGTGTCTGCGGGA3′ and 5′AGGACGGTGCGGTGAGAGTG3′, followed by restriction digestion using the HinfI enzyme. A single band of 198bp characterized the wild-type C allele for codon 677, while the presence of 3 bands at 198 bp, 175 bp, and 23 bp or 175 bp and 23 bp characterized the heterozygous (CT) and homozygous (TT) variant status, respectively.

Statistical analysis was performed using the statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). Genetic markers for HWE were tested (Table 1). Differences in the allele and genotype distribution between the groups were assessed by χ2-test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Two inheritance models were analyzed (the dominant model and the multiplicative model), and a comparison of the frequencies of the heterozygous and homozygous dominant genotypes of the studied gene polymorphisms. The recessive and additive models were not calculable due to the homozygous recessive genotype frequency of zero in both cases and controls. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Ethics Committee at the Omdurman Maternity Hospital.

Table 1.

The distribution of polymorphic markers of the MTHFR C677T, FII G20210A and FVL G1691A polymorphisms in RPL women (cases) and non-RPL women (control).

Gene	SNP/ mutations	Genotype	Cases	HWE	χ^2	*P*	Control	HWE	χ^2	*P*	Allele	Frequency of alleles	
												Cases	Control
FII	rs1799963 G20210A	GG	0.970	0.970	0.00	1	1.000	1.000	0.00	1	G	0.985	1.000
		GA	0.030	0.030			0.000	0.000			A	0.015	0.000
		AA	0.000	0.000			0.000	0.000					
FVL	G1619A	GG	0.920	0.922	0.00	1	0.936	0.937	0.00	1	G	0.960	0.968
		GA	0.080	0.077			0.064	0.062			A	0.040	0.032
		AA	0.000	0.002			0.000	0.001					
MTHFR	rs1801133 C677T	CC	0.970	0.970	0.00	1	1.000	1.000	0.00	1	C	0.985	1.000
		CT	0.030	0.030			0.000	0.000			T	0.015	0.000
		TT	0.000	0.000			0.000	0.000					

Results

The distribution of polymorphic markers of the *MTHFR* C677T, *FII* G20210A and the *FVL* G1691A polymorphisms in the case group and control group was in HWE (Table 1).

For the *FII* G20210A, the genotype distribution in the case group and control group was as follows: GG=97.0%, GA=3.0%, AA=0% and GG=94.0%, GA=0%, AA=0%, respectively. In the case group, the allelic distribution was as follows: G=98.5%, A=1.5%. In the control group, the A allele was absent, and the frequency of the G allele was 100%.

For the *MTHFR* C677T, the genotypic and allelic frequencies in the case group were 97%, 3%, and 0%, respectively, for the CC, CT, and TT genotypes, and 98.5% and 1.5%, respectively, for the C and T alleles. In the control group, the genotype distribution was as follows: CC-100% CT-0%, TT-0%; the T allele was absent, and the frequency of the C allele was 100%.

For the *FVL* G1691A, the genotype distribution in the case group and control group was as follows: GG=92.0%, GA=8.0%, AA=0% and GG=93.6%, GA=6.4%, AA=0%, respectively. For G and A alleles, the frequencies were 96.0% and 4.0%, respectively, for the case group, and 96.8% and 3.2%, respectively, for the control group.

Our analysis did not reveal a significant positive association between the *MTHFR* C677T, *FII* G20210A, and *FVL* G1691A polymorphisms and the risk of RPL across the dominant model (C677T: OR=6.78, 95% CI = 0.35 – 133.14, *P*=0.09; G20210A: OR=6.78, 95% CI = 0.35 – 133.14, *P*=0.09; G1619A: OR=1.28, 95% CI = 0.43 – 3.82, *P*=0.66), multiplicative model (C677T: OR=6.68, 95% CI = 0.34 – 130.22, *P*=0.09; G20210A: OR =6.68, 95% CI = 0.34 – 130.22, *P*=0.09; G1619A: OR =1.26, 95% CI = 0.43 – 3.71, *P*=0.67), and a comparison of the frequencies of the heterozygous and homozygous dominant genotypes (C677T: OR =6.78, 95% CI = 0.34 – 133.14, *P*=0.207; G20210A: OR=6.78, 95% CI = 0.34 – 133.14, *P*=0.207; G1619A: OR=1.28, 95% CI = 0.42 – 3.82, *P*=0.664) (Tables 2 and 3).

Discussion

The present study continued previously published research on the prevalence of genetic polymorphisms among Sudanese women with RPL.[5] The findings align with prior research outcomes, indicating no association between the *MTHFR* C677T, *FVL* G1619A and *FII* G20210A, and RPL. In a study by Serrano et al.,[36] which involved 100 participants with RPL, it was concluded that neither *FII* G20210A nor *FVL* G1619A is linked to recurrent miscarriage before the 10th week of pregnancy. However, in a study by Ahmed et al.,[37] in Sudanese women with preeclampsia, the *FVL* G1619A mutation was found in 9.6% of the cases, compared with 0.6% of the controls (*P*<0.001; OR=18.60, 95% CI = 2.38-136.1), and homozygous AA genotype was found in 2.2% of patients with severe preeclampsia and was not detected in the controls.

Contradictory results were reported by other extensive prospective studies, reporting that thrombophilia-associated mutations associated with hypercoagulability are not elevated in women experiencing RPL.[38,39]

Cardona et al.[40] evaluated whether inherited thrombophilia is associated with RPL in a Colombian subpopulation. The frequency of thrombophilia-associated SNPs (*FII* G20210A and *FVL* G1691A), APC resistance, and anticoagulant protein deficiencies were low overall, except for the *MTHFR* C677T. The differences between patients with RPL and healthy multiparous women (controls) had no statistical significance. This study also confirmed the low prevalence of inherited thrombophilia in non-Caucasian populations. These findings concurred with other research by Abu-Asab et al.[41] The authors failed to find a significant

Table 2.

Genetic predisposition to RPL (the genetic models)

Inheritance model	Allele, Genotype	Cases	Control	χ^2	*P*	OR (95%CI)	
		n=100	n=94			OR	95%CI
MTHFR C677T							
Multiplicative model (χ^2 test, df=1)	C	0.985	1.000	2.84	0.09	0.15	0.01 – 2.92
	T	0.015	0.000			6.68	0.34 – 130.22
Dominant model (χ^2 test, df=1)	CC	0.970	1.000	2.86	0.09	0.15	0.01 – 2.89
	CT + TT	0.030	0.000			6.78	0.35 – 133.14
FVL G1619A							
Inheritance model	Allele, Genotype	Cases	Control	χ^2	*P*	OR (95%CI)	
		n=100	n=94			OR	95%CI
Multiplicative model (χ^2 test, df=1)	G	0.960	0.968	0.18	0.67	0.79	0.27 – 2.32
	A	0.040	0.032			1.26	0.43 – 3.71
Dominant model (χ^2 test, df=1)	GG	0.920	0.936	0.19	0.66	0.78	0.26 – 2.35
	GA + AA	0.080	0.064			1.28	0.43 – 3.82
FII G20210A							
Inheritance model	Allele, Genotype	Cases	Control	χ^2	*P*	OR (95%CI)	
		n=100	n=94			OR	95%CI
Multiplicative model (χ^2 test, df=1)	G	0.985	1.000	2.84	0.09	0.15	0.01 – 2.92
	A	0.015	0.000			6.68	0.34 – 130.22
Dominant model (χ^2 test, df=1)	GG	0.970	1.000	2.86	0.09	0.15	0.01 – 2.89
	GA + AA	0.030	0.000			6.78	0.35 – 133.14

Table 3.

Comparison of the frequencies of the heterozygous and homozygous dominant genotypes of the studied gene polymorphisms.

Gene	Heterozygous genotype	Homozygous dominant genotype	*P*-value	OR (95%CI)	
				OR	95%CI
FII G20210A	GA	GG	0.207	6.78	0.34 to 133.14
Case	3	97			
Control	0	94			
FVL G1619A	GA	GG	0.664	1.28	0.42 to 3.82
Case	8	92			
Control	6	88			
MTHFR C677T	CT	CC	0.207	6.78	0.34 to 133.14
Case	3	97			
Control	0	94			

association between the *FVL* G1691A, *FII* G20210 and *MTHFR* C677T polymorphisms, and RPL in either the first or second trimester in 329 Palestinian women with RPL. In contrast, Abdelsalam et al.,[42] reported a significant increase in the prevalence of *FVL* G1691A and *MTHFR* C677T mutations in the RPL patients, compared to controls without

involvement of the *FII* gene. A study by Al-Achkar et al.[43] involving Syrian women showed that RPL women with homozygous TT genotype of *MTHFR* C677T had a high risk of RPL.

Eldeen et al.[44] investigated the distribution of the analyzed polymorphic markers in Saudi women in the Northern area of Saudi Arabia. They found a significantly higher frequency of the *FVL* G1691A AA genotype and the *FII* G20210A GA genotype in RPL women than in healthy controls. For the *MTHFR* C677T, there was no significant difference in the distribution of genotypes and alleles among the RPL patients and controls.

In general, the complex interaction of genetic factors in the context of RPL requires continued research into the genetic predisposition of individual populations to reproductive problems. Variations observed across populations and studies highlight the multifaceted nature of genetic influence on pregnancy outcomes.[45,46]

Conclusion

The research findings suggest that the *MTHFR* C677T, *FVL* G1691A, and *FII* G20210A variants do not significantly contribute to the increased susceptibility to RPL in this specific population of Sudanese women. Continued scientific inquiry is crucial for developing more nuanced and personalized strategies for the diagnosis and prevention of RPL, ultimately improving women's reproductive health.

Competing Interests

The authors declare that they have no competing interests.

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State of Pituitary-Ovarian Axis of the Neuroendocrine Regulation System in Women of Reproductive Age with Ovarian Hyperandrogenism

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Abstract

Background: This study aimed to evaluate the state of the pituitary-ovarian axis of the neuroendocrine regulation system in women of reproductive age with ovarian hyperandrogenism (OH) of the main ethnic groups of the Baikal region.

***Methods and Results*:** Groups of women with OH of Buryat (n=35) and Caucasian (n=97) ethnic groups were formed. Data from somatically healthy women of Buryat (n=42) and Caucasian (n=87) ethnic groups were used for comparison. A comparative characterization of clinical data and indicators of the pituitary-ovarian link of the neuroendocrine regulation system was carried out. ELISA methods were used to determine thyroid-stimulating hormone (TSH), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17-OH-progesterone (17-OH-Pg), and sex hormone-binding globulin (SHBG). Testosterone levels were analyzed by liquid chromatography-mass spectrometry.

Buryat ethnic group women with OH had higher values of body weight, BMI, waist and hip circumference, and % fat compared to the corresponding control group. In the group of Caucasian women with OH, higher values of height, systolic blood pressure, and diastolic blood pressure were registered compared to the control group. In Buryat women with OH, higher TSH, anti-Mullerian hormone (AMH), free androgen index (FAI), and dehydroepiandrosterone sulfate (DHEA-S) values were found compared to controls. In the group of Caucasian women with OH, higher values of PRL, AMH, TSH, FAI, 17-OH-Pg, and DHEA-S were registered compared to the control. In Buryat women with OH, LH values were higher only in phase 2 compared to controls. In Caucasian women with OH, LH values increased both in phase 1 and phase 2 compared to controls.

***Conclusion*.** A comprehensive analysis of the state of the neuroendocrine regulation system in women of reproductive age with OH showed certain changes in the level of a number of hormones relative to control groups, most pronounced in the group of Caucasian women. At the same time, there were no differences in the studied indicators between ethnic groups with OH. The data obtained indicate the necessity of assessing and controlling the state of the neuroendocrine regulation system in female patients with OH for PCOS prevention and treatment. The ethnic component may have a certain contribution to the realization of further risks of the disease. **(International Journal of Biomedicine. 2024;14(1):66-71.)**

Keywords: pituitary-ovarian axis • ethnos • ovarian hyperandrogenism • polycystic ovary syndrome

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Abbreviations

AMG, anti-Mullerian hormone; **BMI,** body mass index; **BP**, blood pressure; **DBP**, diastolic blood pressure; **DHEA-S**, dehydroepiandrosterone sulfate; **FAI**, free androgen index; **FSH,** follicle-stimulating hormone; **HC**, hip volume; **HR**, heart rate; **LH,** luteinizing hormone; **MS**, metabolic syndrome; **OH,** ovarian hyperandrogenism; **PCOS**, polycystic ovary syndrome; **PRL,** prolactin; **TSH,** thyroid-stimulating hormone; **17-OH-Pg**, 17-OH-progesterone; **SBP**, systolic blood pressure; **SHBG**, sex hormone-binding globulin; **WC**, waist circumference.

Introduction

Ovarian hyperandrogenism (OH) is a polygenic endocrine disorder most commonly found in women of reproductive age.[1] The prevalence of OH in the female population ranges from 8-21%.[2,3] OH has a serious impact on the reproductive system and contributes to the development of infertility.[4] Neuroendocrine, metabolic, psychological, and other factors play a significant role in the OH pathogenesis.[5,6] Androgen excess is a characteristic feature of OH, determining many of its phenotypic features.[7] The clinical course of OH depends on a number of factors, including the ethnicity of the features.[5]

Several studies demonstrated global differences in the phenotypes of polycystic ovary syndrome (PCOS), of which OH is a major feature, in women of different racial and ethnic groups. For example, Middle Eastern, Mediterranean, Indian, and South Asian women with PCOS have a higher prevalence and/or severity of hirsutism than East Asian or Caucasian women.[8-10] Thirty studies were evaluated, and metabolic outcomes in women with PCOS from different nationalities worldwide were compared in a systematic review by Chan et al.[11] South Asian, Indian, and Norwegian women with PCOS, in particular, are at increased risk for metabolic syndrome (MS), Hispanic and Mexican women are at high risk for insulin resistance, and black women in the United States are at increased risk for hypertension compared with white women.[11-13] In a cross-sectional study of over 1000 women with PCOS in 5 countries, Chan et al.[11] found a significant difference in the prevalence of MS and the clustering of its components in different racial/ethnic groups. In Russia, there were practically no studies on the prevalence of OH; there are only single works.[10]

In the pathogenesis of OH, the leading role is played by changes in the pituitary-ovarian link of the neuroendocrine regulation system, including circadian rhythm disorders, isolated secretion of luteinizing hormone (LH), hyperinsulinemia, insulin resistance, ovarian theca-cell dysfunction, and hyperandrogenism.[10,14] The outcome of pathogenetic disorders in OH is progesterone deficiency, the main source of steroid hormone production.[14]

The Baikal territory is characterized by multinationals, where representatives of different ethnic groups coexist in the same zone of residence in the same climatic-geographical and social conditions.[15,16] The indigenous ethnic groups are Buryats, Evenks, Yakuts, Tofalars, and mixt-races.[17] Their gene pool is of special interest to researchers since it was formed over a long period of time under conditions of a sharply continental climate.[18] Buryats belong to the largest indigenous ethnic group - 3.3% of the total population of the Irkutsk region. Currently, there are negative trends in the growth of morbidity of this ethnic group, in particular, the increase in the number of diseases of the reproductive system - infertility, miscarriage, and endometriosis.[19-21] However, the pathogenetic mechanisms of these diseases remain unclear. In particular, the mechanisms of disorders of the pituitary-ovarian link in OH in the ethnic aspect are not fully disclosed.

This study aimed to evaluate the state of the pituitary-ovarian axis of the neuroendocrine regulation system in women of reproductive age with OH of the main ethnic groups of the Baikal region.

Material and Methods

The objects of the study were women of reproductive age, subject to annual preventive examination at the place of work, living in the Irkutsk region and Buryatia.

The following groups were formed: groups of women with OH of Buryat (n=35; mean age - 30.7±5.81 years) and Caucasian (n=97; mean age - 29.6±5.79 years) ethnic groups and groups of somatically healthy women without signs of OH (control groups) of Buryat (n=42; mean age - 36.28±5.45 years) and Caucasian (n=87; mean age - 34.4±6.1 years) ethnic groups.

Belonging to a specific ethnic group was determined by taking into account the duration of residence in a territory (at least one generation), genealogical history (women with two generations of parents of the same nationality), self-identification and taking into account the phenotypic characteristics of women.

Inclusion criteria for the control groups: menstrual cycle length of 21-34 days; modified Ferriman-Galvey score < 3, no alopecia or acne; ovarian volume < 10 cm^3 or a number of follicles with a diameter of 2-9 mm < 12 on transvaginal ultrasound; systolic BP <140 mmHg, diastolic BP <90 mmHg; fasting glucose level ≤ 6.1 mmol/L; PRL ≤ 727 IU/mL, TSH ≤ 4 IU/mL, 17-OH-Pg ≤ 6.91 nmol/L, and DHEA-S ≤ 430 μg/dL.

Exclusion criteria for the control groups: current pregnancy or lactation; history of hysterectomy, bilateral ovariectomy, endometrial ablation, and/or uterine artery embolization; current or previous (within 3 months) taking hormonal drugs (thyroid hormones, glucocorticoids, as well as insulin sensitizers); chronic diseases in the history (cardiovascular, oncologic, genitourinary diseases, diabetes mellitus, hypertension, etc.); BMI ≥ 30 kg/m^2; hormonal drugs (thyroid hormones, glucocorticosteroids, insulin sensitizers); chronic diseases in the history (cardiovascular, cancer, genitourinary diseases, diabetes mellitus, hypertension, etc.).

Inclusion criteria for women with OH: OH according to the current international diagnostic criteria ESHRE/ASRM[1] based on the use of two of the following three parameters (1 - oligo- or anovulation; 2 - clinical or/and biochemical hyperandrogenism; 3 - signs of polycystic ovaries according to pelvic ultrasound).

Exclusion criteria for women with OH: hyperprolactinemia; hypothyroidism; current pregnancy or lactation; taking hormonal drugs; removal of uterus and/or appendages from both sides; endometrial ablation and/or uterine artery embolization.

An anthropometric examination was carried out (measurement of height, weight, BMI, waist circumference (WC), hip volume (HC), % fat, % visceral fat). Waist circumference (WC) was measured with a measuring tape to the nearest 0.5 cm in a standing position at the end of

exhalation. The location of the tape was strictly horizontal at the level of the crista iliaca. Blood pressure (BP) was measured in a sitting position on the subject's right shoulder with an Omron automatic tonometer after a 5-minute rest.

Determination of the concentrations of TSH, PRL, LH, FSH, AMH, 17-OH-Pg, and sex hormone-binding globulin (SHBG) was carried out by the method of competitive enzyme-linked immunosorbent assay using Alkor-Bio test systems on the enzyme immunoassay analyzer ELx808 "Bio Tek" (USA). The level of DHEA-S was determined using a set of reagents on a Siemens Immulite 1000 immunochemical analyzer (USA).

The study was carried out in accordance with the Helsinki Declaration of the World Medical Association (1964, ed. 2013) and approved by the Committee on Biomedical Ethics under the Scientific Centre for Family Health and Human Reproduction (Extract from the meeting No. 2.1 as of 24.02.2016).

Statistical analysis was performed using STATISTICA v.10.0 software package (Stat-Soft Inc, USA). The normality of the 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 median (Me) and interquartile range (IQR; 25th to 75th percentiles). Differences in continuous variables departing from the normal distribution were tested by the Mann-Whitney U-test. A probability value of $P<0.05$ was considered statistically significant.

This work was carried out using the equipment of the Center for the Development of Advanced Personalized Health Technologies, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk.

Results and Discussion

In the first stage, a comparative characterization of clinical data in women with OH of the Caucasian and Buryat ethnic groups was performed (Table 1). Buryat ethnic group women with OH had higher values of body weight (P=0.007), BMI (P=0.003), WC (P=0.004), HC (P=0.010), % fat (P=0.030) compared to the corresponding control group (Table 1).

The group of Caucasian women with OH had higher values of height (P=0.03), SBP (P=0.030), and DBP ($P<0.0001$) compared to controls. Ethnic differences in control groups were expressed in increased body weight (P=0.011), BMI (P=0.024), HC (P=0.026), and WC/HC (P=0.004) in Caucasians compared to Buryats. No ethnic differences were recorded in the groups with OH ($P>0.05$) (Table 1).

Next, the state of the neuroendocrine regulation system in the studied groups was analyzed (Table 2). In Buryat women with OH, statistically significant differences in AMH ($P<0.001$), testosterone ($P<0.0001$), FAI ($P<0.0001$), DHEA-S (P=0.003) were found in the direction of increase compared to the control. The group of Caucasian women with OH had higher values of PRL (P=0.090), AMH ($P<0.0001$), testosterone ($P<0.0001$), FAI ($P<0.0001$), 17-OH-Pg ($P<0.0001$), and DHEA-S ($P<0.0001$) compared to controls (Table 2). Ethnic differences in controls were high PRL values ($P<0.0001$) and low testosterone levels (P=0.011) in Buryat women compared to Caucasians. At the same time, there were no differences in the studied indicators between ethnic groups with OH ($P>0.05$) (Table 2).

The level of gonadotropins was analyzed depending on the menstrual cycle phase (Table 3).

Table 1.

Clinical characteristics of reproductive-age women with OH, depending on ethnicity.

Parameters	Control groups		OH groups		P
	Buryats (1)	Caucasians (2)	Buryats (3)	Caucasians (4)	
	n=42	n=87	n=35	n=97	
	Me (25%;75%)		Me (25%;75%)		
Height, cm	160 (158;165)	163 (158;167)	162 (159;167)	165 (161;168)	P_{2-4}
Weight, kg	61.15 (55;66)	65.5 (57.1; 74.6)	69.7 (57;82)	67.2 (59.4;76.1)	P_{1-2} P_{1-3}
BMI, kg/м^2	23.32 (20.8;25.8)	25.02 (22.31; 27.88)	26.1 (21.4;31.6)	24.96 (21.77;28.87)	P_{1-2} P_{1-3}
WC, cm	75.5 (68;81)	77 (68;82)	82 (71;91)	75 (67;86)	P_{1-3}
HC, cm	95 (92;100)	99 (93;104)	102 (93;106)	100 (93;105)	P_{1-2} P_{1-3}
WC/HC	0.78 (0.75;0.83)	0.76 (0.73;0.82)	0.8 (0.77;0.84)	0.76 (0.71;0.82)	P_{1-2}
WC/height	0.47 (0.42;0.5)	0.47 (0.41;0.5)	0.48 (0.43;0.56)	0.46 (0.4;0.52)	
% fat	35.45 (30.1;40.7)	37.2 (30.2; 40.9)	40 (31.9;47.4)	35.8 (29.35;41.85)	P_{1-3}
Visceral fat, %	5 (4;7)	6 (4.0;7.0)	6 (4.0;7.0)	5 (4.0;7.0)	
SBP, mmHg	117 (110;127)	117 (112;123)	120 (113;131)	121 (113;132)	P_{2-4}
DBP, mmHg	75.5 (69;82)	74 (70;79)	78 (70;82)	80 (71;83)	P_{2-4}
HR, bpm	74.5 (69;82)	74 (68;82)	76 (69;81)	76 (70;85)	

P - statistically significant differences between groups (P<0.05)

Table 2.

The state of the neuroendocrine regulation system in reproductive-age women with OH, depending on ethnicity.

Parameters	Control groups		OH groups		*P*
	Buryats (1)	Caucasians (2)	Buryats (3)	Caucasians (4)	
	n=42	n=87	n=35	n=97	
	Me (25%;75%)		Me (25%;75%)		
TSH, IU/mL	1.8 (1.2;2.1)	1.4 (1.0;2.0)	1.4 (0.8;1.9)	1.6 (1.1;2.2)	
PRL, IU/mL	422.5 (329 ;491)	251 (197;336)	325 (242;490)	312 (224;437)	P_{1-2} P_{2-4}
AMH, ng/mL	1.6 (1.0;2.9)	2.1 (1.0 ;4.4)	4.7 (2.8 ;8.0)	6.7 (3.3 ;13.4)	P_{1-3} P_{2-4}
Testosterone, nmol/L	187.6 (104.7;293.5)	247.5 (166.9;366.5)	369.7 (297.5; 475.1)	381.15 (264.05;560.74)	P_{1-2} P_{1-3} P_{2-4}
SHBG, nmol/L	60.3 (54.1;79)	68.7 (46.7;104.2)	45.3 (31.4;89.6)	57.6 (36.3;90.9)	
FAI ,%	0.98 (0.63;1.44)	1.3 (0.58;2.2)	2.38 (1.16;4.68)	2.63 (1.23;4.32)	P_{1-3} P_{2-4}
17-OH-Pg, nmol/L	3.4 (1.7;8.1)	4.3 (2.1;7.1)	4.8 (2.4;6.4)	6.2 (4.6;8.9)	P_{2-4}
DHEA-S, μg/dL	141.5 (92.8;182.0)	161 (117;214)	190 (134;254)	231 (173;337)	P_{1-3} P_{2-4}

P - statistically significant differences between groups (P<0.05)

Table 3.

Level of gonadotropic hormones in reproductive-age women with OH in Phases 1 and 2 of the menstrual cycle, depending on ethnicity.

Parameters	Control groups				OH groups				*P*
	Buryats		Caucasians		Buryats		Caucasians		
	n=42		n=87		n=35		n=97		
	Me (25%;75%)				Me (25%;75%)				
	(1) MC-P1	(2) MC-P2	(3) MC-P1	(4) MC-P2	(5) MC-P1	(6) MC-P2	(7) MC-P1	(8) MC-P2	
LH, IU/mL	4.9 (4,0; 7,9)	4.0 (3,0; 6,1)	4.3 (3.3;6.4)	4.9 (3.2 ;8.9)	3.8 (3.3 ; 9.7)	8.9 (4.4 ; 14.4)	7.0 (5.4; 7.6	8.0 (4.3; 13.2)	P_{2-6} P_{3-7} P_{4-8}
FSH, IU/mL	6.5 (5.3; 8.0)	4.6 (2.7; 6.9)	6.7 (5.5;7.6)	5.0 (3.5 ;7.9)	5.9 (5.8; 7.0)	5.3 (3.1; 7.6)	5.6 (5.2; 6.8	4.9 (3.3; 6.3)	

MC-P2, Menstrual cycle: Phase 2 (P2); MC-P1, Menstrual cycle: Phase 1 (P1); P - statistically significant differences between groups (P<0.05)

In Buryat women with OH, LH values were higher only in Phase 2 (*P*=0.005) compared to the control. In Caucasian women with OH, LH values increased both in Phase 1 (*P*=0.002) and in Phase 2 (*P*=0.003) compared to controls (Table 3).

Comparative characterization of clinical data in women of control groups of Caucasian and Buryat ethnic groups showed higher values of anthropometric parameters in Caucasians. This concerned the increased indicators - BMI, weight, HC, WC/HC. In women with OH, no ethnic peculiarities with regard to these parameters were observed, however, the group of women of Buryat ethnicity was characterized by increased values of BMI, weight, WC, HC, % fat in comparison with the control.

In Caucasian women with OH, the changes in relation to the control data were insignificant (height, BP). Increased anthropometric indices in indigenous women with OH probably reflect the significance of the metabolic component in Buryat women.[17,19] Undoubtedly, it may be of importance in relation to clinical manifestations of OH.

The state of the neuroendocrine regulation system significantly influences the course of OH. The main indices of a neuroendocrine regulation system in the studied groups were within the reference values. In the control group of Buryat women, the levels of PRL demonstrated higher values, and testosterone decreased in comparison with Caucasians. This fact can be explained by the mechanism of increased conversion of testosterone into estradiol, corresponding to an increase in estradiol under conditions of optimal aromatase activity in granulosa cells. Estradiol, in turn, may contribute to enhanced synthesis of PRL. Despite the increased PRL values in Buryat women in the control group, no interethnic differences were recorded in the groups with OH. The difference in PRL values was observed only in the group of Caucasian women with OH relative to the control.[22]

The level of circulating AMH increased in both Buryat and Caucasian women with OH. This may be due to the increased secretion of AMH by granulosa cells in OH and to an increase in the number of mature follicles in the ovaries.[23] Increased AMH level, in turn, leads to a decrease in estrogen

content, the deficiency of which, according to the principle of positive feedback, contributes to the activation of gonadotropic function of the pituitary gland with a natural increase in the level of LH.[9] In our study, we found a significant increase in the LH content in the group of Caucasian women with OH, both in the first and second phases of the menstrual cycle, while in Buryat women, it was only in the second phase. No interethnic differences were revealed with regard to this indicator. These changes may be due to excessive LH receptivity of granulosa cells synthesizing progesterone. However, relative estrogen deficiency leads to low luteinization of this cell type, which results in decreased serum progesterone concentration.[14,24] At the same time, androgens in women with OH inhibit the negative feedback effect of progesterone on gonadotropin-releasing hormone pulse frequency.[25]

High levels of androgens of both adrenal (DHEA-S, 17-OH-Pg) and ovarian (testosterone) genesis play an important role in this process. It should be noted that in both groups with OH (to a greater extent in Caucasian women), there was an increase in FAI. This index reflects the level of free testosterone in the blood, as only it is able to bind to tissue receptors to exert its effect. It is believed that free testosterone is the best marker for assessing androgen status as one of the manifestations of hyperandrogenism of ovarian genesis.[26]

Several studies found that OH in 20-30% of women is due to excess adrenal androgens such as DHEA-S, which can alter cytochrome P450c 17 alpha-hydroxylase activity and increase peripheral cortisol metabolism, resulting in negative feedback dysregulation of adrenocorticotropic hormone.[7]

Hypersecretion of androgens by the adrenal reticular zone after conversion to estrogens by the surrounding adipose tissue and/or liver by feedback increases LH secretion, which promotes androgen synthesis by the ovaries. Consequently, androgens synthesized by the adrenal glands may cause changes in ovarian steroid synthesis. Conversely, elevated levels of exogenous androgens may have a positive feedback loop at the hypothalamic-pituitary level, and elevated LH levels, in turn, promote steroidogenesis in the ovaries.[27] Clinically, ovarian dysfunction is mainly expressed in ovulatory dysfunction, theca-cell, and oocyte dysfunction.

Conclusion

A comprehensive analysis of the state of the neuroendocrine regulation system in women of reproductive age with OH showed certain changes in the level of a number of hormones relative to control groups, most pronounced in the group of Caucasian women. At the same time, there were no differences in the studied indicators between ethnic groups with OH. The data obtained indicate the necessity of assessing and controlling the state of the neuroendocrine regulation system in female patients with OH for PCOS prevention and treatment. The ethnic component may have a certain contribution to the realization of further risks of the disease.

Competing Interests

The authors declare that they have no competing interests.

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^{68}Ga-PSMA PET/CT in Initial Diagnosis and Bone Metastasis Evaluation in Saudi Patients with High-Grade Prostate Cancer

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Abstract

We present the first study performed in Saudi Arabia to evaluate ^{68}Ga-PSMA PET/CT in prostate cancer (PCa) initial diagnosis and its added value in bone metastases (BM) diagnosis in such patients. Twenty-six male patients underwent prostate histopathological examination and all imaging studies (^{68}Ga-PSMA PET/CT and CT); all of them were confirmed with high-grade PCa. Patients' mean PSA levels and Gleason score were 5.12±1.12 and 7.0±0.9, respectively. ^{68}Ga-PSMA PET/CT (20/26; sensitivity of 76.9%) was superior to traditional CT (18/26; sensitivity of 69.2%) in PCa detection. There was a non-significant association (*P*=0.332) between patients' age and BM. Based on bone scintigraphy (BS), in patients without BM (n=16), ^{68}Ga-PSMA PET/CT detected metastasis-suspicious lesions in six patients (37.5%) and negative results in ten patients (62.5%). ^{68}Ga-PSMA PET/CT showed no false-negative cases among patients with confirmed BM using BS.

In conclusion, ^{68}Ga-PSMA PET/CT performed well in PCa initial diagnosis in Saudi male patients with high-grade tumors. ^{68}Ga-PSMA PET/CT also accurately detected BM in all PCa patients with confirmed BM by BS. Larger prospective studies are urgently required to compare ^{68}Ga-PSMA PET/CT diagnostic performance with other standard modalities in PCa and BM diagnosis. **(International Journal of Biomedicine. 2024;14(1):72-76.)**

Keywords: prostate cancer • ^{68}Ga-PSMA PET/CT • diagnosis • bone metastasis • sensitivity

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Abbreviations

BM, bone metastases; **BS**, bone scintigraphy; **PCa**, prostate cancer; **PSA**, prostate-specific antigen; **PET/CT**, positron emission tomography/computed tomography

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed tumor Among men worldwide, with about 1414000 new cases in 2020, and the fifth leading cause of cancer-related death, with about 375304 related deaths in 2020.[1] Owing to the economic growth and population aging, it is worth noting that the PCa burden is assumed to increase.[2] PCa incidence varies from nation to nation. In the Kingdom of Saudi Arabia (KSA), PCa poses a burden to public health, and its estimated age-standardized incidence rate was 7.7/100,000 men, and its age-standardized mortality rate was 5.1/100,000 men.[3]

Early PCa diagnosis plays a vital role in its overall management.[4] Traditionally, PCa diagnosis depends on serum prostate-specific antigen (PSA) testing and digital rectal examination (DRE) with confirmation on transrectal

ultrasound (TRUS)-guided biopsy.[5] However, the PSA test is far from specificity due to the reported false-positive results in many benign inflammations, including benign prostatic hyperplasia (BPH) and prostatitis.[6] The gold standard PCa diagnostic method, TRUS-guided biopsy, has inherent disadvantages, including the risk of potentially life-threatening infections. It is an invasive procedure, and it can miss lesions in the apical and anterior prostate and can yield false-negative findings.[7] In light of these disadvantages, there is an urgent need for alternative, accurate, and noninvasive methods for PCa lesion detection.[4]

Another noninvasive method, multiparametric MRI (mpMRI), has shown considerable promise in PCa staging and diagnosis.[8] Although reports have found that mpMRI has a high sensitivity for PCa detection,[9] its use is limited by the extreme range of negative predictive values, moderate interreader reproducibility, and moderate specificity.[10,9] Thus, there is still a need to use and/or develop alternative noninvasive methods.

In virtually all PCas, prostate-specific membrane antigen (PSMA), a transmembrane glycoprotein (type II), is overexpressed.[11] It is a glutamate carboxypeptidase II metalloproteinase mainly presenting in PCa tissues.[11] In patients with intermediate-high risk PCa, gallium-68–labeled PSMA (^{68}Ga-PSMA) positron emission tomography/computed tomography (PET/CT) has been reported to have a potential role in recurrent PCa detection as well as PCa staging with a high degree of accuracy.[11] In advanced disease stages, bone metastases (BM) are present. BM imaging is important not only for characterization and localization but also to assess their number and size and to follow up on the disease after and during treatment.[12]

Therefore, this study was designated to evaluate the diagnostic accuracy of ^{68}Ga-PSMA PET/CT in PCa initial diagnosis in Saudi patients with histopathologically proven high-grade PCa and to assess the ^{68}Ga-PSMA PET/CT ability to detect BM in those patients.

Materials and Methods

Study protocol and ethical considerations

This retrospective study was conducted in the Medical Imaging - Nuclear Medicine Department of King Abdulaziz Medical City (KAMC) at the Prince Sultan Military Medical City (PSMMC), Riyadh, Saudi Arabia, from December 2019 to December 2020, and included 26 patients. The ethical approval of the study protocol was obtained from the Institutional Review Board (IRB) at the Princess Norah bint Abdulrahman University. All data was confidently obtained from the patient's medical records, and thus, the need to obtain informed consent was waived.

Patients

The study included male patients aged ≥40 with proven high stages PCa by histopathological findings as the gold standard. Patients with other benign diseases like BPH who have the same PCa pathological symptoms and patients who underwent post-prostatectomy were excluded. All patients were screened using traditional CT scans and ^{68}Ga-PSMA PET/CT. Age, indication, Gleason score, and lab tests were also involved.

^{68}Ga-PSMA PET/CT

Images were obtained using ^{68}Ga-PSMA. 68Ga-PSMA ligand complex solution was applied to all patients through an intravenous bolus (mean 6.1, SD 154.5, 6.1-27.4 MBq; range, 87–241 MBq). After tracer injection (range, 50-70 minutes), PET acquisition began at a mean time of 58.5±9.5 minutes. All patients underwent ^{68}Ga-PSMA PET/CT on a Biograph mCT scanner (Siemens, Germany). For attenuation correction, low-dose CT covered by PET (from skull to mid-thigh) was taken place. After CT scan completion, PET data were gained for three minutes/bed position. After CT scan completion, PET data were gained for three minutes/bed position. After CT scan completion, PET data were gained for three minutes/bed position. Emission data were corrected for attenuation, scatter, dead time, and randoms. They were reconstructed iteratively by an ordered-subsets expectation maximization algorithm (8 subsets, 4 iterations) followed by a post-reconstruction smoothing Gaussian filter (5 mm in full width at one-half maximum).

The dedicated software (Syngo; Siemens) was used to review PET/CT images. Two different nuclear medicine physicians quantitatively and visually analyzed images.

Briefly, the assay involved obtaining a circular semi-automated volume of interest (VOI) from the prostate bed. Concerning the assessment of distant bone and lymph node metastases, any focal uptake > surrounding background activity that did not correspond to physiological tracer uptake was suggestive of tumor pathology.

^{99m}Tc bone scintigraphy

In agreement with the guidelines of the European Association of Nuclear Medicine/Society of Nuclear Medicine (EANM),[13] bone scintigraphy (BS) was conducted. Two-three hours after 9.4 MBq/kg body weight ^{99m}Tc-labelled methylene disphosphonate intravenous injection, a planar whole body BS scan was performed using a 2-headed gamma camera (Symbia T16, Siemens, Germany). At the discretion of the treating physician, supplemental single-photon emission CT (SPECT)/CT, covering 1- or 2-bed positions was conducted. SPECT/CT parameters were 16 views with 10 seconds/view, as previously described.[14] Images were reconstructed using iterative reconstruction with scatter correction. Low-dose CT was performed for anatomical co-registration and attenuation correction. In clinical guideline recommendations, BS and any supplementary SPECT/low-dose CT are represented as the standard bone evaluation. Another contrast-enhanced CT could be conducted after BS to assess soft tissue and lymph node metastasis.

Statistical analysis

The diagnostic values for CT and ^{68}Ga-PSMA PET/CT were evaluated and compared. Diagnostic sensitivity for each modality was calculated. Statistical analysis was performed using the statistical software package SPSS version 20.0 (SPSS Inc, Armonk, NY: IBM Corp) and GraphPad prism (ver. 8.0, San Diego, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean ± standard deviation (SD) for continuous variables.

Inter-group comparisons were performed using Student's t-test. Group comparisons with respect to categorical variables are 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

Twenty-six male patients underwent prostate histopathological examination and all imaging studies; all of them were with histopathologically confirmed high-grade PCa. Patient characteristics are described in Table 1. The mean PSA levels and Gleason score were 5.12±1.12 ng/mL and 7.0±0.9, respectively.

Table 1.

Characteristics of the study patients.

Variable	Mean	SD
Age (years)	70.08	12.17
<50 (number; percentage)	2 (7.7)	—
51 to 60 (number; percentage)	2 (7.7)	—
61 to 70 (number; percentage)	10 (38.5)	—
71 to 80 (number; percentage)	6 (23.1)	—
>80 (number; percentage)	6 (23.1)	—
PSA (ng/mL)	5.12	1.12
Gleason score	7.0	0.9

^{68}Ga-PSMA PET/CT was superior to traditional CT in PCa detection

^{68}Ga-PSMA PET/CT and CT examinations were performed for each patient, and the histologic findings were recorded. As shown in Table 2, the diagnostic ability of ^{68}Ga-PSMA PET/CT (20/26; sensitivity of 76.9%) was superior to CT (18/26; sensitivity of 69.2%) in identifying PCa.

Table 2.

Diagnostic values for different imaging modalities.

Imaging method	PCa patients	TP	FN	Sensitivity
Computed tomography	26	18	8	69.2%
^{68}Ga-PSMA PET/CT	26	20	6	76.9%

TP: true positive; FN: false negative.

^{68}Ga-PSMA PET/CT for evaluating bone metastasis

A cross-tabulation for patients' age and the values of non-metastatic and metastatic cancer after a diagnosis of patients with PCa revealed that there was a non-significant association ($P=0.332$) between age categories and bone metastasis (Table 3).

Based on BS, in patients without BM (n=16), ^{68}Ga-PSMA PET/CT detected metastasis-suspicious lesions in six patients (37.5%) and negative results in ten patients (62.5%) (Table 4). ^{68}Ga-PSMA PET/CT showed no false-negative cases among patients with confirmed BM using BS (Table 4).

Table 3.

Age categories and BS metastasis cross-tabulation.

			BS metastasis		*P*-value
			Positive	Negative	
			n=10	n=16	
Age (years)	>50	n=2	2 (100%)	0	0.332
	51-60	n=2	0	2 (100%)	
	61-70	n=10	4 (40%)	6 (60%)	
	71-80	n=6	2 (33.3%)	4 (66.7%)	
	>81	n=6	2 (33.3%)	4 (66.7%)	

Table 4.

^{68}Ga-PSMA PET/CT assessment.

			^{68}Ga-PSMA PET/CT		*P*-value
			Positive	Negative	
			n=20	n=6	
BS metastasis	Positive	n=10	10	0	0.0025
	Negative	n=16	6	10	

Discussion

PET/CT using PSMA ligands has gained increasing attention for diagnosing PCa and evaluating the extent of the disease.[15] It is recommended for high-risk cases with various advantages, including the ability to perform multimodal hybrid imaging, great specificity, and improved target-to-background ratio.[16] Reports demonstrating ^{68}Ga-PSMA PET/CT for initial detection of suspected PCa are scarce.[4] This study is the first conducted in Saudi Arabia to investigate ^{68}Ga-PSMA PET/CT diagnostic accuracy for PCa initial diagnosis in Saudi patients with histopathologically proven high-grade PCa. Also, this study evaluated the added value of ^{68}Ga-PSMA PET/CT in BM diagnosis in PCa patients who recently underwent BS.

In the present research, as a gold standard, the biopsy showed hormonal and functional results for positive PCa in all patients (n=26). Also, CT showed an anatomical result to locate PCa in 18/26 patients (sensitivity - 69.2%). The 68Ga-PSMA PET/CT was superior to CT and showed functional and

anatomical results to detect PCa in 20/26 (sensitivity - 76.9%).

Similar to our results, Lopci et al.[17] found that ^{68}Ga-PSMA PET/CT was capable of detecting malignancy and accurately identifying clinically relevant PCa in patients with high suspicion of cancer.

In a prospective Australian multicenter study, ^{68}Ga-PSMA PET/CT impact on PCa management was greater in cases with biochemical recurrence after radiation treatment or definitive surgery than in cases undergoing primary staging.[18] Interestingly, ^{68}Ga-PSMA PET/CT detected distant metastatic disease in 16% of patients, locoregional lymph nodes in 39%, and unsuspected disease in the prostate bed in 27%.[18]

A recent meta-analysis and systematic review including 7 studies that comprised 389 patients revealed that PCa initial diagnosis using ^{68}Ga-PSMA PET/CT had pooled specificity, sensitivity, negative likelihood ratio, and positive likelihood ratio of 66%, 97%, 0.05, and 2.86, respectively.[4] This finding indicates a 20-fold decrease in PCa odds being found in cases with negative PET results, thus making ^{68}Ga-PSMA PET/CT a potential rule-out test in patients with PCa suspicious biochemical or clinical findings and allowing unnecessary biopsies to be avoided.[4]

A PCa's typical characteristic is its tendency to metastasize to the bones.[19] Accurate and timely detection of bone involvement is critical in PCa management.[19] The highly sensitive, widely available, and cost-effective BS technique remains the standard for bone metastase diagnosis and is recommended by international guidelines.[20] However, given its poor performance compared with modern imaging techniques using tumor-specific tracers, its lack of specificity, and its poor performance in diagnosing small metastases in the bone marrow that have not resulted in enough osteoblastic response, it is debatable if classical BS is the suitable method for bone metastases diagnosis.[21]

In this study, ^{68}Ga-PSMA PET/CT diagnosed BM in 100% of patients with positive BS findings. However, ^{68}Ga-PSMA PET/CT also identified a notable proportion of cases in whom PSMA-avid lesions in the ribs were false positive. In line with this finding, research comparing planar BS and ^{68}Ga-PSMA PET in PCa cases demonstrated that ^{68}Ga-PSMA PET demonstrated a significantly lower equivocal lesions number, showed higher diagnostic accuracy for evaluating involved bone regions and had higher performance in diagnosing BM status of the patients.[22] Furthermore compared to BS, a similar study in 30 PCa cases reported that ^{68}Ga-PSMA PET/CT detected a significantly higher number of BM.[23] Sachpekidis et al.[19] reported that ^{68}Ga-PSMA PET/CT is a useful diagnostic method in BM detection in PCa. Compared to low-dose CT, ^{68}Ga-PSMA PET/CT visualizes more BM.[19] Authors also found that many parameters of ^{68}Ga-PSMA PET significantly correlate with plasma PSA levels.[19]

Conclusion

Using histology examination as the reference standard, compared to CT, ^{68}Ga-PSMA PET/CT performed well in PCa initial diagnosis in Saudi male patients with high-grade tumors. Also, ^{68}Ga-PSMA PET/CT accurately detected BM in all PCa patients with confirmed BM by BS. Furthermore, whereas ^{68}Ga-PSMA PET/CT commonly detected BM in patients with negative BS results, BS rarely detected BM in patients with negative ^{68}Ga-PSMA PET/CT results. Given these findings, ^{68}Ga-PSMA PET/CT is a promising technique in PCa detection and may be a potential additive in identifying BM in PCa patients. In this setting, larger prospective studies are urgently required to compare mpMRI diagnostic performance with ^{68}Ga-PSMA PET/CT.

Competing Interests

The authors declare that they have no competing interests.

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Dental Cone-Beam Computed Tomography: Are the Eye Lens and Thyroid at Risk?

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Abstract

Background: The purpose of this study was to assess radiation dose to the eye lens (EL) and thyroid gland (TG) from 22 protocols used in maxillofacial imaging with cone-beam computed tomography (CBCT).

Methods and Results: NanoDot optically stimulated luminescence dosimeters were used to assess scattered radiation to the EL and TG using a phantom. The dosimeters were secured at four sites around areas of interest. Mean eye radiation dose was significantly associated with field of view (FOV) size (r=0.830, *P*<0.001). Meanwhile, the mean thyroid radiation dose was found to be significantly associated only with exposure time (r=0.464, *P*=0.030). Mandible centralization was observed to be the most significant predictor for a greater effective thyroid dose; mandible FOV centralization had 0.236 odds of a higher thyroid dose than maxilla FOV centralization.

Conclusion: FOV size significantly impacted EL dose. Thyroid exposure was affected by FOV centralization and exposure time. Centering the FOV on the mandible resulted in a greater effective dose due to the proximity of the TG to the primary beam. **(International Journal of Biomedicine. 2024;14(1):77-82.)**

Keywords: cone-beam computed tomography • radiation dose • thyroid gland • eye lens

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Abbreviations

CT, computed tomography; **CBCT**, cone-beam computed tomography; **CTDI**, CT dose index; **DAP**, dose-area product; **EL**, eye lens; **FOV**, field of view; **OSL**, optically stimulated luminescence; **TG**, thyroid gland.

Introduction

The utilization of cone-beam computed tomography (CBCT) in dental practice has increased dramatically in the last decade to assess maxillofacial structures for diagnostic, treatment planning, and follow-up purposes. It is estimated that almost 4 million CBCT examinations are performed annually in the United States of America alone.[1] CBCT has become a useful tool for dentists worldwide and is gaining popularity in orthodontic clinics, where most patients are children or adolescents.[2] Of all the imaging techniques used in dentistry, CBCT is the newest and most closely associated with the highest radiation dose.[3] This modality consists of a cone-shaped beam rotating around the patient's head to acquire raw two-dimensional images reconstructed from several projections to form a three-dimensional volume.[4] The cumulative doses from CBCT machines can range from 5 μSv to 1073 μSv.[5] Ionizing radiation, which is used in CBCT, is associated with an increased risk of developing leukemia and other cancers over a patient's life span. Although CBCT is an extremely useful tool, the associated radiation risk is a significant public health hazard.[6]

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To express the risks associated with ionizing radiation exposures, the International Commission on Radiological Protection (ICRP) recommends using the effective dose, which considers the biological effect on radiosensitive tissue/organs using weighting factors depending on the degree of organ sensitivity.[7] Other exposure indicators specific to CT are the CT dose index (CTDI) or the dose-area product (DAP), which can be used to calculate the CBCT dose.[3]

The FOV size, image resolution, and other exposure parameters are important in the radiation dose received during CBCT examinations. According to the FDA, "radiation doses from dental CBCT exams are generally lower than other CT exams; dental CBCT exams typically deliver more radiation than conventional dental X-ray exams."[8]

The effective dose from CBCT examinations was reported to be between 46 µSv and 1073 µSv in adult phantom dosimetry studies. For child phantoms, however, standard protocols resulted in an effective dose that varied from 13 µS to 769 µS.[9,10] This large range in radiation doses is mainly due to the various exposure parameters that can be adjusted before each examination. Though the guidelines suggest that the dose from the CBCT modality is equivalent to doses from 2 to 10 panoramic radiographs, it has been reported that this dose can range from 2 to 200 panoramic radiographs.[11] This large variation emphasizes the need for practice standardization and justification of use.

The American Association of Endodontists (AAE) and the American Academy of Oral and Maxillofacial Radiology (AAOMR) issued a joint position statement on dose considerations for CBCT, which includes the use of the smallest possible FOV size, largest voxel size, lowest current setting (mA), shortest exposure time, and pulsed exposure modes when possible.[12] This statement "recommended that the use of CBCT in endodontics be limited to certain complex conditions" to ensure that the benefit outweighs the risks associated with the exposure.[12] In addition to direct exposure, the scattered radiation is of concern, especially in head and neck imaging, where the eye lens (Els) and thyroid gland (TG) could receive an unnecessary dose.[13] The ICRP has dropped the yearly occupational eye dose considerably from 150 mSv to 20 mSv after epidemiological evidence proved damage to EL with radiation exposure. This has lowered the threshold for this sensitive organ, compared to the past.[14] The scattered radiation associated with CBCT usage can be affected by technique, including FOV size and centralization, which can decrease photon scattering and overall patient dose reduction.[15]

During abdominal CT, the dose of the scattered radiation reaching the TG was reported to be 214 µSv and the EL to be 57 µSv.[16] For comparison, the dose of the scattered radiation during a digital mammography screening could be 25 µSv and 2.5 µSv to the thyroid and lens, respectively.[17] A study by Alwasiah et al.[18] conducted in 2021 reported the mean absorbed dose to the eyes during a brain CT to be 33.6 mGy. Moreover, the authors indicated that these numbers are alarming, especially since damage could be induced in the eyes due to radiation doses "as low as 0.2 Gy and 0.5 Gy."

Since factors such as FOV size and the location of radiosensitive organs impact patient radiation dose, using a larger FOV exposes more tissue to radiation, resulting in more scattering to adjacent areas. FOV centralization (depending on the protocol used) also impacts the dose. A volume–dose model proposed by Pauwel et al.[19] in 2014 used various FOV sizes and centralizations to optimize patient doses and reduce scattering to radiosensitive organs. The results of this study demonstrated a significant dose reduction (up to 69%) when using the same FOV for the mandible instead of the maxilla. Additionally, in the mandible position, a dose reduction of more than 30% was achievable when changing the FOV from 17cm×2cm to 14cm×5cm. The authors also measured a higher scattered dose to the TG when using mandibular scans due to anatomic proximity. Most importantly, FOV should not be positioned inferiorly to achieve a reduction of the EL dose.[19] This, in turn, could increase the thyroid dose. Therefore, a reduction of EL dose is only achievable using a smaller FOV or decreasing mAs.

Studies propose patients use small leaded glasses during CBCT examination to spare the EL from unnecessary exposure.[20] In addition, since the thyroid is another area adjacent to the primary beam in CBCT, a high dose to the thyroid could result in radiation-induced damage. Epidemiological studies have provided some limited evidence of an increased risk of thyroid tumors resulting from dental radiography. During CBCT, the radiation dose to the TG can be reduced by 18% to 40% when using a front thyroid collar and up to 43% when using a front/back thyroid collar.[21] The use of leaded glasses and a thyroid collar during maxillofacial scans decreased the organ dose to the eye's lens by 62% and to the thyroid by 26%, respectively. Additionally, doses to the thyroid could also be reduced by 70% using collimation. In mandibular scans, using leaded glasses and thyroid collars decreased the dose to the eye's lens by 13% and to the thyroid by 33%. That study reported that doses to the EL were five times greater when leaded glasses were not used.[21]

This study aimed to assess radiation dose to the EL and TG during CBCT examinations using protocols developed for dental purposes. Optically stimulated luminescence (OSL) dosimeters, commonly used for dosimetry and to determine the radiation dose in diagnostic and therapeutic imaging modalities, were used in this study to measure the absorbed dose during CBCT dental examinations.[22,23]

Materials and Methods

This cross-sectional dosimetry study was conducted at the Oral Radiology Department of King Abdulaziz University Dental Hospital between September 2020 and September 2021. The study followed the methods of Jadu et al.,[24] except the nanodots were fixed securely to the phantom using fabricated straps at only four sites: the right and left eye surfaces and on the right and left side of the neck at the TG level.

Two similar CBCT machines (iCAT Imaging Sciences International, Hatfield, PA, USA) were used for data collection. Additionally, 22 different protocols covering the range of CBCT use for dentistry were used. The details of the protocols are outlined in Table 1. The exposure parameters for each protocol were as follows: FOV size, voxel size, which represents the image resolution, time, DAP, and FOV

centralization. The kVp and mA for all the protocols were constant at 120 and 5, respectively.

The effective radiation doses (E) to the eyes and thyroid were calculated by multiplying the average absorbed radiation dose by the radiation- and tissue-weighted factors according to the following equation:

$$E\ (\mu Sv) = \sum W_T D_T X1$$

where W_T is the tissue (T) weighted factor, and the sum of all tissue-weighted factors is 1. The issue-weighted factors were based on the most recent ICRP guidelines.[14] D_T is the average absorbed dose measured in a particular organ or tissue, and the radiation-weighted factor for X-radiation is 1.

This study did not require ethical approval since no human subjects were enrolled. The experiment was conducted using a phantom. The data collected were analyzed and presented using IBM SPSS version 23 (IBM Corp., Armonk, N.Y., USA) and GraphPad Prism version 8 (GraphPad Software, Inc., San Diego, CA, USA).

Results

This study evaluated the effective radiation dose on the ELs and TG during CBCT examinations using a radiation phantom and 22 different protocols for dental purposes (Table 1).

The mean difference between the absorbed radiation dose to the right and left sides of each organ was assessed (Table 2). The results revealed no significant mean differences (P>0.05) between the average doses to the right and left eyes and between the right and left thyroid lobes, suggesting that the eyes and the thyroid can be considered unitary organs in further analyses.

The association between the mean organ dose and the imaging parameters of the different protocols was then evaluated (Table 3). The results revealed that the mean eye radiation dose was significantly associated with the FOV size (r=0.830, P<0.001), DAP (r=0.668, P=0.001), and voxel size (r=0.489, P=0.021). Meanwhile, the mean thyroid radiation dose was found to be significantly associated only with exposure time (r=0.464, P=0.030).

The association between the effective dose to the eyes and thyroid and the FOV centralization was investigated using a paired sample t-test. More specifically, a significant mean difference of -0.0437 (P=0.046) was found between the mean eye dose (0.24±0.0 mGy, N=3) and the thyroid dose (0.68±0.2 mGy, N=3) when the FOV was centered on the mandible and between the eye dose (0.76±0.4 mGy, N=12) and the thyroid dose (0.38±0.1 mGy, N=12) when the FOV was centered on the occlusal plane. No significant differences were observed between the mean eye and thyroid doses when the FOV was centered on the maxilla (P>0.05).

The significant imaging factors associated with the mean effective dose to the EL and TG were also determined (Table 4). The results revealed that only the FOV size was found to significantly predict the mean effective dose to the EL (SE=0.001, 95% CI: lower bound = 0.000, upper bound = 0.005, P=0.030) according to the general linear model (GLM) at the P<0.05 level, resulting in a 0.003 unit increase in the EL effective dose with every cm increase in FOV size.

Table 1.

The exposure parameters used for each of the 22 cone beam CT dental protocols explored.

No.	Indication	FOV (cm)	Vox (mm)	Time (sec)	DAP	FOV centralization
1	Single arch implant protocol (Protocol 1)	16 × 6	0.3	4.8	168.5	maxilla
2	Single arch implant protocol (Protocol 2)	16 × 6	0.3	8.9	302.9	maxilla
3	Single arch implant protocol (Protocol 3)	16 × 6	0.3	4.8	168.5	mandible
4	Single arch implant protocol (Protocol 4)	16 × 6	0.4	4.8	168.5	maxilla
5	Both arches implant (Protocol 1)	8 × 8	0.3	4.8	134.8	occlusal plane
6	Both arches implant (Protocol 2)	16 × 8	0.4	4.8	219.6	occlusal plane
7	Both arches implant (Protocol 3)	16 × 10	0.4	4.8	278.1	occlusal plane
8	Both arches implant (Protocol 4)	16 × 10	0.3	4.8	278.1	occlusal plane
9	Both arches implant (Protocol 5)	16 ×10	0.4	8.9	501.3	occlusal plane
10	Root resorption/root fracture/root canals assessment	8 × 8	0.25	14.7	275.1	occlusal plane
11	Apical periodontitis/ apical surgery	8 × 8	0.25	14.7	275.1	occlusal plane
12	Impacted third molars (single arches)	16 × 6	0.4	4.8	168.5	mandible
13	Impacted third molars (both arches)	16 ×10	0.4	4.8	278.1	occlusal plane
14	Impacted canines and supernumerary teeth	8 × 8	0.3	8.9	239	maxilla
15	Orthodontic planning	16 × 10	0.4	4.8	278.1	occlusal plane
16	Orthognathic surgery	16 × 13	0.4	4.8	349.4	occlusal plane
17	Cleft palate	16 × 13	0.4	4.8	349.4	occlusal plane
18	Craniofacial anomaly	23 × 17	0.4	4.8	458.6	occlusal plane
19	TMJ (closed)	16 × 8	0.25	14.7	444.3	maxilla
20	Pathosis (single arch)	16 × 8	0.3	4.8	219.6	occlusal plane
21	Pathosis (both arch)	16 × 10	0.3	4.8	278.1	occlusal plane
22	Maxillofacial trauma	16 × 13	0.3	4.8	349.4	occlusal plane

FOV, field of view; VOX, voxel; DAP, dose-area product; TMJ, temporomandibular joint.

Table 2.

Paired sample association of eyes lenses and thyroid lobes (N = 22).

Dose mGy		Mean ±SD	Mean Difference	95% CI of the Difference		P-value
				Lower	Upper	
Pair 1	RE	0.55 ± 0.3	-0.055	-0.116	0.005	0.072
	LE	0.60 ± 0.4				
Pair 2	RT	0.42 ± 0.2	0.011	-0.019	0.040	0.471
	LT	0.41 ± 0.2				

SD, standard deviation; CI, confidence interval; RE, right eye; LE, left eye; RT, right thyroid lobe; LT, left thyroid lobe. A P-value of < 0.05 was considered statistically significant.

Table 3.

Correlation between the mean organ dose and exposure parameters of the various protocols (N = 22).

Variables		Average dose mGy	
		Eye	Thyroid
FOV size	r	0.830	0.208
	P-value	<0.001	0.354
Vox size	r	0.489	0.184
	P-value	0.021	0.413
Time	r	0.103	0.464
	P-value	0.648	0.030
DAP	r	0.668	0.124
	P-value	0.001	0.582

FOV, field of view; VOX, voxel; DAP, dose-area product.
A P-value of < 0.05 was considered statistically significant.

More specifically, mandible centralization was observed to be the most significant predictor for a greater effective thyroid dose; mandible FOV centralization had 0.236 odds of a higher thyroid dose when compared to maxilla FOV centralization. In contrast, maxilla FOV centralization demonstrated an inverse relationship with dose. Another predictor was imaging time, for which a 0.021 increase in the thyroid effective dose with every unit increase in time was observed (Table 4).

Table 4.

Association between the imaging parameters and mean eyes radiation dose and thyroid gland dose (mGy)

Average EL dose (mGy)					
Parameter	B	SE	95% CI		*P*-value
			Lower Bound	Upper Bound	
Intercept	-0.134	0.451	-1.095	0.827	0.770
FOV size (cm)	0.003	0.001	0.000	0.005	0.030[a]
VOX size (mm)	0.699	0.993	-1.419	2.816	0.493
Time (sec)					
DAP	0.001	0.001	-0.001	0.002	0.351
FOV centralization=Maxilla	0.124	0.185	-0.270	0.519	0.512
FOV centralization=Mandible	0.033	0.200	-0.393	0.460	0.870

Average TG dose (mGy)					
Parameter	B	SE	95% CI		*P*-value
			Lower Bound	Upper Bound	
Intercept	0.266	0.040	0.182	0.351	<0.001[a]
Centralization=Maxilla	-0.101	0.045	-0.195	-0.008	0.036[a]
Centralization=Mandible	0.236	0.057	0.117	0.355	0.001[a]
Time	0.021	0.006	0.009	0.034	0.002[a]
[a]-Significant using General Linear Model (GLM) at <0.05 level.					

CI, confidence interval; B, B coefficient; SE, standard error.

Discussion

CBCT use in Saudi Arabia is neither monitored nor regulated. Local efforts are ongoing by national authorities to establish diagnostic reference levels, policies for practice justification and standardization, optimization of exposure, and quality assurance. Given the increased use of CBCT, there is a clear need for thorough justification criteria. This is especially important because of the current practice of "self-referral," in which a dentist performs CBCT examinations for patients based on their own clinical assessment.[3] Currently, CBCT use is highly dependent on self-awareness.

The Safety and Efficacy of a New and Emerging Dental X-Ray Modality (SEDENTEXCT) project, aiming to provide evidence-based guidelines for dental and maxillofacial use of CBCT, resulted in the publication of several dosimetry studies using Monte Carlo modeling of phantoms to estimate the effective doses and organs that contribute to these doses.[25] The results of these studies have confirmed that 19% of the average relative contribution of organ doses in CBCT maxillofacial examinations is from thyroid exposure.[26] Hence, radiation risk from CBCT examinations for dental purposes is generally higher than intraoral and panoramic modalities but lower than multidetector CT examinations of the same area.[25]

The purpose of this study was to assess radiation dose to the eye lens (EL) and thyroid gland (TG) from 22 protocols used in maxillofacial imaging with cone-beam computed tomography (CBCT).

Of all the imaging factors examined in this dosimetry study, the factor most significantly impacted the EL dose was the FOV size. This result is expected since the EL is more likely to be in the direct path of the primary X-ray beam in larger FOVs that extend above the maxilla—such as those used for orthodontic purposes, for example. Several previous studies have confirmed this finding and reported EL dose reductions that range between 26% and 67% with smaller FOVs.[19,27-29] Remarkably, no association was noted between EL doses and FOV centralization. It would have been plausible to record higher EL doses in CBCT examinations centered on the maxilla as opposed to the mandible; however, this was not the case. We hypothesize that this result is due to the anatomic distance from the eyes to both jaws being relatively similar. Hence, no significant difference in EL dose was detected when the FOV centralization was changed.

Cataract is a well-known and documented deterministic effect of eye lens radiation exposure during interventional procedures.[14,30] Not many studies quantify the radiation risk to the eye lens from diagnostic procedures such as CBCT. Yuan et al. evaluated the potential radiation risk to the EL from diagnostic CT imaging. Since the use of CBCT is growing, the authors cautioned that similar risks can be anticipated in patients undergoing CBCT, especially when the primary X-ray beam is closer to the eye.[31] There is growing evidence that the EL is likely to be affected by the levels of radiation used for diagnostic purposes, and this has prompted the change in the threshold for EL radiation-induced damage from 2.0 Gy to 0.5 Gy.[30] Consequently, caution must be exercised whenever the dental CBCT examination area is close to or includes the eyes.

The TG was most significantly affected by 2 imaging factors: FOV centralization and exposure time. The effect of FOV centralization on the effective dose has been well-documented in previous studies, such as the one by Jadu et al.[24] In agreement with the results of our study, the authors found that centering the FOV on the mandible led to a greater effective dose due to the anatomic proximity of the TG to the primary X-ray beam and, thus, a greater contribution of the thyroid dose to the overall whole-body effective dose. Jadu et al.[24] also found that the voxel size (i.e., the image resolution) affected the effective dose significantly; however, this was not the case in our study. This variation in results may be due to differences in the methods used to calculate the effective dose.

The effective doses to TG were also significantly influenced by the exposure time, with greater doses associated with longer exposure times. Despite the lack of publications that support this result, it seems plausible that a longer exposure time will result in a greater dose absorbed by the TG.

However, this result should be interpreted cautiously, as it cannot stand alone without considering the other exposure parameters.

The TG is of particular interest in dental imaging due to its proximity to the areas usually imaged and its sensitivity to the stochastic effects of radiation. The radiosensitivity of TG is especially relevant for children and adolescents. In fact, TG is still considered the most radiosensitive organ in the head and neck.[32] This has prompted several authors to strongly recommend using thyroid shields and collars, especially in children and adults until the age of 50.[32-34]

The CBCT maxillofacial imaging protocols are designed to ensure that the diagnostic purpose of the examination is met while exposing the patient to a relatively reasonable dose of radiation. This concept is known as the "as low as diagnostically achievable" (ALADA) principle. To follow this principle, there is usually a compromise between the various imaging parameters used. For example, protocol number 10 in Table 1 is used for assessing root resorption, root fractures, and root canals and, hence, utilizes a smaller voxel size to ensure that the images produced are of sufficiently high resolution to distinguish the delicate structures of the roots. This protocol is coupled with a smaller FOV size to offset the radiation dose to the patient to compensate for the increased radiation associated with these high-resolution images. Alternatively, larger FOV examinations are usually coupled with lower-resolution images (greater voxel size) to moderate the radiation risk to the patient. This explains the association we noted with only one or two—and not several—of the numerous imaging factors. This finding also highlights the importance of carefully selecting these parameters to balance the diagnostic task and radiation risk to the patient.

The results of this study may vary between different CBCT machines depending on other imaging factors, such as kVp, mA, filtration, rotation arc, and pulsed vs. continuous exposures. Future directions should include more CBCT dosimetry studies to improve our understanding and control of this significant public health risk. In addition, the use of CBCT should be monitored, and patient doses should be tracked and reported to establish reference levels for benchmarking and practice optimization.[35] Future research should especially focus on dosimetry involving vulnerable populations, such as children and adolescents, who often receive CBCT examinations for dental purposes.

Conclusion

We showed that radiation doses to the eye lens and thyroid gland from CBCT examinations of the maxillofacial region were most significantly affected by the FOV size and FOV centralization, respectively. Therefore, these parameters should be chosen carefully for the various CBCT dental indications. Every attempt to shield these sensitive organs using lead eyeglasses and thyroid collars should be made.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Diagnostic Performance of ^{18}F-fluorocholine PET/CT Compared to ^{99m}Tc-Sestamibi Scintigraphy in Diagnosis of Parathyroid Adenoma

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Abstract

Background: In most hyperparathyroidism cases, the disease is related to parathyroid adenoma (PTA). Owing to the inconsistencies of currently approved imaging methods, novel methods for detecting PTA are being evaluated. This study aimed to compare ^{18}F-fluorocholine PET/CT with ^{99m}Tc-sestamibi SPECT/CT in identifying PTA in Saudi patients.

Methods and Results: The study included 40 adult patients with PTA diagnosed by histopathological findings. ^{99m}Tc-sestamibi SPECT/CT and ^{18}F-fluorocholine PET/CT examinations were performed for each patient, and parathyroid hormone (PTH) levels and histologic findings were recorded. The diagnostic ability of ^{18}F-fluorocholine PET/CT (AUC=0.720; P=0.029) was superior to ^{99m}Tc-sestamibi SPECT/CT (AUC=0.623; P=0.214) in identifying PTA. The sensitivity and accuracy of ^{18}F-fluorocholine PET/CT (81.5% and 75.0%, respectively) were significantly (P<0.05) higher than that of ^{99m}Tc-sestamibi SPECT/CT (63.0% and 62.5%, respectively). ^{18}F-fluorocholine PET/CT findings were correlated significantly (P=0.023) with PTH results.

Conclusion: ^{18}F-fluorocholine PET/CT is a diagnostic imaging method superior to conventional modality ^{99m}Tc-sestamibi SPECT/CT in the detection of PTA and, thus, allows for accurate preoperative localization.**(International Journal of Biomedicine. 2024;14(1):83-87.)**

Keywords: ^{18}F-fluorocholine PET/CT • parathyroid adenoma • hyperparathyroidism

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Abbreviations

4DCT, four-dimensional computed tomography; **FCH**, fluorocholine; **HPT**, hyperparathyroidism; **PTA**, parathyroid adenoma; **PET/CT**, positron emission tomography/computed tomography; **SPECT**, single photon emission computed tomography; **PTH**, parathyroid hormone.

Introduction

Worldwide, around 1% of the general population is affected by hyperparathyroidism (HPT), characterized by excess parathyroid hormone (PTH) release and hypercalcemia.(1) In up to 85% of cases, this disorder results from parathyroid adenoma (PTA). Parathyroid carcinoma and hyperplasia are less common causes.(2) In most patients, HPT is asymptomatic and is detected incidentally during routine blood tests. In patients with symptomatic disorders, cardiovascular, neural, gastrointestinal, urinary, and musculoskeletal systems are affected.(3)

In both asymptomatic and symptomatic cases, surgical treatment (parathyroidectomy) of the hyperfunctioning

gland remains the only curative method.[4] For a minimally invasive parathyroidectomy, preoperative localization of the hyperfunctioning gland is needed, and this, in turn, is related to a decreased risk of disability and complications after surgery.[4] Preoperative adenoma localization is complex, and imaging methods and recommendations vary significantly. ^{99m}Tc-sestamibi SPECT/CT and neck ultrasonography are the most frequently used methods. Other imaging modalities are available to facilitate localization, including MRI and four-dimensional computed tomography (4DCT).[5] These methods can be used alone or in combination; both methods have disadvantages and advantages. Moreover, their diagnostic accuracy significantly varies depending on the skill of individual sonographers, the adenoma size, and the location of the affected glands.[4,5]

Grimaldi et al.,[6] in their study, emphasize that ^{18}F-FCH PET/CT is a promising modality in challenging presurgical localization of hyperfunctioning parathyroid glands, such as inconclusive standard imaging, recurrence after surgery, or suspected multiple gland disease. In several small studies, ^{18}F-FCH PET/CT has demonstrated promising results, possibly leading to an expanded role for this tracer.[7,8] In clinical practice, it has become clear that ^{18}F-FCH PET/CT may have diagnostic ability superior to other modalities and may become the gold standard diagnostic method for HPT.

In this study, we aimed to compare the diagnostic performance of ^{18}F-FCH PET/CT with ^{99m}Tc-sestamibi SPECT/CT for preoperative identification of PTA. Patients were identified with the gold standard histologic examination.

Materials and Methods

Study Design

This retrospective study, which included 40 patients, was performed from January 2017 to December 2021 at the endocrinology and nuclear medicine unit of 3 hospitals in Riyadh (Saudi Arabia): King Abdulaziz Medical City Hospital (KAMC), Prince Sultan Military Medical City (PSMMC), and King Abdullah bin Abdulaziz University Hospital (KAAUH). The ethical approval of the study protocol was obtained from the Institutional Review Board (IRB) at King Abdulaziz Medical City Hospital (KAMC), Prince Sultan Military Medical City (PSMMC), and Princess Norah University. All data was obtained from the patient's medical records, and thus, the need to obtain informed consent was waived.

The study included adult patients with PTA diagnosed by histopathological findings, a gold standard, during the period between January 2017 and December 2021. All patients had at least one image to identify a hyperfunctional parathyroid gland with PTA using ^{99m}Tc-Sestamibi SPECT/CT and ^{18}F-FCH PET/CT scan. Lab tests, age and gender of the patient were also involved. A total of 40 patients fulfilled the inclusion criteria. Patients who had previously undergone thyroid surgery and patients who had other pathologic conditions that could modify phosphocalcic metabolism, such as progressive neoplasia, multiple endocrine neoplasia, sarcoidosis, hyper- or hypovitaminosis D, or chronic renal failure, were excluded. Also, patients with renal stones, hypothyroidism, and osteoporosis were excluded.

^{99m}Tc-sestamibi SPECT/CT

An early parathyroid scan was obtained 15 minutes after ^{99m}Tc-sestamibi (555 MBq15mCi) injection. After 2 hours and 30 minutes after the injection, another delayed parathyroid scan was obtained. Compared to the background, the parathyroid scan positive result was elevated ^{99m}Tc-sestamibi uptake on the delayed image. Immediately after the delayed ^{99m}Tc-sestamibi scan, a delayed ^{99m}Tc-sestamibi SPECT/CT scan was performed using a Hawkeye 4 apparatus(GE Healthcare, USA).For 10 and 20 minutes, CT and SPECT images were taken. CT images were obtained using a standard filter with 512×512 matrices, 2.5mA, 140kV, and 5.0mm slice thickness. SPECT images were acquired with 128×128 matrices, 1.59 zoom, with step-and-shoot scan mode. Automatically by intrinsic software, the CT and SPECT images were fused. A positive result for ^{99m}Tc-sestamibi SPECT/CT was a focal elevated uptake lesion rather than the surrounding thyroid gland. ^{99m}Tc-sestamibi SPECT/CT images were assessed in the blinded condition by physicians' agreement.

^{18}F-FCH PET/CT

^{18}F-FCH PET/CT was taken one hour after intravenous injection of ^{18}F-FCH (150-185 MBq) using a Discovery PET/CT 710 Elite imager (GE Healthcare, USA). The strategy was in the following order: CT tomogram, low-dose attenuation-correction CT scan, PET acquisition, and additional IV contrast-enhanced diagnostic CT scan. Instead of the attenuation-correction CT scan, a diagnostic CT scan was obtained if the contrast medium was contraindicated. The acquisition protocol included a 12 0kV tension, display field-of-view of 70 cm, 1.25 mm interval, 2.5 mm thickness, and automatic mA regulation. The acquisition was centered on the cervicothoracic region. With the Q-Clear algorithm (GE Healthcare), PET image iterative reconstruction was performed to improve the signal-to-noise ratio using a β of 600. Contrast-enhanced CT permits more precise anatomic localization; thus, iodinated contrast medium was injected 80 seconds before the CT acquisition for fused-image analysis and to optimize the CT. Mediastinal or neck hyper uptake matching a scanner image of hyperplasia or adenoma was detected. SUVmax adjusted for lean body mass was determined to quantify the uptake intensity.

Statistical analysis was performed using statistical software package SPSS version 20.0 (Armonk, NY: IBM Corp.) and GraphPad Prism (version 8.0; GraphPad Prism Software Inc., San Diego, CA, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Receiver operating characteristic (ROC) curve analyses were performed. We calculated the sensitivity, specificity, positive predictive value, and negative predictive value to determine the diagnostic value of signs. A probability value of $P<0.05$ was considered statistically significant.

Results

Forty patients (7 males and 33 females) underwent histopathological examination and all imaging studies; 27 had histopathologically confirmed PTA, and 13 were without PTA. There was no significant difference between patients with and without PTA, except for PTH (143 [40-185] vs. 44 [29-121] ng/L, *P*=0.000) (Table 1).

Table 1.

Characteristics of patients.

Variable	Patients		*P*-value
	With PTA	Without PTA	
Number	27	13	
Male/Female	4/23	3/10	0.235
Age, years	48.4±16.3	47.9±15.3	0.314
Weight, kg	74.1±20.6	75.1±22.3	0.216
Height, m	155.7±11.7	160.1±16.1	0.344
Calcium, mmol/L	2.57±0.34	2.31±0.6	0.092
PTH, ng/L	143 (40-185)	44 (29-121)	0.000

^{99m}Tc-sestamibi SPECT/CT (Figure 1) and ^{18}F-FCH PET/CT (Figure 2) examinations were performed for each patient, and PTH levels and histologic findings were recorded. The diagnostic ability of ^{18}F-FCH PET/CT (Figure 3A; AUC=0.720; P=0.029) was superior to ^{99m}Tc-sestamibi SPECT/CT (Figure 3B; AUC=0.623; P=0.214) in identifying PTA. The sensitivity of ^{18}F-FCH PET/CT was significantly (*P*<0.05) higher than that of ^{99m}Tc-sestamibi SPECT/CT (conventional imaging method), with specificity similar to that of ^{99m}Tc-sestamibi SPECT/CT. Diagnostic performances of both methods are given in Table 2. In contrast to ^{99m}Tc-sestamibi SPECT/CT (*P*=0.252), ^{18}F-FCH PET/CT findings were correlated significantly (*P*=0.023) with PTH results (Table 3).

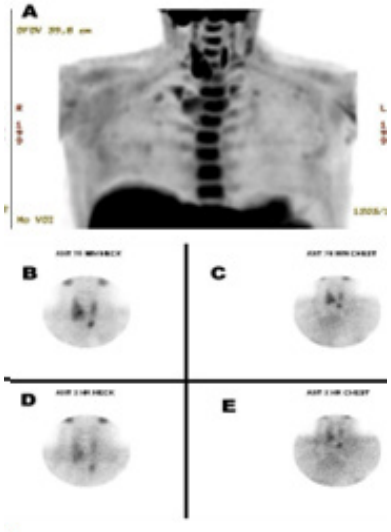

Fig. 1. *^{99m}Tc-sestamibi SPECT/CT.*

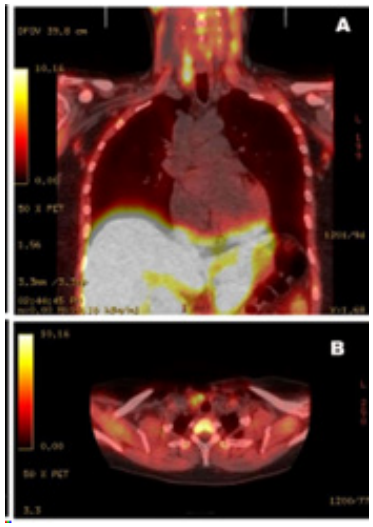

Fig 2. *^{18}F-FCH PET/CT.*

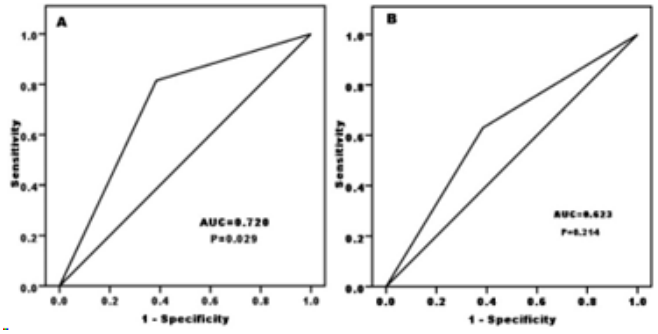

Fig. 3. *(A):^{18}F-FCH PET/CT (AUC=0.720, P=0.029); (B): ^{99m}Tc-sestamibi (SPECT/CT AUC=0.623, P=0.214)*

Table 2.

Diagnostic values (%) for different imaging modalities.

Imaging method	TP	TN	FP	FN	SN	SP	PPV	NPV	Acc
^{99m}Tc-sestamibi SPECT/CT	17	8	5	10	63.0	61.5	77.3	44.4	62.5
^{18}F-FCH PET/CT	22	8	5	5	81.5	61.5	81.5	61.5	75.0

Acc, accuracy; TP, true positive; TN, true negative; FP, false positive; FN, false negative; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

Table 3.

^{99m}Tc-MIBI SPECT/CT and ^{18}F-FCH PET/CT results × PTH ng/L cross tabulation

		PTH, ng/L		*P*-value
		Positive	Negative	
		n=27	n=13	
^{99m}Tc-MIBI SPECT/CT	Positive	17	5	0.252
	Negative	10	8	
^{18}F-FCH PET/CT	Positive	22	5	0.023
	Negative	5	8	

Discussion

Many literature studies support and encourage ^{18}F-FCH PET/CT utility.[9] However, data on ^{18}F-FCH PET/CT utility in detecting and localizing PTA and HPT remain relatively sparse, and comparison of ^{18}F-FCH PET/CT to traditional imaging modalities is limited.[4]

Our ROC analysis revealed that ^{18}F-FCH PET/CT had a superior diagnostic ability (AUC=0.720; *P*=0.029) to ^{99m}Tc-sestamibi SPECT/CT (AUC=0.623; *P*=0.214) in identifying PTA. The sensitivity and accuracy of ^{18}F-FCH PET/CT (81.5% and 75.0%, respectively) were significantly (*P*<0.05) higher than that of ^{99m}Tc-sestamibi SPECT/CT (63.0% and 62.5%, respectively). In contrast to ^{99m}Tc-sestamibi SPECT/CT (*P*=0.252), ^{18}F-FCH PET/CT findings were correlated significantly (*P*=0.023) with PTH results.

Our results agree with Thanseer et al.,[10] who reported that ^{18}F-FCH PET/CT detected 52 of 54 patients with histopathologically confirmed PTA (sensitivity of 96.3%). This was superior to ^{99m}Tc-sestamibi SPECT/CT (sensitivity of 80.7%). Bossert et al. found that abnormal parathyroid gland detection rates were 71% for ^{18}F-FCH PET/CT compared to 15% and 68% for ^{99m}Tc-sestamibi scintigraphy and neck US, respectively.[11] Amadou et al.[12] evaluated ^{18}F-FCH PET/CT to guide surgery compared to other imaging modalities, including ^{99m}Tc-sestamibi SPECT/CT in patients with primary HPT and prior neck surgery. They found that ^{18}F-FCH PET/CT is a promising method in the challenging population of reoperative primary HPT patients.

More recently, Boudousq et al.,[13] in a total of 149 pathologic parathyroids, found that ^{18}F-FCH PET/CT detected 148 of 149 pathologic parathyroids with only one false-negative and 4 false-positives. The sensitivity and accuracy of ^{18}F-FCH PET/CT (99.3% and 98%) were superior to ^{99m}Tc-sestamibi SPECT/CT (65.1% and 81%), neck ultrasonography (75.2% and 84%), and their combination (89.9% and 91%). Also, Dudoignon et al.[14] compared ^{18}F-FCH PET/CT with conventional imaging in primary HPT. On a patient basis, sensitivity and accuracy for detecting abnormal parathyroid glands were 76%/76% for ^{18}F-FCH PET/CT, 33%/33% for neck ultrasonography, and 71%/71% for ^{99m}Tc-MIBI scintigraphy.

In 103 patients with primary HPT, Cuderman et al.[8] compared ^{18}F-FCH PET/CT with conventional scintigraphic methods, consisting of ^{99m}Tc-sestamibi dual-phase imaging, ^{99m}Tc-sestamibi/pertechnetate subtraction imaging and ^{99m}Tc-sestamibi SPECT/CT. The diagnostic performance of ^{18}F-FCH PET/CT surpassed these combined or separate conventional scintigraphy. Its sensitivity was 92% compared to 39%-56% for conventional imaging methods. Also, in differentiating multiple from single hyperfunctioning glands, they found that ^{18}F-FCH PET/CT was the most valuable method (sensitivity of 88%).

In addition to diagnostic values that are better than those of ^{99m}Tc-MIBI scintigraphy, ^{18}F-FCH PET/CT has other advantages that justify its systematic use in patients with clinically suspected HPT for the initial evaluation. Compared to ^{99m}Tc-MIBI scintigraphy, ^{18}F-FCH PET/CT generated a lower radiation dose and is more efficient.[13] Due to a higher spatial resolution, ^{18}F-FCH PET/CT generates better image quality and increased sensitivity and allows the detection of smaller lesions.[13,15] Also, it requires shorter acquisition times, one hour after injecting the tracer, compared to >2 hours for ^{99m}Tc-MIBI scintigraphy, and thus, the patient experiences less discomfort and spends less time under the camera.[13]

In conclusion, our results, for the first time in Saudi Arabia, clearly demonstrated that ^{18}F-FCH PET/CT is a diagnostic imaging method superior to conventional modality ^{99m}Tc-sestamibi SPECT/CT in the detection of PTA and, thus, allows for accurate preoperative localization. These findings suggested using ^{18}F-FCH PET/CT as a first-line evaluation in preference to other conventional modalities, including ^{99m}Tc-sestamibi SPECT/CT and neck ultrasonography.

Competing Interests

The authors declare that they have no competing interests.

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Comparison of Ictal SPECT with ^{99m}Tc-HMPAO versus MRI for the Epileptic Seizure Onset Zone Detection in Saudi Patients

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Abstract

Background: In the presurgical evaluation of patients with drug-resistant epilepsy, magnetic resonance imaging (MRI) and ictal brain perfusion SPECT with the chemical microspheres ^{99m}Tc-HMPAO are widely used for diagnosing the seizure onset zone (SOZ). For both modalities, there is theoretical controversy over favoring one over the other. This study aimed to compare the performance of ^{99m}Tc-HMPAO SPECT with MRI for SOZ identification in EEG-proved epileptic Saudi patients.

Methods and Results: For this observational retrospective study, the database of the nuclear medicine departments at the Prince Sultan Military Medical City (PSMMC) and King Abdullah bin Abdulaziz University Hospital (KAAUH) were searched for male and female patients with suspected unifocal epilepsy in whom ictal brain perfusion SPECT and MRI had been performed for presurgical evaluation. A total of 14 adult epileptic patients above 18 years were included who have undergone SPECT scans using 99mTc-HMPAO and MRI between Jan 2014 and Dec 2021. ^{99m}Tc-HMPAO SPECT and MRI scans were performed simultaneously for each patient, and there was almost general agreement that ^{99m}Tc-HMPAO SPECT accurately localized and detected the SOZ in 12/14 patients (sensitivity 85.71%). It was superior to MRI, which detected and localized the SOZ in only 7/14 patients (sensitivity 50.0%). Unfortunately, the specificity of ictal brain perfusion SPECT, by using ^{99m}Tc-HMPAO in seizure localization in epileptic patients, was not detected due to the shortage of data that led to all the EEG findings being 100% positive.

Conclusion: This study reported that ictal SPECT using ^{99m}Tc-HMPAO provides more valuable information about SOZ localization than MRI. However, future studies with a larger sample size are needed to assess the specificity of ^{99m}Tc-HMPAO SPECT in detecting the SOZ.**(International Journal of Biomedicine. 2024;14(1):88-92.)**

Keywords: epilepsy • seizure• perfusion • ^{99m}Tc-HMPAO • ictal SPECT

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Abbreviations

EEG, electroencephalography; **ETE**, extratemporal epilepsy; **EZ**, epileptogenic zone; **MRI**, magnetic resonance imaging; **NE**, neocortical epilepsy; **SPECT**, single photon emission computed tomography; **SOZ**, seizure onset zone; **^{99m}Tc-HMPAO,** ^{99m}Tc-labeled tracers hexamethyl propyleneamine oxim; **TLE**, temporal lobe epilepsy.

Introduction

Epilepsy is a chronic medical disorder or condition characterized by unprovoked recurrence of seizures, which are paroxysmal events owing to neuron hyperexcitability with synchronicity or abnormal neuronal discharges.[1,2] This neurological disease results in abnormal elevations of brain activity, unusual behaviors and sensations, loss of

consciousness, and repetitive seizures.[3] Globally, with no socio-demographic boundary, it affects about 50 million individuals, and former reports have estimated epilepsy point prevalence to be 4-10/1000 individuals.[4,5]

In cases with suspected focal epilepsy, brain surgery is a therapeutic choice for patients who do not respond efficiently to drug treatment.[6,7] To all patients with focal epilepsy, it was concluded that "assessment for surgical selection should be offered where the first 2 antiepileptic drugs have failed."[7,8] The aim of epilepsy surgery is to remove the epileptogenic zone (EZ), defined as the cortex area that needs to be disconnected or removed to completely abolish seizures.[9] For surgery planning, since the EZ clearly is not an operational concept, the combination of 5 varied operationally detected cortical zones based on noninvasive tools is usually used in presurgical evaluation:[10] 1) the "functional deficit zone" that is not functioning normally in the interictal period and can be derived by neuropsychological and neurological evaluation, 2) the "epileptogenic lesion" defined as a single discrete macroscopic lesion causing the seizures that is visible on the structural MRI, 3) the "symptomatogenic zone" that can be localized by initial seizure semiology and that causes the initial ictal symptoms, 4) the "irritative zone" that generates interictal spikes and can be localized by interictal scalp-EEG, and 5) the SOZ that initiates seizures and can be localized by ictal scalp-EEG.(7) If all 5 zones can be reliably delineated and all point to the same brain region as EZ, additional presurgical investigation is usually not required.[2] If one or more of the zones point to different brain regions or cannot be reliably detected, more investigations might be helpful, including nuclear imaging,[11,12] to localize the SOZ or to plan the placement of intracranial EEG electrodes to achieve this aim.[7]

^{99m}Tc-HMPAO brain SPECT is a nuclear imaging tool commonly used in epilepsy cases for presurgical evaluation with discrepant or uncertain standard pointers.[13,14] ^{99m}Tc-HMPAO, after intravenous injection, is fully extracted from arterial blood (like a chemical microsphere) to tissue through a single capillary passage and then is locally retained in the tissue.[7] In tissue, its fixation is because of glutathione-dependent metabolism to hydrophilic forms and binding to non-diffusible cell components.[15] The aim of the ictal brain SPECT is to detect SOZ by its regional hyperperfusion.[16] Compared to regional hypoperfusion between seizures, regional hyperperfusion is not only more sensitive for SOZ detection, but is also more specific, specifically in cases with any lesion types (including non-epileptogenic lesions) that may, independent of epileptic activity, cause regional hypoperfusion.[7,17]

Against this introduction, the aim of the current study was to evaluate and compare the performance, effectiveness, and diagnostic accuracy of ictal brain perfusion SPECT with ^{99m}Tc-HMPAO and MRI in localizing the SOZ in epileptic Saudi patients.

Materials and Methods

For this observational retrospective study, the database of the nuclear medicine departments at the Prince Sultan Military Medical City (PSMMC) and King Abdullah bin Abdulaziz University Hospital (KAAUH) were searched for male and female patients with suspected unifocal epilepsy in whom ictal brain perfusion SPECT and MRI had been performed for presurgical evaluation. All patients were diagnosed with EEG, and patients were excluded when the tracer injection latency, after electrical seizure onset, was >120 seconds.[18] Also, any adolescent or pediatric epileptic patients and any patients who have had SPECT scans without MRI scans were excluded. A total of 14 adult epileptic patients above 18 years were included who have undergone SPECT scans using ^{99m}Tc-HMPAO and MRI between Jan 2014 and Dec 2021.

Sample size

The statistical calculation was done by using an online calculator.[19] The obtained theoretical sample size, a 95% confidence interval, and a total population size of 30 from multiple hospitals were inserted in the online calculator. Therefore, according to the online calculator, the theoretical sample size is 28 epileptic patients who have undergone a brain perfusion Ictal ^{99m}Tc-HMPAO SPECT

Ictal brain perfusion SPECT

While the patient was undergoing video-EEG monitoring, ictal tracer injections were administered during a seizure in the inpatient epilepsy unit. The patient was transported to a quiet and dimly lit room. Within one hour, patients were positioned supine and their heads secured to the table to ensure all the brain and cerebellum were included in the image.[20] SPECT was acquired with a double-head gamma camera (Siemens Symbia T2, Germany) equipped with low-energy ultra-high-resolution collimators and angular steps of 2.8-3.0°. The total acquisition time was around 30 minutes, and the rotation radius was 15.3±1.6cm. Five million count events, or more, should be detected. Furthermore, SPECT images are reconstructed by iterative reconstruction using a low pass (e.g. Butterworth) filter with the brain processing protocol, generating the trans-axial, sagittal, and coronal slices.[20] The resulting SPECT images were normalized stereotactically using the statistical parametric mapping software (version SPM12). All images were interpreted twice.

Magnetic resonance imaging

This study used MRI as another non-invasive method to detect epilepsy. Sequences of MRI images were combined to enhance sensitivity and specificity in identifying probable anatomical abnormalities that are caused by seizure disorders. MRI protocol, along with the procedures for adults with epilepsy, was done at 3T. If the physicians viewed the tumor, they gave contrast and did pre- and post-T1 AX (COR T2 / FLAIR perpendicular to the long axis of the hippocampus). Pulse sequence was: 1) Coronal T2 whole brain 2mm/0.4, 2) Coronal FLAIR, 3) Axial T1 3D with reformats, 4) Axial DWI, 5) Axial GRE (SWI), 6) Axial T2, 7) Axial FLAIR and 8) Coronal (optionally).

Statistical analysis was performed using statistical software package SPSS version 21.0 (Armonk, NY: IBM Corp.). Baseline characteristics were summarized as frequencies and percentages for categorical variables. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Sensitivity, Specificity, and Predictive Values for the study tests were calculated.

Results

Baseline characteristics of the study cases are presented in Table 1. ^{99m}Tc-HMPAO SPECT and MRI scans were performed simultaneously for each patient, and there was almost general agreement that ^{99m}Tc-HMPAO SPECT accurately localized and detected the SOZ in 12/14 patients (sensitivity 85.71%; Table 2). It was superior to MRI, which detected and localized the SOZ in only 7/14 patients (sensitivity 50.0%; Table 3).

Table 1.

Baseline characteristics of the study patients.

Variable	Value
Total number of patients	14
Gender (male/female)	8/6
Age	32.57±14.86
Height (cm)	160.14±8.97
Weight (kg)	71.54±17.03
Positive EEG (number/percentage)	14 (100%)

Table 2.

Cross-tabulation of the EEG × ^{99m}Tc-HMPAO SPECT results

			^{99m}Tc-HMPAO SPECT	
			Positive	Negative
			n=12	n=2
EEG	Positive	n=14	12	2
	Negative	n=0	0	0

Table 3.

Cross-tabulation of the EEG × MRI scan results.

			MRI	
			Positive	Negative
			n=7	n=7
EEG	Positive	n=14	7	7
	Negative	n=0	0	0

Most of the patients (6/7) detected by MRI were also detected accurately by ^{99m}Tc-HMPAO SPECT. But ^{99m}Tc-HMPAO SPECT detected 6/7 cases that MRI did not detect (Table 4).

Table 4.

Cross-tabulation of the ^{99m}Tc-HMPAO SPECT × MRI scan results.

			^{99m}Tc-HMPAO SPECT	
			Positive	Negative
			n=12	n=2
MRI	Positive	n=7	6	1
	Negative	n=7	6	1

Discussion

In the epileptic brain, SPECT, PET, and MRI are all used to image abnormalities.[21,22] Comparison of these modalities is difficult because they assess different aspects of the epileptic process, perfusion, metabolism, and structure.[23] In the localization of epileptogenic foci, these methods have been studied extensively as individual techniques, but only a few comparative studies have been performed.[24] Few studies have been conducted to compare these techniques in the presurgical diagnosis of lesional and nonlesional epilepsy.[21,25,26] SPECT with ^{99m}Tc-HMPAO can help in localizing the SOZ, as epileptic activity is related to a significant elevation in blood flow in affected cortical areas.[27] This study aimed to compare the diagnostic performance and clinical utility of ^{99m}Tc-HMPAO SPECT with MRI in localizing the SOZ in epileptic Saudi patients, all of which are positive (100%) epileptic diagnosis and verified by EEG.

The primary finding of the current study is that ^{99m}Tc-HMPAO SPECT (sensitivity 85.71%) was superior to MRI (sensitivity 50.0%) in presurgical detecting and localizing of the SOZ in epileptic Saudi patients. Unfortunately, the specificity of ictal brain perfusion SPECT, by using ^{99m}Tc-HMPAO in seizure localization in epileptic patients, was not detected due to the shortage of data that led to all the EEG findings being 100% positive.

Our results agreed with previous studies. Lee et al.[25] evaluated the sensitivity of ^{99m}Tc-HMPAO SPECT for localizing the SOZ among 40 patients (17 with temporal lobe epilepsy (TLE) and 23 with neocortical epilepsy (NE)). They reported that ^{99m}Tc-HMPAO had 89% sensitivity in localizing the TLE and 70% sensitivity among patients with neocortical epilepsy. A review by Spencer et al.[23] suggested that compared to MRI, SPECT has higher sensitivity in extratemporal epilepsy (ETE) detection (60% vs. 43%). Also, SPECT was superior to MRI in TLE diagnosis (66% for SPECT and 55% for MRI).[23] They suggested that the highest diagnostic specificity and sensitivity were obtained by ictal imaging with SPECT (81% in ETE, 90% in TLE). In patients with occipital lobe epilepsy, ^{99m}Tc-HMPAO ictal SPECT was reported to be helpful in lateralizing EZs, even in patients with ambiguous MRI results.[28]

A study by Won et al.[24] included 118 patients who performed surgery for intractable epilepsy, and they retrospectively compared the ability of MRI with ictal ^{99m}Tc-HMPAO SPECT and invasive EEG to localize the epileptogenic focus. Results revealed that MRI was concordant with ictal SPECT in 58% of patients. Using pathologic diagnosis as the standard, MRI and ictal SPECT correctly lateralized the lesion in 72% and 73% of patients, respectively. Among patients who underwent EEG, MRI was concordant with EEG in 47% compared to 58% in the case of ictal SPECT. Among patients with normal MRI results, ictal SPECT accurately lateralized lesions in 55%.[24]

There were some limitations of the study that should be mentioned. This study was retrospective, which led to a lack of data on some patient variables. To avoid retrospective inclusion-related potential selection bias, only very liberal eligibility criteria were applied. Another limitation is the

small number of cases. This is because, in the hospitals of Riyadh, researchers expect that the data will be collected from another ictal detecting technique known as PET. Thus, the data are only collected from one hospital containing a few cases (n=14), which restricted this study from reaching the calculated sample size 28. Finally, the specificity could not be calculated due to the fact that the EEG data did not exhibit negative results for epilepsy, perhaps because of the shortness of sample size.

Conclusion

This study reported that ictal SPECT using ^{99m}Tc-HMPAO provides valuable information about SOZ localization with higher sensitivity, 85.17%, and diagnostic accuracy, 85.17%, compared to MRI. Although invasive EEG detected 100% of patients, ^{99m}Tc-HMPAO SPECT is not a redundant method, and combining refined imaging techniques findings holds great promise in epilepsy diagnosis and localization corresponding to MRI. Future studies with a larger sample size are needed to assess the specificity of ^{99m}Tc-HMPAO SPECT in detecting the SOZ, and more studies should be considered.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

The ethical approval of the study protocol was obtained from the Institutional Review Board (IRB) at Princess Nourah bint Abdulrahman University. All data were gathered from patients' medical records, and all patients' information was confidentially coded.

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Assessment of Sperm Morphometry in Evaluating Male Infertility

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Abstract

Background: Infertility is a complex issue affecting 15% of couples of reproductive age, with men accounting for 40%-50% of infertility cases. Semen analysis comprises various descriptive measures of sperm and seminal fluid to determine semen quality. Transforming qualitative descriptions of sperm deformities and shape changes into quantitative terms can aid in identifying sub-visual abnormalities. This study aimed to evaluate sperm morphometry parameters in both infertile and fertile men.

Methods and Results: The study enrolled a total of 101 participants, divided into three groups: Group A included 38 subfertile patients with varicocele, Group B included 33 patients with idiopathic infertility (23 with asthenozoospermia and 10 with oligozoospermia), and Group C (the control group) included 30 healthy fertile men. The mean age of patients was 31.6±5.81, 31.3±6.0, and 29.47±4.27 years in Groups A, B, and C, respectively (P>0.05). Scrotal duplex examinations were performed to identify the presence of varicocele. Semen samples were collected following WHO Manual (2010). Semen dynamic and morphological analyses were conducted using CASA (Computer-Assisted Semen Analysis, MIRALAB, ISO9001, ISO13485). We found that sperm concentration, total sperm count, sperm progressive motility, and sperm progressive+non-progressive motility were significantly lower in Group A and Group B than in Group C (P=0.000 in all cases); however, there were no differences between Group A and Group B regarding these parameters. The sperm morphology index was significantly lower in Group A than in Group C (P=0.0024); no differences were found between Group B and Group C and Group B and Group A. The mean value of the sperm deformity index was significantly lower in Group A than in Group C (P=0.004).

Conclusion: Our study highlights the significant association between sperm morphology and male infertility in varicocele and idiopathic subfertile males.**(International Journal of Biomedicine. 2024;14(1):93-98.)**

Keywords: infertility • semen quality • varicocele

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Abbreviations

MAI, multiple anomalies index; **TZI**, teratozoospermia index; **SDI**, sperm deformity index.

Introduction

Infertility is a condition of the reproductive system characterized by the inability to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual activity. Infertility is a complex issue affecting 15% of couples of reproductive age,[1] with men accounting for 40%-50% of infertility cases.[2,3] Various factors, including occupational hazards, exposure to reproductive toxicants, chemotherapy, radiation therapy, heat exposure, physical labor, lifestyle variables (wearing tight underwear, poor diet), genital injuries, hereditary traits, testicular maldescent, infections, and iatrogenic causes, can contribute to decreased male fertility.[4-6]

The most prevalent form of male infertility is idiopathic male infertility, which is characterized by the presence of one or more abnormal semen parameters without a clear explanation.[7] Following closely is varicocele, accounting for 35% to 50% of men with primary infertility and up to 81% with secondary infertility.[8] The negative impact of varicocele on spermatogenesis can be attributed to several factors, including elevated testicular temperature, increased intratesticular pressure, hypoxia due to reduced blood supply, reflux of toxic compounds from the adrenal glands, and hormonal profile abnormalities.[9,10]

Semen analysis involves a set of descriptive measurements of spermatozoa and seminal fluid parameters used to assess semen quality.[11] Determining sperm morphology, however, poses challenges due to subjective factors and inconsistency. A comprehensive assessment of sperm shape necessitates an evaluation of the head, neck, midsection, and tail. In normal sperm, the head should be oval and symmetrical, and tail insertion should be axial, in line with the long axis of the head. Abnormal sperm variations include those with oversized, undersized, round, asymmetrical, or amorphous heads, as well as those with tapering, bulging midpieces, multiple heads or tails, or amorphous heads.[12] Typically, clinical laboratories apply sperm morphology parameters established by the WHO or the "strict morphology" criteria developed by Dr. Kruger.[13]

To enhance the quantitative identification of sperm shape, morphometric methods to measure sperm under normal conditions can establish a reference for quantitative terms, replacing qualitative descriptions with more precise numerical terms. Quantitative stereological methods allow investigators of seminal samples to derive three-dimensional concepts from two-dimensional microscopic fields. This enriches the biophysical assessment of sperm by calculating absolute and relative volumetric parameters, which conventional microscopic assessment cannot provide.[14,15] Converting qualitative descriptions of sperm deformities and shape changes into quantitative numerical terms can be particularly valuable in identifying sub-visual shape changes and abnormalities.[16,17] Using quantitative numerical descriptions for qualitative characteristics can facilitate the comparison of different treatment modalities and determine their respective advantages. Mathematical descriptions of sperm movement allow for a more precise expression of the type of movement and velocity, which can be challenging to convey using ambiguous qualitative terms.[18,19]

A recent study by Rrumbullaku et al.[20] demonstrated a significant increase in the percentage of tapered spermatozoa, spermatozoa containing cytoplasmic droplets, and spermatozoa with bent tails in varicocele patients, compared to controls. In our study, we evaluated sperm morphometry in varicocele, non-varicocele infertile patients, and controls using Computer-Assisted Semen Analysis (CASA, MIRA LAB, ISO9001, ISO13485). This approach promises to provide a more accurate and quantitative assessment of sperm morphology, shedding light on potential sub-visual abnormalities and shape changes that could be contributing to male infertility.

This study aimed to evaluate sperm morphometry parameters in both infertile and fertile men.

Materials and Methods

Study Setting and Participants

This prospective study took place at the Andrology Unit of Alazhar University Hospital (Assiut) and was conducted with the approval of the relevant authorities. Informed consent was obtained from all participants. The study enrolled a total of 101 participants, divided into three groups: Group A included 38 subfertile patients with varicocele, Group B included 33 patients with idiopathic infertility (23 with asthenozoospermia and 10 with oligozoospermia), and Group C (the control group) included 30 healthy fertile men.

Data Collection

Participants underwent a comprehensive assessment, including the following aspects:

History: This included information such as patient age, age of puberty onset, age of varicocele onset (if applicable), sexual history, number of children, lifestyle habits (smoking, alcohol, drug use), medical history (using cytotoxic, teratogenic, or antiandrogen drugs), surgical history, spinal cord trauma, prostatectomy, sexually transmitted diseases, and epididymitis or epididymo-orchitis.

Examination: General and genital examinations were conducted, encompassing secondary sexual characteristics, body musculature, tall span index, gynecomastia, and body mass index, as well as a thorough examination of the penis, scrotum, epididymis, vas deferens, and spermatic cord.

Scrotal Duplex: Scrotal duplex examinations were performed to identify the presence of varicocele.

Semen Analysis: Semen samples were collected following WHO Manual (2010), with a recommended abstinence period of 2-5 days. Samples were collected by masturbation in sterile containers without the use of lubricants or soap. Samples were incubated at 37°C until complete liquefaction occurred (30-60 minutes). Dynamic and morphological analyses were conducted using CASA (Computer-Assisted Semen Analysis, MIRALAB, ISO9001, ISO13485) to assess sperm parameters.

Exclusion Criteria: Patients with conditions such as erectile dysfunction, benign prostatic hyperplasia, psychological disorders, genetic sex disorders, azoospermia, necrozoospermia, severe debilitating diseases, malnutrition, or use of cytotoxic, teratogenic, or antiandrogen drugs were excluded from the study.

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY:

IBM Corp). For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Multiple comparisons were performed with one-way ANOVA and Tukey HSD post-hoc test. Group comparisons with respect to categorical variables are performed using chi-square tests A probability value of $P<0.05$ was considered statistically significant.

Results

The mean age of patients was 31.6±5.81, 31.3±6.0, and 29.47±4.27 years in Groups A, B, and C, respectively ($P>0.05$). Primary infertility was diagnosed in 27(71.1%) patients of Group A and 21(63.6%) patients of Group B (Table 1). In Group A, the majority of patients had varicocele grade II (55.3%), followed by grade III (39.5%) and grade I (5.3%) ($P=0.0006$) (Table 2). In Group B, 10(30.3%) patients had oligoasthenozoospermia, and 23(69.7%) patients had asthenozoospermia.

Table 1.

Type of infertility in the patients of the study groups.

Type of infertility	Group A (n= 38)	Group B (n=33)	Statistics
Primary	27 (71.1%)	21 (63.6%)	$\chi2=0.444$ df=1 P=0.505
Secondary	11 (28.9)	12 (14.1%)	

Table 2.

Distribution of patients in Group A according to the varicocele grade.

Grade of varicocele	Group A (n=38)	Statistics
Grade I	2 (5.3)	$\chi2=14.895$ df=2 P=0.0006
Grade II	21 (55.3%)	
Grade III	15 (39.5%)	

In terms of semen parameters, the mean semen volume was significantly lower in Group A than in Group C (2.68±0.99 mL vs. 3.31±1.05 mL, $P=0.0219$); however, mean pH did not show significant differences between study groups, but the mean liquefaction time of semen was slightly higher in Groups A and B than in Group C, without statistical significance (Table 3). We found that sperm concentration, total sperm count, sperm progressive motility, and sperm progressive+non-progressive motility were significantly lower in Group A and Group B than in Group C; however, there were no differences between Group A and Group B regarding these parameters (Table 4). The sperm morphology index was significantly lower in Group A than in Group C ($P=0.0024$); no differences were found between Group B and Group C and Group B and Group A (Table 5). According to the anatomic-morphological characteristics of sperm, Group A was characterized by significantly smaller dimensions of the length and width of the head, its area, and perimeter, as well as the acrosome coverage, compared to both Group C and Group B (Table 6). The mean value of MAI and TZI did not significantly differ between study groups ($P=0.2573$ and $P=0.2480$, respectively). However, the mean value of SDI was significantly lower in Group A than in Group C ($P=0.004$) (Table 7).

Table 3.

Semen analysis (macroscopic examination) in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Volume, mL	2.68±0.99	3.17±0.81	3.31±1.05	F=4.2025 P=0.0177 P_{1-2}=0.0837 P^{1-3}=0.0219 P_{2-3}=0.8303
pH	7.51±0.12	7.52±0.11	7.50± 0.11	F=0.2426 P=0.7851
Liquefaction time, min	27.89±14.73	28.21±11.49	21.73±5.17	F=3.1638 P=0.0466 P_{1-2}=0.9924 P^{1-3}=0.0783 P_{2-3}=0.0715

Table 4.

Semen analysis (microscopic examination) in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Sperm concentration, 10^6/ml	23.63±24.97	20.73±24.02	55.88±22.71	F=20.7786 P=0.0000 P_{1-2}=0.8678 P^{1-3}=0.0000 P_{2-3}=0.0000
Total sperm count, 10^6/ml	62.57±76.51	79.81±110.29	178.79±88.66	F=14.8199 P=0.0000 P_{1-2}=0.7128 P^{1-3}=0.0000 P_{2-3}=0.0001
Progressive motility, %	18.39±18.01	14.36±13.44	52.91±12.63	F=61.7070 P=0.0000 P_{1-2}=0.5041 P^{1-3}=0.0000 P_{2-3}=0.0001
Progressive + non-progressive motility, %	32.18±23.04	26.05±15.57	69.08±13.70	F=50.7048 P=0.0000 P_{1-2}=0.3411 P^{1-3}=0.0000 P_{2-3}=0.0001

Table 5.

The sperm morphology index in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Sperm morphology index, %	23.18±18.63	28.16±13.40	35.93±11.58	F=5.9615 P=0.0036 P_{1-2}=0.3543 P^{1-3}=0.0024 P_{2-3}=0.1095

Table 6.

The anatomic-morphological characteristics of sperm in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Head length, µm	3.60±2.04	4.74±0.23	4.78±0.21	F=9.9490 P=0.0001 P_{1-2}=0.0008 P_{1-3}=0.0007 P_{2-3}=0.9913
Head width, µm	2.27±1.29	2.95±0.15	2.94±0.15	F=8.4143 P=0.0004 P_{1-2}=0.0016 P_{1-3}=0.0026 P_{2-3}=0.9520
Length/ width ratio	1.22±0.69	1.62±0.10	1.63±0.09	F=10.4762 P=0.0001 P_{1-2}=0.0005 P_{1-3}=0.0005 P_{2-3}=0.9968
Head area, μm^2	8.44±4.80	11.00±0.84	11.13±0.70	F=8.9930 P=0.0003 P_{1-2}=0.0016 P_{1-3}=0.0012 P_{2-3}=0.9839
Head perimeter, µm	9.81±5.55	12.87±0.55	12.93±0.44	F=7.5856 P=0.0009 P_{1-2}=0.0009 P_{1-3}=0.0254 P_{2-3}=0.5975
Acrosome coverage, %	29.86±19.43	38.97±8.56	33.72±13.69	F=3.3092 P=0.0407 P_{1-2}=0.0311 P_{1-3}=0.5405 P_{2-3}=0.3464

Table 7.

Sperm morphology indices in the study groups.

	Group A (1)	Group B (2)	Group C (3)	Statistics
MAI	2.01±0.37	2.11±0.33	1.97±0.34	F=1.3764 P=0.2573
TZI	1.06±0.15	1.05±0.14	1.01±0.06	F=1.4142 P=0.2480
SDI	0.73±0.24	0.68±0.20	0.57±0.13	F=5.5087 P=0.0054 P_{1-2}=0.5454 P_{1-3}=0.0040 P_{2-3}=0.0787

Discussion

Varicocele is a common condition found in 15% of the general population and 19%-41% of infertile males,[1-3,21] making it the second most prevalent cause of infertility after idiopathic infertility. Despite the considerable frequency of varicocele in subfertile individuals and proven spermatogenic failure, the specific mechanisms behind varicocele's negative impact on fertility remain unclear.[22] Nevertheless, it affects all sperm characteristics, including count, motility, and morphology.[23]

Sperm morphology, a reflection of intricate cellular changes during spermiogenesis, has been identified by some experts as a particularly robust predictor of fertility.[12,24] This association between sperm morphology and fertility has been established in numerous species, emphasizing the critical role of sperm morphology in fertility assessment.[25] Beyond mere motility, sperm morphology encapsulates vital genetic and DNA characteristics.[26]

Our investigation uncovered a strong link between infertility and sperm morphology across three distinct groups: healthy fertile males, subfertile individuals with varicocele, and those with idiopathic infertility. Healthy fertile males show normal semen characteristics (volume, count, motility, and morphology), as reported by Aziz et al.[27] and Ahmad et al.[28] Based on semen characteristics and the existence of a varicocele, subfertile individuals were divided into groups, as previously investigated by Pasqualotto et al.[29] and Blumer et al.[30]

We found that sperm concentration, total sperm count, sperm progressive motility, and sperm progressive+non-progressive motility were significantly lower in patients with varicocele and idiopathic infertile males than in healthy fertile controls (P=0.000 in all cases). This aligns with findings reported by Vivas-Acevedo et al.,[31] highlighting decreased sperm motility in infertile males with varicoceles. However, it is worth noting that Saleh and Agarwal[32] found no substantial disparities in sperm motility between infertile males and fertile controls.

Furthermore, our study revealed that the mean sperm morphology index was significantly lower in subfertile patients with varicocele than in healthy fertile men (P=0.0024). These observations align with the results of Tawadrous et al.,[33] Mostafa et al.,[34] and Vivas-Acevedo,[35] who reported similar findings.

Conversely, the WHO study indicated that infertile males with varicocele exhibited reduced sperm concentration but did not provide specific evidence concerning motility and morphology.[36] Some researchers postulate that the observed low sperm concentration may be attributed to the elevated rate of germ apoptosis often found in men. In contrast, diminished motility may be linked to a high concentration of reactive oxygen species or anti-sperm antibodies.[37,38]

Semen analysis normally evaluates only the dimensions of the sperm head (WHO, 1999)[12] because head morphological anomalies significantly affect male fertility.[39] However, despite WHO recommendations to consider additional aspects of sperm morphology, little attention has been given to the diameters of the midpiece and flagellum.[40]

Our study revealed significant deviations in head lengths, perimeters, and acrosome coverage in patients of the studied groups. Subfertile patients with varicocele were characterized by significantly smaller dimensions of the length and width of the head, its area, and perimeter, as well as the acrosome coverage than in fertile men.

These findings echo the results of Vazquez Levin[41] and Schatte,[42] who identified a lower frequency of morphologically normal forms in varicocele patients when stringent criteria were applied. In contrast, Saleh and Agarwal[32] observed no significant differences in sperm morphology between infertile individuals and fertile controls. MacLeod in 1965[43] identified the "stress pattern," characterized by elongated tapering sperm heads and amorphous spermatozoa linked with varicocele.

However, Rodrigues-Rigau et al.[44] found no notable changes in sperm shape between males with and without varicocele. WHO (1999) also observed a substantial negative correlation between average head length and the proportion of sperm with "normal" morphology.[12]

Our study also revealed a significantly reduced sperm deformity index in subfertile patients with varicocele, compared to fertile men, suggesting that increased abnormality in head length and perimeters is one of the possible causes of infertility due to varicocele. Wang et al.[45] observed that a 1°C increase in testicular temperature inhibits spermatogenesis by 14%, resulting in a drop in sperm production. Additionally, exposure to extreme temperatures alters the shape of sperm, resulting in a rise in sperm with aberrant morphology. Within 6-8 months of exposure to high temperatures, the average percentage of sperm with aberrant morphology increases from 30% to 60%. The researchers hypothesized that heating the testes decreased the quantity and the quality of sperm production.[45] Activation of the *p53* gene, a tumor-suppressor gene expressed in testes, is a well-known mechanism for explaining spermatogenic dysfunction caused by heat.[46,47] It is most highly expressed in pachytene spermatocytes.[48] High scrotal temperatures result in condensation of nuclear chromatin, which activates *p53* and halts the cell cycle. This hinders the clonal expansion of germ cells with DNA damage. Morgentaler et al.[49] hypothesized that *p53* may be involved in heat-induced germ-cell death. *p53* is situated on the nuclear membrane of normal germ cells and is responsible for germ-cell quality control. With heat-induced nuclear damage, it translocates to the nucleoplasm and triggers germ-cell death.[50]

In conclusion, our study highlights the significant association between sperm morphology and male infertility in varicocele and idiopathic subfertile males. Further research is needed to explore the relationship between sperm morphometry, sperm function, and fertility across different species. Additionally, understanding the therapeutic implications of sperm morphology could aid in selecting semen samples with the least aberrant morphometry for subfertile men.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Andrology

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Preoperative and Intraoperative Scrotal Duplex Ultrasound in the Assessment of Varicocele: A Comparative Study

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Abstract

Background: This study aimed to compare the accuracy of preoperative and intraoperative scrotal duplex ultrasound (SDU) in assessing varicocele, a common cause of male subfertility.

Methods and Results: The study was conducted on 20 male patients scheduled for subinguinal varicocelectomy at a tertiary care hospital from March 2022 to March 2023. We used grey scale ultrasound and Doppler ultrasound to evaluate testicular size, vein diameter, arterial flow parameters, and retrograde flow. We found that the preoperative SDU was a good and accurate method for assessing the grading of varicocele, and there was no significant difference in vein diameter or venous reflux between preoperative and intraoperative assessments. However, we observed a significant increase in the number of veins and testicular volume during intraoperative SDU compared to preoperative SDU.

Conclusion: Scrotal duplex ultrasound is a reliable tool for determining testicular volume and provides objective, accurate, and reproducible measurements of testicular volume.**(International Journal of Biomedicine. 2024;14(1):99-103.)**

Keywords: varicocele • infertility • varicocelectomy • scrotal duplex ultrasound

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Abbreviations

SDU, scrotal duplex ultrasound; **CDU**, color Doppler ultrasound

Introduction

Varicocele is an uncontrolled growth and dilation of the scrotal pampiniform plexus that drains both testes. Varicoceles are clinically important and the most prevalent cause of aberrant semen assay, decreased motility, abnormal shape, and a low number of sperms.[1] Varicocele is a prevalent issue in the field of reproductive medicine. It affects about 15% of healthy males and up to 35% of males with primary subfertility. About 75% of males with secondary subfertility are affected by it.[2]

The exact cause of varicoceles is unknown, but this disorder may be caused by blood flowing backward in the internal spermatic vein, which causes swollen veins that can be felt in the scrotum.[3] Typically, varicoceles are asymptomatic soft swelling on the scrotal left side, if big enough, the patient may characterize varicocele as a “bag of worms”. Bilateral and varicoceles on the right side are possible. Patients may experience heaviness or discomfort in the scrotum. Varicoceles are typically identified during an infertility examination.[4]

Large varicoceles have a ‘bag of worms’ look and are easily recognized. Medium varicoceles are palpable without the patient pressing down. Small varicoceles are seen with a powerful Valsalva maneuver.[5] Following physical examination, varicocele can be confirmed by duplex

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ultrasound, which shows pampiniform plexus vein dilatation (with a diameter typically greater than 3 mm).[6]

Ultrasonography is the examination of choice for investigating varicoceles; it is considered the most practical and the most accurate noninvasive technique that permits precise diagnosis of varicoceles (even subclinical type). Duplex ultrasound can be done at rest and during the Valsalva maneuver.[7] The scrotal veins' dilatation, direction, and flow augmentation during the Valsalva maneuver and period of reflux are evaluated. In this way, color Doppler ultrasound (CDU) is useful in identifying patients for varicocele surgery.[8]

Varicocele can be evaluated as follows: Grade 1, mild reflux (2s) during the Valsalva maneuver; Grade 2, reflux (>2s) during the Valsalva maneuver, but not continuous; and Grade 3, reflux at rest or constantly throughout the Valsalva maneuver.[9]

Varicocele, according to Sarteschi et al.,[10] can be categorized into five classes based on the features of reflux and its duration, as well as alterations during the Valsalva maneuver: Grade 1 is distinguished by the identification of longer reflux in the inguinal blood vessels solely during the Valsalva maneuver, whereas scrotal varicose veins were not detectable in the prior gray-scale research. Grade 2 is marked by a modest posterior varicosity that reaches the superior pole of the testes and whose diameter rises whenever the Valsalva maneuver is performed. The CDU test indicates the existence of venous reflux in the supra testicular area only during the Valsalva maneuver. Grade 3 is defined by vessels that seem dilated to the testicular inferior pole while the patient is upright, but no ectasia is found when the patient is supine. CDU exhibits accurate venous reflux only during the Valsalva maneuver. Grade 4 is determined when the veins seem tortuous and dilated, regardless of the patient's posture, since dilatation rises when standing and during Valsalva. Augmentation of venous reflux following the Valsalva maneuver is the criteria that distinguishes grade 4 from Grade 2 and Grade 5. At this age, testicular hypotrophy is prevalent. Grade 5 is marked by obvious venous ectasia in the standing position of the patient. Considerable baseline venous reflux is seen in CDU, which does not rise after performing the Valsalva maneuver.

This study aimed to compare the accuracy of preoperative and intraoperative scrotal duplex ultrasound (SDU) in assessing varicocele.

Patients and Methods

The study was conducted in accordance with the ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013). The study protocol was reviewed and approved by the Ethics Committee of the Al-Azhar University, Faculty of Medicine in Assiut, Egypt. All participants provided written informed consent.

This prospective cohort study was conducted on 20 male patients scheduled for subinguinal varicocelectomy. It was conducted at the Dermatology and Andrology Department in Azhar-Assiut University Hospital (tertiary care hospital) and carried out from March 2023 to November 2023.

All patients were exposed to a comprehensive medical history, including personal data, such as name, age, marital status, address, employment, number of children, and the smoking index. History of medical diseases (e.g., diabetes mellitus), drug intake, trauma, or surgical operation was also evaluated.

All patients were subjected to general and local examinations in a room with a suitable temperature. The patient did the Valsalva maneuver while standing up. Varicocele severity was clinically categorized as Grade 1 (palpable solely during the Valsalva maneuver), Grade 2 (palpable in upright posture), and Grade 3 (visible varicocele by the naked eye).[11]

Clinically, we obtained the testicular volumes after pulling the scrotal skin in a warm room and comparing the testicles to 12 solid ellipsoid models (Prader orchidometer) varying in volume 1 - 25 cm^3 (1 to 6, 10, 12,15, 20, and 25 cm^3).[12]

As regards Sonographic Technique & Analysis, firstly, we calculated the testicular volume according to the prolate ellipsoid formula (length (L) × width (W) × height (H) × 0.52), expressed in cm^3. For volume computation and statistical analysis, the greatest obtainable measurement for each testicular dimension was employed. Morphological evaluation was performed of both testes, epididymis, spermatic cord, scrotal wall, and pampiniform plexus of veins as regards the number of veins and the maximum diameter of dilated veins. Secondly, the venous component was performed on all patients, including the diameter of the biggest vein of the pampiniform plexus and retrograde flow under both relaxed and Valsalva conditions.[13]

All patients conducted a varicocelectomy operation. First, we prepared the duplex ultrasound for the operation (we used an ultrasound probe cover filled with gel to prevent direct contact between the probe and examined parts and avoid infection). After adequate spinal anesthesia induction, We put the patient on his back. We marked the external inguinal ring placement on the skin. We started with a 3 cm oblique skin incision centered over the external inguinal ring. An artery was used to expand the incision, which was subsequently deepened utilizing Camper's and Scarpa's fascia. The spermatic cord was then grabbed with a Babcock clamp, delivered, and put over a moist dressing, and the testis was pulled out from the scrotum and placed over the same wet dressing.[14] The internal and external spermatic fascia is incised, and the structures of the cord are examined using grey scale ultrasound and Doppler ultrasound to detect the number of veins, venous reflux, vein diameter, and testicular volume.[13]

Statistical analysis was performed using the statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables and mean ± standard deviation (SD) for continuous variables. Inter-group comparisons were performed using Student's t-test. Group comparisons concerning categorical variables were performed using the chi-square test. *P*-values less than 0.05 were considered significant.

Results

The patients' ages ranged from 23 to 45 years, with a mean age of 34.60 ± 8.28 years. Fourteen (70%) patients presented with primary infertility, 6(30%) patients with secondary infertility, 17(85%) patients had bilateral varicocele, and 3(15%) patients had unilateral varicocele. Grade 2 varicocele was presented in 6(30%) patients, and Grade 3 varicocele was presented in 14(70%) patients (Table 1).

Table 1.

Demographic characteristics of the studied patients.

Age of patients, years	
(Range) Mean ± SD	(23-45) 33.8± 8.28
Type of infertility, n (%)	
Primary	14 (70)
Secondary	6 (30)
Side of varicocele, n (%)	
Unilateral	3 (15)
Bilateral	17 (85)
Grade of varicocele, n (%)	
Grade 2	6 (30)
Grade 3	14 (70)

There was a significant increase in testicular volume as well, in the number of veins, pre- and intraoperative SDU (P<0.05), on the other hand, there was no significant difference in vein diameter pre- and intraoperative SDU (P>0.05) (Table 2).

Table 2.

Testicular and venous parameters in preoperative and intraoperative SDU.

	Preoperative SDU	Intraoperative SDU	P-value
	(Range) Mean ± SD		
Testicular volume, cm^3			
Right testes	(8-18) 12.23 ± 1.62	(9-20) 14.83 ± 1.93	0.000
Left testes	(7-16) 12.01 ± 1.95	(7-15) 13.95 ± 1.96	0.003
Number of veins			
Right testes	(0-5) 3.75 ± 1.28	(3-7) 4.50 ± 1.0	0.046
Left testes	(4-10) 5.35 ± 1.56	(5-10) 6.65 ± 1.22	0.006
Vein diameter, mm			
Right testes	(1.2-3.8) 2.52 ±0.63	(2-4) 2.63 ± 0.56	0.563
Left testes	(2.7-5.7) 3.95 ± 0.79	(3-6) 4.09 ± 0.80	0.581

As regards venous reflux, there was no significant difference in venous reflux pre and intra-operative SDU on both sides (Table 3).

Table 3.

Venous reflux in preoperative and intraoperative SDU.

	Preoperative SDU	Intraoperative SDU	P-value
	n (%)		
Right side			
Severe reflux	1 (5)	0 (0)	0.737
Moderate reflux	7 (35)	8 (40)	
Minimal reflux	4 (20)	3 (15)	
No reflux	8 (40)	9 (45)	
Left side			
Severe reflux	15 (75)	14 (70)	0.727
Moderate reflux	5 (25)	6 (30)	
Minimal reflux	0 (0)	0 (0)	
No reflux	0 (0)	0 (0)	

Discussion

Ultrasonography is considered to be the most accurate and useful technique for diagnosing varicocele. The presence of veins with a diameter greater than 2 mm is an established US diagnostic criterion for varicocele.[13] To date, there is a lack of or few published studies that are concerned with the benefit of intraoperative Doppler US in the reassessment of grading of varicocele. Numerous researchers have used various assessment criteria to compare varicocele grade on physical assessment and vein diameter on color Doppler and observed venous reflux.

In a study by Gonda et al.,[15] sonography was considered positive for subclinical varicocele in 95% of patients. Chiou et al.[16] developed a scoring system that included the maximum diameter of the veins (score 0 to 3), the presence of a venous plexus and the sum of the diameters of the veins in the plexus (score 0 to 3), and the change in blood flow during the Valsalva maneuver (score 0 to 3). Using a total score of ≥4 to define the presence of a CDU-positive varicocele, investigators observed a sensitivity of 93% and specificity of 85% compared with physical examination (55%).

Kocakoc et al.[17] have highlighted that the flow volume of reflux assessment, which reflects a combination of the vein diameters, duration and velocity of reflux, is deemed more valuable than measuring venous diameters alone.

In our study, we found that there was no significant difference in the vein diameter and venous reflux between preoperative and intraoperative SDU assessments. Meanwhile, we noticed a significant increase in the number of veins and testicular volume during intraoperative SDU compared to preoperative SDU.

Özkaptan et al.[18] reported a strong correlation between using Doppler US and the number of veins in their study. The findings of prior studies support our own. These researchers noted more veins with the use of Doppler ultrasonography. Intraoperative Doppler ultrasonography enables more exact

identification of tiny veins during a varicocelectomy; more veins of the thick network of adhering veins around the artery can be removed effectively under the direction of Doppler ultrasonography.

In a study by Juho et al.,[19] a total of 24 male patients underwent subinguinal varicocelectomy with intraoperative vascular Doppler ultrasonography because of symptomatic varicocele or infertility. The authors concluded that subinguinal varicocelectomy with intraoperative vascular Doppler ultrasonography is an effective treatment for symptomatic varicocele.

Testicular volume can be assessed clinically using various orchidometers, calipers, and rulers or by ultrasound measuring the length, width, and height of the testicles followed by multiplication by a constant. The theoretical advantage of ultrasound over orchidometers or testicular models for assessing testicular volume is its ability to distinguish the testis from adjacent soft tissue, providing more accurate volumes.[20] Hsieh et al.[20] and Paltiel [21] found that among the commonly used ultrasound formulas, the empirical formula of Lambert ($L \times W \times H \times 0.71$) provided better accuracy.

In a study by Mbaeri et al.,[22] the mean testicular volume of the 121 testes was 10.60±3.5 ml and 13.26±5.2 ml for water displacement and Prader orchidometer measurements, respectively. The study showed that measuring the testicular volume with a Prader orchidometer overestimated the actual testicular volume by 25.10%. Behre and colleagues[23] showed that comparing Prader orchidometer measurements performed by four clinical investigators and ultrasonography in 256 patients revealed a significant correlation of 0.91. Still, the correlation degree depended on the investigator's clinical experience. Schiff et al.[24] concluded that in the hands of an experienced examiner, orchidometer measurements can provide an accurate, rapid, and inexpensive assessment of testicular volume. In a study by Sakamoto et al.,[25] Prader orchidometry morphometrically and functionally overestimated the testicular volume compared to ultrasound. Fuse et al.[26] showed that the testicular volume measured by slide calipers on scrotal skin was also found to be incorrect. The testicular volume measured by ultrasonography was closer to and correlates well with the actual volume and was considered the best method.

Thus, it can be concluded that ultrasonography is a reliable tool for determining testicular volume and provides objective, accurate, and reproducible measurements of testicular volume.

The current study had some limitations, such as a small sample size. The larger sample size will help determine the importance of preoperative and intraoperative SDU in the evaluation of varicoceles.

Conclusion

The preoperative SDU is a good and accurate method for assessing the grading of varicocele. There is no significant difference in vein diameter or venous reflux between preoperative and intraoperative assessments. However, a significant increase in the number of veins and testicular volume during intraoperative SDU compared to preoperative SDU is found.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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ORIGINAL ARTICLE

Population Genetics

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The Association of Leptin Receptor Gene Q223R Polymorphism with Obesity in the Yakut Population

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Abstract

Background: This study aimed to compare the frequencies of alleles and genotypes of the *LEPR* Q223R SNP in the Yakut population in samples with normal BMI and obesity and compare the data obtained with other populations worldwide.

Methods and Results: The study included 336 DNA samples from volunteers of Yakut nationality (117 women and 219 men) without chronic diseases, whose average age was 47.4±0.06 years. All volunteers were divided into two groups: Group 1 (n=151) with normal BMI and Group 2 (n=185) with obesity (BMI ≥30 kg/m^2). Group 2 was divided into two subgroups: Group 2A (n=156) with BMI ≥30 kg/m^2 plus abdominal obesity and Group 2B (n=29) with BMI ≥30 kg/m^2 and without abdominal obesity.

The study of the *LEPR* Q223R SNP was performed using the PCR-RFLP method. The frequency of the G allele of the *LEPR* Q223R SNP was 79.5% in Group 1 and 82.7% in Group 2. Analysis showed a high frequency of genotype GG: 64.2% and 69.7% in Group 1 and Group 2, respectively. The frequency of the GA genotype was 30.5% in Group 1 and 25.9% in Group 2. The frequency of alleles and genotypes does not differ in the sample of Yakuts with normal BMI and those with obesity. There are also no differences in the frequency of alleles and genotypes based on gender and the presence of abdominal obesity.

The high frequency of the G allele in the Yakut population is close to that observed in East Asian populations (86.9%). There was no statistical difference in allele frequencies in comparison with the populations of Han Chinese from Beijing, Japanese from Tokyo, and Vietnamese from Ho Chi Minh City. In European, African, American, and South Asian populations, the G allele occurs with a frequency of 43.7% to 59.2%.

Conclusion: The *LEPR* Q223R SNP does not affect BMI in the Yakut population. In this study, Q223R allele frequencies were like allele frequencies in East Asian populations but not in Caucasians, reflecting racial diversity in the allele distribution of this polymorphism.**(International Journal of Biomedicine. 2024;14(1):104-109.)**

Keywords: body mass index • *LEPR* gene • Q223R SNP • Yakut population

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Abbreviations

AO, abdominal obesity; **BMI**, body mass index; **PCR**, polymerase chain reaction; **SNP**, single nucleotide polymorphism.

Introduction

Over the past few decades, the prevalence and incidence of obesity have increased rapidly worldwide and reached epidemic proportions. Obesity is associated with many adverse consequences, such as type 2 diabetes, hypercholesterolemia, hypertension, or coronary heart disease, and is directly associated with increased mortality and reduced life

expectancy.[1] With the completion of the Human Genome Project and the first large genome-wide association studies, an increasing number of risk alleles associated with obesity have been identified, some in genes not previously known to be associated with obesity. However, genome-wide association studies have identified a small percentage of genetic variations significantly associated with obesity or body mass index (BMI) for most ethnic groups.

The mechanisms and genetic basis of the influence of diet and habitat remain unclear. One widely studied candidate gene for obesity, the leptin receptor (*LEPR*) gene, is located in the biological pathway to obesity (leptin-insulin pathway). Leptin is a hormone primarily produced by the adipose tissue in proportion to the size of fat stores. Besides adipose tissue, leptin is also produced by other tissues, such as the stomach, placenta, and mammary gland. It is known to have pleiotropic effects, including regulating several neuropeptides involved in appetite control and thermogenesis. Numerous studies have tested two nonsynonymous single nucleotide polymorphisms of the *LEPR* gene (Q223R and K109R) for association with obesity and type 2 diabetes, with inconclusive results.[2,3] Recently, many studies have been published on the association between *LEPR* variants and obesity, including studies of the interaction of these variants with gender or other factors. Of these two polymorphisms, the Q223R (rs1137101) SNP occurs as a result of a non-conservative A to G substitution at codon 223 resulting in a glutamine to arginine amino acid change. This functional variant reduces leptin binding and thus impairs leptin signaling. Data from studies of the Q223R polymorphism are very contradictory, considering the results obtained in patients from different ethnic groups. This makes further studies of polymorphic loci of the *LEPR* gene relevant.

This study aimed to compare the frequencies of alleles and genotypes of the *LEPR* Q223R SNP in the Yakut population in samples with normal BMI and obesity and compare the data obtained with other populations worldwide.

Materials and Methods

The study included 336 DNA samples from volunteers of Yakut nationality (117 women and 219 men) without chronic diseases, whose average age was 47.4±0.06 years.

BMI was calculated and categorized as underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30 kg/m^2), based on WHO classification.[4] Abdominal obesity (AO) was diagnosed when the waist circumference (WC) exceeded 88 cm in women and 102 cm in men.

All volunteers were divided into two groups: Group 1 (n=151) with normal BMI and Group 2 (n=185) with obesity. Group 2 was divided into two subgroups: Group 2A (n=156) with BMI ≥30 kg/m^2 plus OA and Group 2B (n=29) with BMI ≥30 kg/m^2 and without OA.

Genomic DNA samples were isolated from whole blood using a commercial kit for DNA isolation (Newteryx, Yakutsk, Russia). The study of the *LEPR* Q223R SNP was performed using the PCR-RFLP method. Amplification of the gene region containing the polymorphic variant was carried out with standard pairs of primers. Forward: 5′-ACCCTTTAAGCTGGGTGTCCCAAATAG-3 and Reverse: 5′ - AATGTCAGTTCAGCCCATAAATATGG -3′. PCR temperature conditions were as follows: 94°C for 4 min, followed by 35 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. The RFLP mixture with a volume of 20 µl consisted of: amplifier - 7 µl, deionized water - 10.9 µl, restriction buffer - 2 µl and restriction endunuclease MspI (2 u.a.).

Interpretation of the genotyping results was performed based on different patterns of gene region bands (Fig 1).

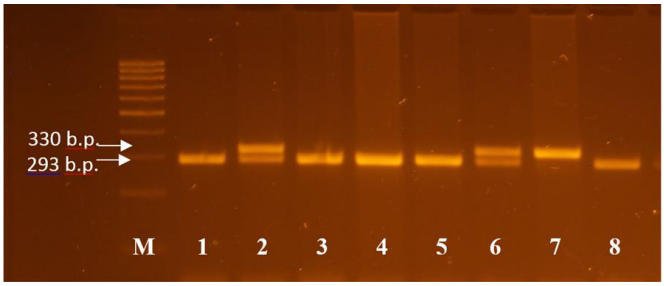

Fig 1. *The electropherogram of the LEPR gene region in a 4% agarose gel after RFLP. 1 - Step 100 marker; 7 – genotype AA (330 bp); 2 and 6 – genotype AG (330, 293 bp); 1, 3,4,5,8 – genotype GG (293 bp).*

Statistical analysis was performed using Microsoft Excel 2010. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Genetic markers for HWE were tested. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Differences in the allele and genotype distribution between the groups were assessed by χ2-test with Yates correction. The Mann-Whitney U-test tested differences in continuous variables. Four inheritance models were analyzed (the dominant model, the codominant model, the recessive model of inheritance, and the multiplicative model). A probability value of P<0.05 was considered statistically significant.

Results

The variability of the *LEPR* Q223R SNP in the Yakut population and the associations of the study SNP with obesity in Yakuts were studied.

The distribution of polymorphic markers of the *LEPR* Q223R SNP in Groups 1 and 2 was in HWE. The frequency of the G allele of the *LEPR* Q223R SNP was 79.5% in Group 1 and 82.7% in Group 2. Analysis showed a high frequency of genotype GG: 64.2% and 69.7% in Group 1 and Group 2, respectively. The frequency of the GA genotype was 30.5% in Group 1 and 25.9% in Group 2. The frequency of alleles and genotypes does not differ in the sample of Yakuts with normal BMI and those with obesity. There are also no differences in the frequency of alleles and genotypes based on gender and the presence of abdominal obesity. Thus, our analysis did not reveal a significant positive association between the *LEPR* Q223R SNP and the risk of obesity across the studied inheritance models (Table 1)

Table 1.

Frequency distribution of alleles and genotypes of the LEPR rs1137101 SNP with inheritance models.

Groups	Alleles		Genotypes					
			Codominant model of inheritance		Dominant model of inheritance		Recessive model of inheritance	
	A [% (n)]	G [% (n)]	AG [% (n)]	GG [% (n)]	AA [% (n)]	AG+GG [% (n)]	AA+AG [% (n)]	GG [% (n)]
Group 1 (normal BMI) (n=151)	20.5 (62)	79.5 (240)	30.5 (46)	64.2 (97)	5.3 (8)	94.7 (143)	35.8 (54)	64.2 (97)
Group 2 (BMI ≥30 kg/m^2) (n=185)	17.3 (64)	82.7 (306)	25.9 (48)	69.7 (129)	4.3 (8)	95.7 (177)	30.3 (56)	69.7 (129)
OR (95% CI)	1.23 (0.8-1.8)		1.04 (0.4-3)	1.33 (0.5-3.7)	1.24 (0.4-3.4)		1.28 (0.8-2)	
P	0.33		0.85	0.77	0.87		0.34	
Men with normal BMI (n=105)	20.5 (43)	79.5 (167)	31.4 (33)	63.8 (67)	4.8 (5)	95.2 (100)	36.2 (38)	63.8 (67)
Men with BMI ≥30 kg/m^2 (n=114)	15.4 (35)	84.6 (193)	27.2 (31)	71.1 (81)	1.8(2)	98.2(112)	28.9(33)	71.1(81)
OR (95% CI)	1.42 (0.9-2.3)		2.35 (0.4-13)	3.02 (0.6-16.1)	2.8(0.5-14.7)		1.39 (0.8-2.5)	
P	0.2		0.55	0.33	0.38		0.32	
Women with normal BMI (n=46)	20.7 (19)	79.3 (73)	28.3 (13)	65.2 (30)	6.5 (3)	93.5 (43)	34.8 (16)	65.2 (30)
Women with BMI ≥30 kg/m^2 (n=71)	20.4 (29)	79.6 (113)	23.9 (17)	67.6 (48)	8.5 (6)	91.5(65)	32.4 (23)	67.6 (48)
OR (95% CI)	1.01 (0.5-1.9)		0.65 (0.1-3.1)	0.8 (0.2-3.4)	0.76 (0.2-3.2)		1.1 (0.5-2.4)	
P	0.9		0.88	0.95	0.98		0.95	
Croup 1 (normal BMI) (n=151)	20.5 (62)	79.5 (240)	30.5(46)	64.2 (97)	5.3 (8)	94.7 (143)	35.8 (54)	64.2 (97)
Group 2A (BMI ≥30 kg/m^2 + OA) (n=156)	17.6 (55)	82.4 (257)	25.0 (39)	69.9 (109)	5.1 (8)	94.9 (148)	30.1 (47)	69.9 (109)
OR (95% CI)	1.21 (0.8-1.8)		0.85 (0.3-2.5)	1.12 (0.4-3.1)	1.03 (0.4-2.8)		1.29 (0.8-2.1)	
P	0.42		0.98	0.97	0.85		0.35	
Group 2B (BMI ≥30 kg/m^2 without OA (n=29)	15.5 (9)	84.5 (49)	31.0 (9)	69.0 (20)	0	100 (29)	31.0 (9)	69.0 (20)
Group 2A (BMI ≥30 kg/m^2 +OA) (n=156)	17.6 (55)	82.4 (257)	25.0 (39)	69.9 (109)	5.1 (8)	94.9 (148)	30.1 (47)	69.9 (109)
OR (95% CI)	0.86 (0.4-1.8)		0.74 (0.3-1.8)	1.04 (0.4-2.5)	0		1.04 (0.4-2.5)	
P	0.84		0.41	0.49	0.45		0.9	

OA - abdominal obesity; BMI - body mass index

Analysis of the association between the *LEPR* Q223R SNP and the BMI values and abdominal obesity is presented in Table 2.

When analyzing the average anthropometric values depending on the genotype, we found that the BMI in carriers of the heterozygous AG genotype was greater than in carriers of the GG genotype. Moreover, in the obese sample, carriers of the AA genotype had the highest BMI. However, differences in BMI indicators depending on genotype in all samples were not statistically significant.

The *LEPR* gene emerged as the most promising candidate gene, likely undergoing natural selection, represented by three variants (*rs1137100*, *rs1137101*, and *rs1805096*) that strongly distinguish East Asian populations from all other populations described in the literature (Table 3).

The high frequency of the G allele in the Yakut population is close to that observed in East Asian populations (86.9%). There was no statistical difference in allele frequencies in comparison with the populations of Han Chinese from Beijing, Japanese from Tokyo, and Vietnamese from Ho Chi Minh City.

In European, African, American, and South Asian populations, the G allele occurs with a frequency of 43.7% to 59.2%.[5] Interestingly, the frequency of the G allele in populations of South and Middle America (43.7%) is similar to populations of Europeans (46.9%) and South Asians (50.3%). These data do not fit into modern views that the Indian and East Asian populations (86.9%) have the same roots.

Table 2.

Average BMI values depending on the LEPR Q223R SNP

Genotypes	n	BMI/ AO	Mann Whitney U test	*P*
BMI ≥30 kg/m^2				
AA	8	34 ± 1.22	215.5 (AA/AG)	>0.05
AG	48	33.4 ± 0.15	3063 (AG/GG)	>0.05
GG	129	33.1 ± 0.07	601 (AA/GG)	>0.05
BMI:18.5-24.9 kg/m^2				
AA	8	22.1 ± 0.69	145 (AA/AG)	>0.05
AG	46	22.7 ± 0.18	2128 (AG/GG)	>0.05
GG	97	22.6 ± 0.12	332.5 (AA/GG)	>0.05
Abdominal obesity				
AA	8	34 ± 1.22	161.5 (AA/AG)	>0.05
AG	39	33.9 ± 0.17	2247 (AG/GG)	>0.05
GG	109	33.5 ± 0.08	475 (AA/GG)	>0.05

Table 3.

Frequency distribution of alleles and genotypes of the LEPR rs1137101 SNP in the Yakut population and the populations of the 1000 Genomes project.

Populations	Alleles (%)		Genotypes (%)			χ^2-test	*P*
	A	G	AA	AG	GG		
Yakutia (this study)	18.8	81.2	4.8	28.0	67.3	-	-
Average frequency in the world	41.6	58.4	19.6	43.8	36.5	51.52	0.00
South and Middle America	56.3	43.7	29.7	53.3	17.0	107.39	0.00
Europe	53.1	46.9	30.2	45.7	24.1	97.92	0.00
South Asia	49.7	50.3	25.6	48.3	26.2	78.97	0.00
Africa	40.8	59.2	15.7	50.1	34.2	42.35	0.00
East Asia	13.1	86.9	1.6	23.0	75.4	9.6	0.00
Chinese Dai inXishuangbanna	11.3	88.7	0.0	22.6	77.4	5.2	0.02
Han Chinese in Beijing	13.1	86.9	2	22.3	75.7	3.11	0.08
Southern Han Chinese	12.4	87.6	1.9	20.9	77.1	4.12	0.04
Japanese in Tokyo	15.4	84.6	1.0	28.8	70.2	1.00	0.32
Kinh in Ho Chi Minh City	13.1	86.9	3.0	20.2	76.8	2.97	0.09

P – significance of differences in allele frequencies between listed populations and this study.

Discussion

Previously, several studies of the *LEPR* Q223R and K109R polymorphisms in the Yakut population were carried out. Ammosova et al.[6] investigated the relationship between the rs1137100 polymorphism of the *LEPR* gene and the lipid spectrum, metabolic syndrome and its components in the Yakuts. They found that the frequency of the G allele was 66.6%. No association with metabolic syndrome and its components has been identified. Asekritova et al.[7] conducted a study of rs1137101 polymorphism in Yakuts. They found that the frequency of the G allele in patients with metabolic syndrome was 87% and in patients without metabolic syndrome 91%. Ievleva[8] came to significant results and found that the risk markers for the implementation of disorders of carbohydrate and energy metabolism against the background of overweight and obesity in Caucasian adolescents (Russians) are the carriage of alleles of the polymorphic loci of the *LEPR* rs1137101 and rs1137100, and in Mongoloid adolescents (Buryats), carriage of the *FTO* rs9939609 and *FTO* rs8050136 alleles. A study in the Malaysian population did not reveal an association between the rs1137101 and rs1137100 polymorphisms and obesity.[9] Okada et al.[10] found an association between the A allele and the incidence of childhood obesity and overweight in the Japanese population. The GENYAL study in Spain assessed 11 SNPs associated with high BMI in children and found a significant association between *LEPR* Q223R and high weight gain.[11] The researchers observed a north-south gradient in Q223R allele frequency in Europeans, with a higher frequency of derived alleles in the north and a lower frequency in the south. The same phenomenon for SNPs of other genes was reported in the Framingham Heart Study[12] and a pan-European analysis.[13] In a study of residents of Sri Lanka, Illangasekera et al.[14] found a connection between the G allele and BMI and waist circumference; they also found that living in an urban area neutralized the protective effect of the non-risk AA genotype in the development of obesity.

Our studies in the Yakut population show a high prevalence of variants of the *PNPLA3* and *FABP2* genes associated with increased BMI and non-alcoholic fatty liver disease.[15] A study by Simcox et al.[16] found that the G allele of the *PNPLA3* gene is associated with adaptation to cold. The authors suggest that the sharply continental climate and specific diet were probably the reason for the high prevalence of variants of genes involved in the metabolism of lipids and carbohydrates, and the accumulation of risk alleles is a consequence of adaptation to living conditions in Yakutia.

LEPR is involved in fat storage, heat dissipation by mitochondria, and body weight regulation. Polymorphisms in the *LEPR* gene that exhibit some signatures of selection in East Asian populations have been reported to be involved in specific metabolic patterns and/or disorders. For example, rs1137100 is responsible for a nonsynonymous substitution (*K109R*) that is found to be associated with an increased respiratory quotient (i.e., increased basal metabolic rate), consistent with its important effect on nonshivering thermogenesis.

There is evidence of adaptation to cold in populations ancestral to anatomically modern humans. Sazini et al.[17]

analyzed genes associated with the function of brown adipose tissue, modifications of which contribute to increased heat dissipation by mitochondria, in the genomes of modern populations of East Asia and Europe, as well as in the genomes of fossil hominids (Neanderthals and Denisovans). They found evidence of positive selection for three SNPs in the *LEPR* gene in East Asians. The G variant of the *LEPR* gene (*rs1137101*), showing signs of positive selection, was found in the Neanderthal and Denisovan genomes, suggesting the evolution of independent mechanisms of adaptation to thermal efficiency in these fossil hominin populations. The variation surrounding *LEPR* rs1137100 appears to have actually been shaped by positive selection in East Asian populations, whereas only the potentially cold-adapted *LEPR* rs1137101 was observed in archaic species. This suggests that convergent evolution of modern and archaic increased thermogenesis mediated through brown adipose tissue or introgression of related archaic cold-adapted alleles into modern genomes is unlikely.[18]

Long-term consumption of fructose has also been shown to lead to leptin resistance. Recently, leptin was found to be associated with autophagy. Autophagy has been demonstrated to be involved in several interesting processes, such as fat storage in adipocytes and the liver.[19]

The mutant allele has also been reported to reduce leptin inhibition of insulin, leading to insulin dysregulation and increased insulin release, thereby accelerating glucose uptake and basal metabolic rate. Accordingly, this variant could potentially be detrimental in hot climates, in which it did maintain a low frequency, becoming increasingly beneficial in colder climates due to the associated increase in basal metabolic rate and, thus, heat dissipation, which is the basis of nonshivering thermogenesis. It appears that changes in the *LEPR* gene (*rs1137101* and *rs1137100*) were an advantage for Asian populations 6000–8000 years ago, corresponding to the introduction of agriculture in Asia. It has been suggested that the *LEPR* gene may be considered a "thrifty" gene, leading to the accumulation of adipose tissue in times of plenty and providing a reserve in times of famine. As an alternative explanation for the positive selection of *LEPR* in Asian populations, Hancock et al.[20] found associations of several *LEPR* variants with climate variables, suggesting a role for climate adaptation in the biological processes underlying cold adaptation and overweight. They suggest that variants such as Q223R and K109R may be harmful in hot equatorial climates and beneficial in colder climates.

In conclusion, our study shows that the *LEPR* Q223R SNP does not affect BMI in the Yakut population. Differences in allele frequencies between populations can be due to various environmental and genetic factors. In this study, Q223R allele frequencies were like allele frequencies in East Asian populations but not in Caucasians, reflecting racial diversity in the allele distribution of this polymorphism.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant.

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ORIGINAL ARTICLE

Population Health

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Knowledge, Attitudes, and Behaviors Toward Proper Nutrition and Lifestyles in Kosovar's Diabetic Patients

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Abstract

***Background*:** The aim of our study was to assess the knowledge and attitudes about type 2 diabetes (T2D) in Kosovars patients, as well as self-efficacy toward eating behavior, and determine the impact of diabetes on patients' daily work and lifestyle practices.

Methods and Results: This analytical cross-sectional survey was performed among 400 T2D patients (203 males and 197 females) to assess their knowledge, attitude, and practice regarding diabetic diet and eating habits. It was conducted from February to May 2023 in Kosovo's rural and urban areas. Knowledge, attitudes, and eating behavior were assessed using the self-administered 28-item sets related to glycemic control, physical activity, risk of complications, foot care, and food and nutrition. The largest proportion (42.3%) was made up of the patients over 60 years of age; 41.5% were 40-60 years of age, and only 16.3% were 30-40 years old. Most of the patients were males (50.8%); more than 87% were married. Interviewed participants did not have enough knowledge about the consumption of foods with more fruits, beverages, and sweets. Generally, participants had poor knowledge about the impact of DM on health. Most diabetics in Kosovo still have suboptimal diet practices, choosing to base their main meals around carbohydrates containing food with a high glycemic index, such as white bread and potatoes most of the time. In our case, 38.8% of participants never exercised during the week because they did not know the importance of exercise to treat their disease. In our study, we found no association between the level of diabetes-related knowledge and age, gender, or the years since the patient was diagnosed. A worrying fact for Kosovar patients is that there are no diabetes counseling centers in the regions where they live.

Conclusion: There is a need to increase awareness of the complications of diabetes and consequently improve nutrition knowledge, attitudes, and practices. The benefits of early detection of T2D through screening in the general population are needed in Kosovo.**(International Journal of Biomedicine. 2024;14(1):110-117.)**

Keywords: knowledge • type 2 diabetes • eating habits • Kosovo adults

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Introduction

Diabetes mellitus is a disease with multifactorial pathogenesis, and modifiable lifestyle factors that include obesity, physical inactivity, diet, and alcohol consumption. Worldwide, the number of people with diabetes is increasing due to population growth, aging, urbanization, and the rising prevalence of obesity and physical inactivity.[1,2]

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2023. By 2030, the world prevalence among adults is projected to increase to 7.7%.[3] Globally, 422 million people are living with diabetes,[4] and the estimate is projected to rise to over 592 million by 2035.[5] Prevalence has been rising more rapidly in low- and middle-income countries than in high-income countries. The largest increases are expected in the older age groups, with numbers more than doubling in the over-60 age group. In addition to known cases of diabetes, undiagnosed diabetes currently affects 1%–2% of US adults.[6] Furthermore, it is estimated that approximately 50% of diabetes cases are considered "uncontrolled." [7]

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In Europe, 61 million adults are living with diabetes. The number of adults with diabetes is expected to reach 67 million by 2030 and 69 million by 2045.[8] A high prevalence of type 2 diabetes (T2D) with considerable variability between European countries and between genders was found.[9,10] T2D most often occurs in persons older than 45 years. Still, it is increasing in children, adolescents, and adults due to increased levels of overweight, physical inactivity, and high-carbohydrate and high-fat diets.[11] In Kosovo, T2D affects more than 90% of diabetics. Diabetes mellitus confers an increased mortality risk and is associated with multiple comorbidities, decreased quality of life, and a significant economic burden.

In 2019, diabetes and kidney disease due to diabetes caused an estimated 2 million deaths.[12] Premature mortality caused by T2D results in the loss of 12 to 14 years of life. This disease is associated with severe complications that affect patients' health, productivity, and quality of life. More than 50% of people with diabetes have cardiovascular disease and account for the sole cause of end-stage renal disease, which requires either dialysis or kidney transplantation. Moreover, diabetes is a leading cause of blindness due to retinal damage in the adult age group, diabetic retinopathy as well as lower limb amputations. Diabetic foot is a life-changing complication for the diabetic patient and is associated with increased morbidity and mortality. In a person with diabetes, ulcers are associated with peripheral arterial disease and peripheral neuropathy, often in combination. It is estimated that at least 10% of diabetic patients will suffer a foot ulcer during their lifetime.

Early diagnosis, intensive treatment, and consistent dietary patterns, along with regular care and follow-ups, are essential and can help to preserve the health of diabetes patients and significantly lower the risk of complications.[13,14] The CDC has identified self-dietary management as a major step in assessing a patient's knowledge of the nutritional aspects, treatment, and complications of diabetes.[15] Previous research has highlighted dietary and lifestyle modifications related to reducing risk.[9,10]

Dietary management in T2D is the cornerstone of care. However, according to many publications, many diabetics worldwide have insufficient dietary knowledge, positive attitudes, and good habits toward the importance of DM care; therefore, self-management is recognized as a central component in disease prevention and treatment.[16-18]

Poor diet quality is a major modifiable risk factor for T2D. Specifically, diets low in legumes, whole grains, fruits and vegetables, and high in sodium, added sugar, alcohol, and red and processed meats are responsible for much of this burden.[19]

According to the World Health Organization, adherence is "the extent to which a person's behavior—taking medication, following a diet, and/or executing lifestyle changes—corresponds with agreed recommendations from a healthcare provider." Dieting to achieve glycemic control in diabetes is integral to improving diabetes outcomes.[20] Many studies from different authors have described dietary practices with very poor dietary profiles and poor knowledge, attitudes, and practices toward diabetes and dietary management.[21-24]

The standard of care for diabetes is education on proper diet, physical activity, and glucose monitoring. Using and managing medications alone (without exercise and diet) does not lower HbA1C. However, the combination of nutrition counseling and exercise results in a 1% reduction in hemoglobin A1C in middle-aged T2D patients.[25] Intake of food in large portions can increase blood glucose levels. Diabetes education leads to better disease control and is widely accepted to be an integral part of comprehensive diabetes care.[26]

Most people living with diabetes mellitus know the dietary recommendations and healthy behavior. Still, they do not comply with the suggestions because they consider that diet food for diabetes patients tends to be unpleasant, so they eat according to their wishes, especially if they have not shown serious problems.[27]

Physical activity also helps control blood sugar levels and lowers the risk of heart disease. Some additional benefits include maintaining a healthy weight, losing weight, if necessary, feeling happier, getting better sleep, improving your memory, controlling blood pressure, lowering LDL ("bad" cholesterol), and raising HDL cholesterol ("good" cholesterol). The goal is to perform moderate-intensity physical activity at least 150 minutes a week. Walking can be one of the most basic forms of exercise, but it is also a very effective form of activity to help lower blood glucose levels. Therefore, many experts have suggested that physical activity in these patients can be an important "weapon" in preventing foot ulcers.[28-30]

"A healthy diet, regular physical activity, maintaining a normal body weight, and avoiding tobacco use are ways to prevent or delay the onset of T2D."[12]

Because of the lack of a National Registry for Diabetes in Kosovo and the lack of official data, according to ICD-10, no conclusion can be drawn about the prevalence of the disease in the country.

The aim of our study was to assess the knowledge and attitudes about T2D in Kosovars patients, as well as self-efficacy toward eating behavior, and determine the impact of diabetes on patients' daily work and lifestyle practices.

Materials and Methods

Study design and participants

This analytical cross-sectional survey was performed among 400 T2D patients to assess their knowledge, attitude, and practice regarding diabetic diet and eating habits. It was conducted from February to May 2023 in Kosovo's rural and urban areas. The qualitative phase used a phenomenological exploratory design, which enabled collection through face-to-face interviews with patients who were scheduled to visit the primary Medicine Centers and in the areas where participants lived.

Participants were over 18 years of age, diagnosed with T2D at least 6 months previously, and were not pregnant. During visits, participants were interviewed in their homes and Family Health Centers. The research protocol included voluntary participation. The subjects were contacted individually and were assured of confidentiality and anonymity.

Data collection

Knowledge, attitudes, and eating behavior were assessed using the self-administered 28-item sets related to glycemic control, physical activity, risk of complications, foot care, and food and nutrition. This questionnaire was designed to take approximately 15 minutes to complete. The dietary knowledge questionnaire used in this study was prepared after thoroughly reviewing the literature.

Questionnaires were administered by third-year students from the Nursing program and physicians in Public Health. Students were trained for data collection before the commencement of the survey. Before distributing the questionnaire, researchers elucidated the aim of the study. Special attention was paid to patients. For those who had problems completing the questionnaires because of age, lack of literacy, or visual impairment, researchers dictated questions to them and recorded their answers without providing hints. The questionnaire was tested, adjusted, and validated through a pilot study on a convenience sample of 20 diabetics (data not reported or included in the study). The questionnaire was divided into three parts:

Part A consisted of questions related to the participant's socio-demographic characteristics and habits.

Part B assessed participants' habits for smoking, use of alcohol, and physical activity, whether they have a counseling center for diabetes, and frequency of visits to diabetic clinics. The study subjects were asked to answer whether they were doing physical activity, the kind of exercise (walking, running), the number of days per week, the duration of disease, comorbidity, and the number of meals.

Part C consisted of diagnosed health conditions, symptoms, and complications of diabetes, such as cardiac, renal, stroke, and diabetic foot infections.

Dietary assessment

In brief, participants were asked to assess their usual dietary intake and all food and beverages consumed within a week according to the types of food. The food frequency questionnaire included information on all the food groups consumed worldwide (i.e., questions about how many times per week they consume dairy products, cereals, fruit, vegetables, meat, meat products, legumes, sweets,etc.)

Statistical analysis was performed using the statistical software package SPSS version 25.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean ± standard deviation (SD) for continuous variables. Levene's Test for Equality of Variances and Independent Samples Test were applied. Odds ratio (OR) with 95% confidence interval (CI) was also calculated. A probability value of $P<0.05$ was considered statistically significant.

Ethical approval for this study was obtained from the Ethical Committee of Medical University of Gjakova (protocol No 001/1094, December 2022). All participants provided written informed consent.

Results

Table 1 presents the socio-demographic characteristics of the participants. There were 203 males and 197 females in our study. The largest proportion (42.3%) was made up of the patients over 60 years of age; 41.5% were 40-60 years of age, and only 16.3% were 30-40 years old. Most of the patients were males (50.8%); more than 87% were married.

Table 1.

Socio-demographic characteristics of the participants.

Demographic data	Number of patients (n=400)	Percentage (%)
Gender		
Males	203	50.8
Females	197	49.2
Age (years)		
30-40 years	65	16.2
40-60 years	166	41.5
over 60 years	169	42.3
Material status		
Married	349	87.2
Unmarried	32	8.0
Divorced	19	4.8
Profession		
Housewife	145	36.2
Unemployed	119	29.8
Employed	136	34.0
Education		
Primary school	189	47.2
Secondary school	136	34.0
University degree	54	13.5
Other	21	5.3
Residence		
Urban	157	39.2
Rural	243	60.8

Regarding education, 47.3% of the participants had only primary education, 34% had secondary education, 13% had university education, and 5.3% had other. Most respondents (60.8%) lived in rural areas, and 39% lived in urban areas. There were significant differences in knowledge and attitude between education levels. There were generally poor practices on a healthy diet, physical activity, diabetes mellitus complications, and setting goals for therapy. We found no association between the level of diabetes-related knowledge and age, gender, or the number of years since the participants were diagnosed.

Diabetes knowledge and attitudes

Generally, participants had poor knowledge about the impact of diabetes mellitus on health. Therefore, they were not aware of how excessive consumption of certain items affects blood glucose levels. Our study showed that most Kosovars make unhealthy choices of food high in sugar, less minerals, and vitamins. Most of them still have a misperception of the amount of food they consume and choose to base their main meals

around carbohydrates most of the time. Therefore, educational efforts should improve knowledge at the population level.

When the participants were asked about the level of their diabetes-related nutrition knowledge, the percentage was significantly lower on healthy eating habits.

Our study showed that over 98% diabetics were not aware of the link between bread and T2D, 98.3% of them eat bread more than four times per week and 88% of them eat legumes more than three times per week.

Interviewed participants did not have enough knowledge about the consumption of foods with more fruits, beverages, and sweets; 70.5% of participants ate fruits more than four times per week, 48.3% consumed beverages more than three times a week, and 50.5% consumed sweets up to three times a week. Red meat and fried food, associated with increased cardiovascular risk, were consumed more by males than by females.

Most diabetics in Kosovo still have suboptimal diet practices, choosing to base their main meals around carbohydrates containing food with a high glycemic index, such as white bread and potatoes most of the time (Table 2).

Table 2.

Practice relating to participant's food intake per week.

Practice relating to participant's food intake per week	Never	Sometimes (1-3 times per week)	Regularly (≥ 4 times per week)
How many times per week do you eat vegetables?	4 (1%)	96 (24%)	300 (75%)
Beverages	122 (30.5%)	193 (48.2%)	85 (21.2%)
Milk	59 (14.8%)	189 (47.2%)	152 (38.0%)
Yogurt	17 (4.2%)	82 (20.5%)	301 (75.3%)
Cheese	19 (4.8%)	82 (20.5%)	299 (74.8%)
Fruits	15 (3.8%)	103 (25.8%)	282 (70.5%)
Sweets	159 (39.8%)	202 (50.5%)	39 (9.8%)
Bread times per week	2 (0.5%)	5 (1.3%)	393 (98.2%)
Potatoes	37 (9.2%)	325 (81.2%)	38 (9.5%)
Legume	35 (8.8%)	352 (88%)	13 (3.2%)
Rise	47 (11.8%)	335 (83.8%)	18 (4.5%)
Pasta	136 (34.0%)	241 (60.2%)	23 (5.8%)
Corn bread	157 (39.2%)	211 (52.8%)	32 (8.0%)
How often do you drink alcohol?	346 (86.5%)	42 (10.5%)	12 (3.0%)

However, an important limitation was the small sample size, which threatened the generalizability of the results. However, this study can still help diabetic patients in Kosovo by providing some insight into the roles that they could play in self-managing diabetes.

Regarding the symptoms participants had and their answers, 61.8% had an infection, followed by 54% who had burning during urination. Problems with itching in hands and feet were found in 27.5%, and 21.8% had frequent colds (Table 3). Among the comorbidities, 64% of the participants had hypertension, followed by hyperlipidemia (43%) and cardiovascular diseases (30.2%). Problems with teeth and a bad smell in the mouth were noted in 38.8% of T2D patients. Overweight and skin problems were found in 40.5% and 31.5% of participants, respectively (Table 4).

Table 3.

Symptoms and conditions commonly observed in study patients.

	Yes (n/%)	No (n/%)
Infection	247 (61.8%)	153 (38.3%)
Acne	27 (6.8%)	373 (93.3%)
Burning during urination	216 (54%)	184 (46%)
Frequent colds	87 (21.8%)	313 (78.3%)
Itching in hands and feet	110 (27.5%)	290 (72.5%)

Table 4.

Co-morbidities among study participants.

	Yes (n/%)	No (n/%)
Hypertension	256 (64.0%)	144 (36%)
Hyperlipidemia	172 (43.0%)	228 (57.0%)
Overweight	162 (40.5%)	238 (59.5%)
Cancer	9 (2.2%)	391 (97.8%)
Thyroid problems	34 (8.5%)	365 (91.2%)
Skin problems	125 (31.5%)	275 (68.8%)
Cardiovascular diseases	121 (30.2%)	279 (69.8%)
Teeth problems and bad smell in the mouth	155 (38.8%)	245 (61.2%)
Foot problem	57 (14.2%)	343 (85.8%)

Among the patients, diabetes awareness and management are still the major challenges stakeholders face worldwide. Knowledge is very important for people living with diabetes mellitus to avoid complications, so an intervention is needed to increase knowledge about the disease, treatment therapy, self-management, diet, and physical activity.

In our study, we found no association between the level of diabetes-related knowledge and age, gender, or the years since the patient was diagnosed.

A worrying fact for Kosovar patients is that there are no diabetes counseling centers in the regions where they live; although the health institutions are close to the patients, because

there is a lack of endocrinologists and nutritionists, they don't go to the doctor. Thus, 50.8% of the participants stated that in the absence of specialists, they turn to the University Clinical Center in Pristina with already developed complications of diabetes; 22% of them receive all the necessary information about the disease from the Internet, and more than 27% of them turn to University Clinical Center for consultation with endocrinologists.

The study results show that 45% of patients had diabetes for less than 5 years, 33.8% for 6 to 10 years, 16% for more than 15 years, and only 5.2% for more than 20 years (Table 5). Diabetes was diagnosed in 44.8% of patients during routine visits; in 41.5% of patients, T2D was diagnosed during a visit to the doctor due to the presence of other diseases and in 13.2% of cases, in preparation for surgery.

Table 5.

Diabetes duration in the patients.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3-5 years	180	45.0	45.0	45.0
	6-10 years	135	33.8	33.8	78.8
	15-20 years	64	16.0	16.0	94.8
	> 20 years	21	5.2	5.2	100.0
	Total	400	100.0	100.0	

Practices

Unhealthy eating habits and physical inactivity are the leading causes and complications among T2D patients. To understand the potential contributions of physical activity, lifestyle, and dietary habits to preventing T2D, beyond their effects on weight loss, it is necessary to determine the physiological basis underlying the lower incidence of T2D.

In our case, 38.8% of participants never exercised during the week because they did not know the importance of exercise to treat their disease; 5.5% exercised once a week, and 8.5% twice a week. Despite the high level of education of the participants in this study, only one participant stated that they exercised up to 8 times a week. At the same time, men were more likely to engage in sports than women. Doctors should be supported by campaigns promoting physical activity among all patients with diabetes throughout the country.

Additionally, a potential reason that Kosovo women engage in less physical activity may be due to differences in social gender roles, as women are expected to take on more household and childcare responsibilities and, therefore, spend less time devoted to physical exercise. An independent sample T-test was conducted to compare the physical activities (exercise) of female and male respondents. There was a significant difference (P=0.000) in scores for males (0.31±0.46) and females (0.15±0.35) with greater physical activity in males than females. The magnitude of differences in the means was significant (P=0.000) (Table 6).

Discussion

Many studies show that low knowledge, poor quality of life, and poor dietary habits are associated with diabetes comorbidity and complications.

Patients with diabetes in Kosovo receive some information from doctors in healthcare settings, but their knowledge about diabetes management, diet, lifestyle, and food intake is insufficient.

Our study found that more than 98% of diabetics in Kosovo were unaware of the link between diabetes and high-carbohydrate meals, fruits, and sweets. More than 51.5% of participants had the habit of eating three times a day, 35% had a habit of eating two times a day, and only 10.8% had a habit of regularly eating - four times a day.

Education and employment levels are important factors in diabetes control. In our study, 47.3% of participants

Table 6.

Comparison by gender and physical activity.

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	fd	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% CI of the Difference	
									Lower	Upper
Do you exercise?	Equal variances assumed	65.329	0.000	-3.925	398	0.000	-0.162	0.041	-0.243	-0.081
	Equal variances not assumed			-3.910	367.587	0.000	-0.162	0.041	-0.243	-0.080

had primary school education, 34% had secondary school education, and 13.5% had tertiary education. In terms of employment status, 29.8% of participants were unemployed, 36.3% were housewives, and 34% were employed. Our data are consistent with a previous study in which 34% of patients were employed and 36.3% of patients with diabetes were unemployed. Lack of work and adequate financial resources limit the ability to adhere to the dietary regime.[31-33]

Many studies show that the link between soft drink consumption with obesity and diabetes results from the use of large amounts of high fructose corn syrup in the production of soft drinks, which raises blood glucose levels and BMI to dangerous levels. In our study, 48.3% of participants consumed soft drinks more than three times per week. Assy and colleagues[34] showed that diet soft drinks contain glycated chemicals that markedly increase insulin resistance.

Food intake is closely associated with obesity, not only in terms of food volume but also in terms of diet composition and quality. High consumption of red meat, sweets and fried foods increases the risk of insulin resistance and T2D.[34-37]

Hansaram et al.[15] found poor patient knowledge regarding diabetic diet with a mean knowledge score of 14.46±4.52 (maximum knowledge score was 34). Similarly, Bano et al.[38] reported that only 19% of the patients had good knowledge, and the remaining 81% had poor knowledge.

Another study conducted at the Abidjan Diabetes Center found that 60.7% of patients did not have good knowledge of the recommended diet for diabetics, and 88.5% of patients did not have regular meals.[39]

In our study, comorbidity was found in almost most of our patients: 64% of the participants had hypertension, followed by hyperlipidemia (43%) and cardiovascular diseases (30.3%). A high percentage of concomitant diseases may be associated with the peculiarities of medical insurance when each patient pays for medical services from his budget, lack of screening at the country level, low level of health education, lack of diabetes consultation centers, the socio-economic situation of the country, etc.

Considering the data obtained, it is recommended that educational programs on diabetes be introduced at the country level. Family physicians must provide appropriate health education to their patients and enforce that their attitudes, knowledge, and practices are consistent. Education is needed on the importance of following a diet that can help treat illness, take proper care of oneself, and improve quality of life. Efforts should also be made to increase knowledge about T2D and the importance of a healthy lifestyle.

Training in self-monitoring skills, frequency, and accuracy of self-monitoring of blood glucose levels, and self-reporting of dietary habits should be integral to diabetes management. Improving glycemic control in poorly controlled populations may provide cost savings because it reduces healthcare costs associated with both short- and long-term complications of diabetes.

Active and effective dietary education can prevent diabetes and its complications. A study conducted by Shah et al.[22] in India found that 63% of patients with T2D did not know what diabetes was, and the majority were also unaware of its complications. According to a study conducted by Bani[40] in Saudi Arabia, most patients (97.3% of men and 93.1% of women) were unaware of the importance of diabetes monitoring.

In our study, patients were not offered any education regarding physical activity and its importance in preventing complications. Many cross-sectional as well as prospective, and retrospective studies have found a significant association between physical inactivity and T2D, so intensive lifestyle interventions on these issues (diet and physical activity) may prevent or delay the development of diabetes among people at high risk.

Conclusion

Our findings may provide practical guidance for interventions in nutrition and education, as the general patterns of behavior that change as a result of dietary intake, physical activity, and self-management can be easily interpreted by the general population as their health status. Diabetes awareness and management continue to be significant challenges faced by healthcare providers and patients in Kosovo. Patients in Kosovo do not go for regular eye, heart, and kidney examinations and only visit the doctor when they are sick, so there is a need to increase awareness of the complications of diabetes and consequently improve nutrition knowledge, attitudes, and practices. There is a gap between the patient's level of knowledge and their practice. The benefits of early detection of T2D through screening in the general population are needed in Kosovo.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Population Health

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Surgical Treatment of Uterine Leiomyomas from 2016-2022 in the Republic of Kazakhstan

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Abstract

Background: Due to the transition to a mandatory health insurance system (MHIS) and also taking into account the impact of the COVID-19 pandemic, the purpose of this study was to evaluate the surgical treatment of uterine leiomyoma in Kazakhstan in recent years, taking into account the ratio of uterine-preserving surgery (UPS) and hysterectomy.

Methods and Results: This epidemiological study was carried out upon the request of the Republican E-Health Center (RCEZ) to assess the dynamics of surgical treatment of uterine leiomyomas (UL) in Almaty at the expense of the Republican budget from 2016 to 2022. Since 2016, by 2022 there was a decrease in the total number of treated cases of UL at the expense of the Republican budget (from 1,478 to 1,063). From 2016 to 2019, there were statistically significantly more hysterectomies, whereas from 2020 to 2022 the number of uterine-preserving surgeries increased statistically significantly. The number of laparoscopic myomectomy and hysteroscopic myomectomy for treating submucous leiomyomas increased statistically significantly from 2016 to 2019. However, there were no statistical differences in the number of treated cases with this technique from 2020 to 2022.

Conclusion: Although high-tech fibroid surgery has been performed more frequently in recent years, these numbers are insufficient today, which justifies the need to train more specialists in advanced technologies. **(International Journal of Biomedicine. 2024;14(1):118-121.)**

Keywords: uterine leiomyomas • myomectomy • uterine-preserving surgery

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Abbreviations

HM, hysteroscopic myomectomy; **LM**, laparoscopic myomectomy; **MHIS**, mandatory health insurance system; **SL**, submucous leiomyomas; **UL**, uterine leiomyomas; **UPS**, uterine-preserving surgery.

Introduction

Due to the high prevalence of submucous leiomyoma (SL) of the uterus, myomectomy is one of the most frequent operations in the structure of gynecological surgery.[1,2] Today, there is a tendency in the world to seek uterine-preserving surgery (UPS) in the absence of cancer risks for women not only of reproductive age.[1-3] Laparoscopic access with temporary occlusion of the uterine arteries is the method of choice with promising results in efficacy, safety, reduction of recurrence, and fertility preservation.[4-7]

Since 2020, the Republic of Kazakhstan has introduced an MHIS (mandatory health insurance system) where uterine leiomyomas (UL) are also treated. During the COVID-19 pandemic, the number of planned hospitalizations for surgical treatment was forced to decrease. The limited budget allocated from the Republican budget for treatment under MHIS and the pandemic impacted the number of planned hospitalizations; today, this procedure is often performed at the expense of fee-based medical services. We consider it important to assess

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the dynamics of surgical treatment of UL at the expense of the Republican budget in recent years, especially the ratio of UPS and hysterectomy, to assess the level of health-services delivery for women with UL. This information is of great importance for understanding the development of a further strategy to improve the effectiveness of health-services delivery.

Materials and Methods

This epidemiological study was carried out upon the request of the Republican E-Health Center (RCEZ). This study aimed to assess the dynamics of surgical treatment of UL in Almaty at the expense of the Republican budget from 2016 to 2022.

Inclusion criteria were surgical treatment cases in in-patient care facilities in Almaty according to the following ICD-10 codes: D25.0 Submucous leiomyoma of uterus, D25.1 Intramural leiomyoma of uterus, D25.2 Subserous leiomyoma of uterus, for the period from 2016 to 2022.

Exclusion criteria were the period up to 2016, surgical treatment of UL in other cities of the Republic of Kazakhstan, and nonsurgical treatment of UL.

Statistical analysis was performed using the statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages. Group comparisons were performed using Pearson's chi-squared test, and the strength of the relationship between the features (effect size) was assessed through the evaluation of Cramer's V values. *P*-values less than 0.05 were considered significant.

Results

According to the data obtained from the RCEP, there was a decrease in the total number of treated UL cases at the expense of the Republican budget (Table 1). There was also a decrease in cases of SL treatment, which was statistically significant when comparing the number of cases in 2022 with 2016, 2017, and 2018 ($P_{(16–22)}$<0.001 (V=0.196, week), $P_{(17–22)}$<0.001 (V=0.215, medium), $P_{(18–22)}$<0.001 (V=0.173, weak)), as well as when comparing the number of cases in 2021 with 2016, 2017, and 2018 ($P_{(16–21)}$<0.001 (V=0.098, insignificant), $P_{(17–21)}$<0.001 (V=0.262, medium), $P_{(18–21)}$<0.001 (V=0.221, medium)) (Table 1). When pairwise comparing cases of SL from other years, no statistically significant differences were found.

From 2016 to 2018, there were no statistically significant dynamics of hysterectomy. Starting in 2019, there was a statistically significant decrease in hysterectomy numbers until 2021; however, in 2022, there was again a statistically significant increase in the number of hysterectomies compared to 2021; nevertheless, in 2022, the number of hysterectomies was significantly less than in other years ($P_{(16-22)}$<0.001 (V=0.154, weak), $P_{(17–22)}$<0.001 (V=0.192, weak), $P_{(18–22)}$ <0.001 (V=0.218, medium), $P_{(19–22)}$<0.001 (V=0.079, insignificant), $P_{(20–22)}$=0.007 (V=0.056, insignificant), $P_{(21–22)}$<0.001 (V=0.149, weak), $P_{(16–21)}$<0.001 (V=0.297, medium), $P_{(17-21)}$<0.001 (V=0.262, medium), $P_{(18–21)}$<0.001 (V=0.335, medium), $P_{(19–21)}$<0.001 (V=0.306, medium), $P_{(20–21)}$=0.016 (V=0.204, medium), $P_{(18–22)}$<0.001 (V=0.218, medium), $P_{(16–20)}$<0.001 (V=0.100, weak), $P_{(17–20)}$<0.001 (V=0.138, weak), $P_{(18–20)}$<0.001 (V=0.165, weak), $P_{(19–20)}$<0.001 (V=0.106, weak), $P_{(19–18)}$=0.003 (V=0.06, insignificant), $P_{(19–18)}$=0.003 (V=0.06, insignificant)) (Table 1).

The number of laparoscopic myomectomy (LM) and hysteroscopic myomectomy (HM), minimally invasive surgical procedures for treating SL, increased statistically significantly when pairwise comparing all cases overall years; however, there were no statistical differences in the number of treated cases using these techniques from 2020 to 2022 ($P_{(19–22)}$ <0.001 (V=0.240, medium), $P_{(18–22)}$<0.001 (V = 0.282, medium), $P_{(17–22)}$<0.001 (V=0.363, medium), $P_{(16–22)}$<0.001 (V=0.371, medium), $P_{(19–21)}$<0.001 (V=0.241, medium), $P_{(18–22)}$<0.001 (V=0.283, medium), $P_{(17–22)}$<0.001 (V=0.362, medium), $P_{(16–22)}$ <0.001 (V=0.364, medium), $P_{(19–20)}$<0.001 (V=0.209, medium), $P_{(18–20)}$<0.001 (V=0.227, medium), $P_{(17–20)}$<0.001 (V=0.308, medium), $P_{(16–20)}$<0.001 (V=0.316, medium), $P_{(16–18)}$<0.001 (V=0.089, insignificant), $P_{(17–18)}$ <0.001 (V=0.099, insignificant), $P_{(16–19)}$<0.001 (V=0.110, weak), $P_{(17–19)}$<0.001 (V=0.112, weak)) (Table 1).

As for other UPS, their number decreased statistically significantly in 2022, compared to previous years. In 2016, the number of other UPS was statistically significantly higher than in other years; there were no statistically significant differences by years from 2017 to 2021 ($P_{(21–22)}$<0.001 (V=0.141, weak), $P_{(20–22)}$=0.013 (V=0.050, insignificant), $P_{(19–22)}$=0.034 (V=0.044, insignificant), $P_{(17–22)}$<0.001 (V=0.093, insignificant), $P_{(16–22)}$<0.001 (V=0.134, weak), $P_{(16–17)}$=0.038 (V=0.039, insignificant), $P_{(16–18)}$<0.001 (V=0.120, weak), $P_{(16–19)}$<0.001 (V=0.089, insignificant), $P_{(16–20)}$<0.001 (V=0.138, weak)) (Table 1).

When comparing the number of hysterectomies and UPS for each year, it was found that from 2016 to 2019, there were statistically significantly more hysterectomies. In contrast, from 2020 to 2022, UPS increased statistically significantly (2016: *P*<0.001 (V=0.072, insignificant); 2017: *P*<0.001 (V=0.147, weak); 2018: *P*<0.001 (V=0.201, medium); 2019: *P*<0.001 (V=0.082, insignificant); 2020: *P*<0.001 (V=0.129, weak); 2021: *P*<0.001 (V=0.516, relatively strong); 2022: *P*<0.001 (V=0.240, medium)) (Table 1). When comparing LM or HM for treating SL with other organ-preserving techniques, other techniques were statistically significantly more often performed until 2019. From 2020 to 2021, other UPS were performed more often without statistical differences. In 2022, LM or HM for treating SL was performed more often than other UPS, but no statistical differences were found (2016: *P*<0.001 (V=0.752, strong); 2017: *P*<0.001 (V=0.738, strong); 2018: *P*<0.001 (V=0.469, relatively strong); 2019: *P*<0.001 (V=408, relatively strong) (Table 1).

Discussion

Since 2016, by 2022 there was a decrease in the total number of treated cases of UL at the expense of the Republican budget (from 1,478 to 1,063), which is associated with the transition to MHIS and with the period of the COVID-19 pandemic, when the number of planned operations had to be reduced due to quarantine.

Table 1.

Surgical treatment of UL at the expense of Mandatory Social Health Insurance (MSHI) funds in Almaty (2016–2022).

Treated cases	Period						
	2016	2017	2018	2019	2020	2021	2022
Total treated cases	1478	1322	1271	1251	1295	1174	1063
SL	629 (42.5%)	585 (44.3%)	507 (39.9%)	317 (25.3%)	369 (28.5%)	230 (19.6%)	251 (23,6%)
P-value	$P_{(16-22)}$<0.001 (V=0.196, weak); $P_{(17-22)}$<0.001 (V=0.215, medium); $P_{(18-22)}$<0.001 (V=0.173. weak); $P_{(16-21)}$<0.001 (V=0.098, insignificant); $P_{(17-21)}$<0.001 (V=0.262. medium); $P_{(18-21)}$<0.001 (V=0.221, medium)						
Hysterectomy	792 (53.6%)	758 (57.3%)	763 (60.03%)	677 (54.1%)	564 (43.5%)	284 (28.1%)	404 (38%)
P-value	$P_{(16-22)}$<0.001 (V=0.154, weak); $P_{(17-22)}$<0.001 (V=0.192, weak); $P_{(18-22)}$<0.001 (V=0.218. medium); $P_{(19-22)}$<0.001 (V=0.079, insignificant); $P_{(20-22)}$=0.007 (V=0.056, insignificant); $P_{(21-22)}$<0.001 (V=0.149, weak); $P_{(16-21)}$<0.001 (V=0.297, medium); $P_{(17-21)}$<0.001 (V=0.262, medium); $P_{(18-21)}$<0.001 (V=0.335, medium); $P_{(19-21)}$<0.001 (V=0.306, medium); $P_{(20-21)}$=0.016 (V=0.204, medium); $P_{(18-22)}$<0.001 (V=0.218, medium); $P_{(16-20)}$<0.001 (V=0.100, weak); $P_{(17-20)}$<0.001 (V=0.138, weak); $P_{(18-20)}$<0.001 (V=0.165, weak); $P_{(19-20)}$<0.001 (V=0.106, weak); $P_{(19-18)}$=0.003 (V=0.06. insignificant); $P_{(19-18)}$=0.003 (V=0.06, insignificant).						
Total UPS	686 (46.4%)	564 (42.7%)	508 (39.97%)	574 (45.9%)	731 (56.5%)	890 (71.9%)	659 (62%)
P-value (Hysterectomy vs. UPS)	2016: *P*<0.001 (V=0.072, insignificant); 2017: *P*<0.001 (V=0.147, weak); 2018: *P*<0.001 (V=0.201, medium); 2019: *P*<0.001 (V=0.082, insignificant); 2020: *P*<0.001 (V=0.129, weak); 2021: *P*<0.001 (V=0.516, relatively strong); 2022: *P*<0.001 (V=0.240, medium).						
Other UPS	601 (40.7%)	490 (37.1%)	373 (29.3%)	404 (32.3%)	359 (27.7%)	490 (41.7%)	300 (28.2%)
P-value	$P_{(21-22)}$<0.001 (V=0.141, weak); $P_{(20-22)}$=0.013 (V=0.050, insignificant); $P_{(19-22)}$=0.034 (V=0.044, insignificant); $P_{(17-22)}$<0.001 (V=0.093, insignificant); $P_{(16-22)}$<0.001 (V=0.134, weak); $P_{(16-17)}$=0.038 (V=0.039, insignificant); $P_{(16-18)}$<0.001 (V=0.120, weak); $P_{(16-19)}$<0.001 (V=0.089, insignificant); $P_{(16-20)}$<0.001 (V=0.138, weak).						
LM or HM for treating SL	85 (5.7%)	74 (5.6%)	135 (10.6%)	170 (13.6%)	372 (28.7%)	400 (34.1%)	359 (33.8%)
P-value	$P_{(19-22)}$<0.001 (V=0.240, medium); $P_{(18-22)}$<0.001 (V=0.282, medium); $P_{(17-22)}$<0.001 (V=0.363, medium); $P_{(16-22)}$<0.001 (V=0.371, medium); $P_{(19-21)}$<0.001 (V=0.241, medium); $P_{(18-22)}$<0.001 (V=0.283, medium); $P_{(17-22)}$<0.001 (V=0.362, medium); $P_{(16-22)}$<0.001 (V=0.364, medium); $P_{(19-20)}$<0.001 (V=0.209, medium); $P_{(18-20)}$<0.001 (V=0.227, medium); $P_{(17-20)}$<0.001 (V=0.308, medium); $P_{(16-20)}$<0.001 (V=0.316, medium); $P_{(16-18)}$<0.001 (V=0.089, insignificant); $P_{(17-18)}$<0.001 (V=0.099, insignificant); $P_{(16-19)}$<0.001 (V=0.110, weak); $P_{(17-19)}$<0.001 (V=0.112, weak).						
P-value (LM or HM vs. other organ-preserving techniques)	2016: *P*<0.001 (V=0.752, strong); 2017: *P*<0.001(V=0.738, strong); 2018: *P*<0.001 (V=0.469, relatively strong); 2019: *P*<0.001 (V=408, relatively strong)						

Surgical treatment of UL according to the mentioned ICD-10 codes (D25.0 Submucous leiomyoma of the uterus; D25.1 Intramural leiomyoma of the uterus; D25.2 Subserous leiomyoma of uterus) was carried out at the expense of MHIS funds. As a rule, a certain amount is allocated for the organization at the beginning of the year and distributed for each month, so it is not possible to perform operations more than the amount issued; therefore, in recent years, this type of surgical treatment has been carried out at the expense of fee-based medical services, either at the expense of the guaranteed volume of free medical care (GVFMC) funds, in the presence of concurrent endometriosis, or bleeding. By 2022, the number of SL cases had decreased from 629 cases per year in 2016 to 251 in 2022, which may also be because the bleeding occurs more often at submucous locations, and assistance is provided at the expense of GVFMC funds, as well as based on fee-based medical services.

Our analysis showed that the number of all UPSs did not change much in dynamics; however, when comparing the number of hysterectomies and UPSs for each year, it was found that from 2016 to 2019, there were statistically significantly more hysterectomies, whereas from 2020 to 2022 the number of UPSs increased statistically significantly.

This trend is positive since today the ability to preserve an organ is a priority in gynecology, even for women who have performed a reproductive function or are in the menopausal

period; this is positive from the position of better blood supply to the ovaries in cases where it is possible to preserve them and preserve the integrity of the connective tissue structures of the pelvis, blood vessels, and nerves.

However, these results should be treated with caution since radical operations with concomitant uterine fibroids can be performed at the expense of GVFMC funds and encoded according to another basic code, which limits the opportunity for objective assessment.

Thus, there is also a positive trend in using high-tech techniques; the number of laparoscopic myomectomy and hysteroscopic myomectomy for treating submucous leiomyomas increased statistically significantly from 2016 to 2019. However, there were no statistical differences in the number of treated cases with this technique from 2020 to 2022. Although high-tech fibroid surgery has been performed more frequently in recent years, these numbers are insufficient today, which justifies the need to train more specialists in advanced technologies.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Adolescent Health

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Levels of Stress, Anxiety and Depression among Students at Alma Mater Europaea Campus College "Rezonanca," Kosovo

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Abstract

Background: This study aims to assess the prevalence of psychological well-being, the level of psychological stress and perceived anxiety among dental students at the Alma Mater Europaea Campus College "Rezonanca."

Methods and Results: We evaluated the stress and anxiety levels of 70 students in the fourth year of dentistry studies at the Alma Mater Europaea Campus College "Rezonanca" in Kosovo in the school year 2022/2023. The distribution of students was equal in terms of gender. Participants were surveyed using the DASS-21 questionnaire. A questionnaire with 25 questions was used to determine the most frequent causes of stress. This questionnaire was distributed to the students at the same time as the DASS-21 questionnaire. The questionnaire had 25 questions about stress. Students had the opportunity to choose the level of stress for the respective statement from 1 to 4. The evaluation was done on a Likert scale. Then, the answers were converted into points by multiplying the given answer by 10.

There was no statistically significant difference among female and male students on the mean points of depression, anxiety, and stress sub-scales. At the normal range of depression scores were 78.6% of students, followed by 67.1% for normal anxiety levels, and 92.9% for normal stress scores. We found no statistically significant difference in the distribution of the severity rating of depression, anxiety, and stress scores between female and male students.

Conclusion: Dental students are exposed to various sources of stress. Stress in DS begins before they are accepted into the dental program and continues until they graduate. To reduce the stress levels among dental students, it is recommended that dental faculties, their clinical mentors, their peers, and colleagues make an effective plan to reduce stress among their students. **(International Journal of Biomedicine. 2024;14(1):122-126.)**

Keywords: anxiety • depression • stress • student

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Introduction

Recent studies have shown that the study process at the faculty of dentistry can be highly stressful for students who should acquire theoretical knowledge and various abilities, such as clinical and communication skills.(1) The Dentistry Department at the Faculty of Medicine at the University of Pristina can enroll a very limited number of students, so the level of competition is extremely high, and only the best students with the highest grades and performance indicators during their high school and on entrance exams are accepted. Competition continues throughout their studies as high grades and above-average performance represent value and success. Dental students feel more stressed about time management, mastering the volume of the study materials presented, and inconsistent feedback from faculty members. Students also must learn the technical skills they should master.(2)

Dental studies can be quite stressful and impact dentistry students' overall physical and mental well-being.(3) The continuous demand to reach perfection and stability while working on a patient seated in a dental chair,

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under the surveillance of other students, clinical mentors, and professional staff, is also one of the main causes of psychological stress. The psychological stress of collaborating with frightened patients can also be the leading cause of influence on the cardiovascular system, manifesting itself with increased heart rate, high blood pressure, sweating, and hand shivering, as well as the feeling of being scolded and experiencing an anxiety attack.

Such stress starts as early as the first year of studies. The prevalence of anxiety among dental students ranges in different countries from 7.7%-65.5%.[4] The perception of stress is due to dental students' tendency toward perfectionism based on their history of high achievement and excellence in previous school years and the fact that excellence is the norm in dental school.

Students with a positive family history of depression and anxiety and individuals who had lost a close relative in the previous years were more likely to have psychological disorders and be anxious than other individuals.[5] Economic difficulties can also affect anxiety levels.[4] There has been shown an association between sociodemographic data and the frequency of symptoms of depression, anxiety, and stress among university students in a medical college. The findings showed that females, university campus residents, pre-clinical students, and students with lower academic achievement had higher anxiety rates than the other groups.[6] Other issues that can have a significant impact on the mental and emotional well-being of dental students and stimulate anxiety among them are long working and study hours, the pressure to master their medical knowledge, competition with their peers, and insufficient time for non-academic activities.

All these factors can be summarized as three main ones: academic performance, pressure to succeed, and plans after graduation.[3] Further studies are required to determine the factors related to mental health, including anxiety, which affects the academic performance of dental and medical students. Stressed students can be helped by either reducing the number of stressors or increasing their coping skills related to stress. Reducing the number of stressors can be achieved in several ways, such as reducing the fear of failure and the pressure of the workload due to exams and course requirements. In addition, the content of the dental curriculum may be reduced, or its design may be changed.[1] Stress cannot be eliminated from dental practice. However, it should be minimized as much as possible to avoid numerous physical and emotional problems. A review of the literature reveals that there are a number of instruments designed to assess self-reported somatic symptoms related to mental health.[7]

Dentists experience considerable amounts of occupational stress beginning with their undergraduate years in dental school that can negatively affect these individuals' personal and professional lives and the quality of their clinical work.

We sought to create an objective scale to assess stress levels in students at dissimilar stages of their education and in practicing physicians. This study aims to assess the prevalence of psychological well-being, the level of psychological stress and perceived anxiety among dental students at the Alma Mater Europaea Campus College "Rezonanca."

Materials and Methods

We evaluated the stress and anxiety levels of 70 students (35 men and 35 women) in the fourth year of dentistry studies at the Alma Mater Europaea Campus College "Rezonanca" in Kosovo in the school year 2022/2023. The distribution of students was equal in terms of gender. Participants were surveyed using the Depression Anxiety Stress Scale 21 (DASS-21) questionnaire. Preliminary tests suggested that the DASS-21 has adequate convergent and discriminant validity.[8] The DASS-21 has demonstrated satisfactory psychometric properties and is comparable to other reliable assessment scales. It includes three self-report scales designed to measure emotional states of depression, anxiety, and stress.

A questionnaire with 25 questions was used to determine the most frequent causes of stress. This questionnaire was distributed to the students at the same time as the DASS-21 questionnaire. The questionnaire had 25 questions about stress. Students had the opportunity to choose the level of stress for the respective statement from 1 to 4. The evaluation was done on a Likert scale. Then, the answers were converted into points by multiplying the given answer by 10. The average calculation was done for each question separately. The question with the highest average was considered more stressful. The questionnaire was divided into seven dimensions: Faculty and administration (questions 12, 18, and 19), Academics (questions 1, 2, 3 and 4), Manual skills (questions 6 and 10), Financial obligations (question 21), Patient care (questions 5, 7, 8 and 11), Personal problems (13,14,15,16,17,22 and 25), and Family (questions 19,20,23 and 24). Each dimension was calculated by taking the average of all dimension questions. Reliability analysis was done for the Albanian language version, and it came out acceptable (Cronbach's alpha of 0.903).

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables and mean ± SD for continuous variables. Inter-group comparisons were performed using Student's t-test. Group comparisons concerning categorical variables are performed using the chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results

There was no statistically significant difference among female and male students on the mean points of depression, anxiety, and stress sub-scales ($P>0.05$) (Table 1). At the normal range of depression scores were 78.6% of students, followed by 67.1% for normal anxiety levels, and 92.9% for normal stress scores ($P>0.05$) (Table 2). We found no statistically significant difference in the distribution of the severity rating of depression, anxiety, and stress scores between female and male students ($P>0.05$) (Tables 3-5).

The five questions with the highest mean scores in order were: "Stress due to exams and grades," "Stress due to patient care responsibilities," "Stress due to difficulties in learning clinical procedures," "Stress due to other personal problems," and "Stress due to class assignments." (Table 6).

Table 1.

Stress, anxiety and depression by gender.

	Total (n=70)	Women (n=35)	Men (n=35)	*P*-value
Depression	6.06±4.74	6.09±4.39	6.03±5.12	0.958
Anxiety	6.34±4.82	6.47±4.11	6.22±5.47	0.830
Stress	7.37±4.83	7.23±4.40	7.50±5.25	0.816

Table 2.

Degrees of stress, anxiety, and depression

Category	Depression		Anxiety		Stress	
	n (%)	*P*-value	n (%)	*P*-value	n (%)	*P*-value
Normal	55 (78.6)	0.000	47 (67.1)	0.000	65 (92.9)	0.000
Mild	9 (12.9)		7 (10.0)		3 (4.3)	
Moderate	6 (8.6)		10 (14.3)		2 (2.9)	
Severe	0 (0)		5 (7.1)		0 (0)	
Extreme severe	0 (0)		1 (1.4)		0 (0)	

Table 3.

Distribution of the severity rating of depression between female and male students

Depression			
Category	Women, n (%)	Men, n(%)	*P*-value
Normal	27 (77.1)	28 (80.0)	0.937
Mild	5 (14.3)	4 (11.4)	
Moderate	3 (8.6)	3 (8.6)	

Table 4.

Distribution of the severity rating of anxiety between female and male students

Anxiety			
Category	Women, n (%)	Men, n (%)	*P*-value
Normal	24 (68.6)	23 (65.7)	0.850
Mild	4 (11.4)	3 (8.6)	
Moderate	5 (14.3)	5 (14.3)	
Severe	2 (5.7)	3 (8.6)	
Extreme severe	0(0.0)	1 (2.8)	

Table 5.

Distribution of the severity rating of stress between female and male students

Stress			
Category	Women, n (%)	Men, n (%)	*P*-value
Normal	32 (91.4)	33 (94.3)	0.840
Mild	2 (5.7)	1 (2.9)	
Moderate	1 (2.8)	1 (2.8)	

Table 6.

Frequencies and percentages of the highest stress score by questions

	Mean
Stress due to class assignments	19.14
Stress due to difficult tasks in class	16.71
Stress due to exams and grades	29.28
Stress due to competition among students	16.43
Stress due to patient care responsibilities	24.57
Stress due to difficulties in learning clinical procedures	20.86
Stress due to the patients' attitude towards me	17.71
Stress due to patients' attitudes towards dentistry	17.28
Stress from the atmosphere created by the clinic's professors	17.43
Stress from the difficulty of teaching precise manual skills in preclinical and practice	17.86
Stress due to the reliability of dental laboratories for the return of cases	18.14
Stress due to administrative responses to student needs	16.28
Stress due to sleeping in a room with a friend	14.00
Stress due to conflicts in the kite	14.00
Stress due to alcohol consumption	13.00
Stress due to drug use	13.57
Stress due to the reevaluation of dentistry as a career choice	15.00
Stress from the fear of being expelled from the faculty	14.28
Stress due to marriage	15.71
Stress due to childcare	15.71
Stress due to financial responsibilities	17.28
Stress due to personal physical health	17.71
Stress due to the health of other family members	18.57
Stress due to student-parent relationship	16.57
Stress because of other personal problems	19.57

The male-to-female student ratio was 50/50 (Table 7) No significant statistical difference was found between men and women in the average stress points according to the dimensions (Table 8). The highest average stress points were "Academics," "Patient care," and "Manual skills." When analyzed separately for men and women, in men, the highest average stress points were "Manual skills," "Academic skills," and "Patient care", while in women, they were "Academics," "Patient care," and "Manual skills" but without statistically significant differences (Table 9).

Table 7.

Percentage by gender

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	35	50.0	50.0	50.0
	Male	35	50.0	50.0	100.0
	Total	70	100.0	100.0	

Table 8.

Frequencies and percentages of the average stress points by questions and gender

	Total n (%)	Men n (%)	Women n (%)
Stress due to class assignments	6 (8.6)	4 (11.4)	2 (5.7)
Stress due to difficult tasks in class	2 (2.9)	1 (2.9)	1 (2.9)
Stress due to exams and grades	29 (41.4)	12 (34.3)	17 (48.6)
Stress due to competition among students	6 (8.6)	3 (8.6)	3 (8.6)
Stress due to patient care responsibilities	19 (27.1)	9 (25.7)	10 (28.6)
Stress due to difficulties in learning clinical procedures	11(15.7)	6 (17.1)	5 (14.3)
Stress due to the patients' attitude towards me	5 (7.1)	3(8.6)	2(17.1)
Stress due to patients' attitudes towards dentistry	5 (7.1)	3 (8.6)	2 (5.7)
Stress from the atmosphere created by the clinic's professors	8 (11.4)	3 (8.6)	5 (14.3)
Stress from the difficulty of teaching precise manual skills in preclinical and practice	10 (14.3)	5 (14.3)	5 (14.3)
Stress due to the reliability of dental laboratories for the return of cases	9 (12.9)	5 (14.3)	4 (11.4)
Stress due to administrative responses to student needs	7 (10.0)	4 (11.4)	3 (8.6)
Stress due to sleeping in a room with a friend	2 (2.9)	1 (2.9)	1 (2.9)
Stress due to conflicts in the kite	3 (4.3)	2 (5.7)	1 (2.9)
Stress due to alcohol consumption	3 (4.3)	2 (5.7)	1 (2.9)
Stress due to drug use	4 (5.7)	1 (2.9)	3 (8.6)
Stress due to the reevaluation of dentistry as a career choice	5 (7.1)	3 (8.6)	2 (5.7)
Stress from the fear of being expelled from the faculty	4 (5.7)	2 (5.7)	2 (5.7)
Stress due to marriage	7 (10.0)	3 (8.6)	4 (11.4)
Stress due to childcare	5 (7.1)	2 (5.7)	3 (8.6)
Stress due to financial responsibilities	6 (8.6)	2 (5.7)	4 (11.4)
Stress due to personal physical health	6 (8.6)	3 (8.6)	3 (8.6)
Stress due to the health of other family members	8 (11.4)	3 (8.6)	5 (14.3)
Stress due to student-parent relationship	4 (5.7)	3 (8.6)	1 (2.9)
Stress because of other personal problems	7 (10.0)	3 (8.6)	4 (11.4)

Table 9.

The dimensions with the average stress points

	Total	Men	Women	*P*
Faculty and administration	16.00±6.75	16.09±7.02	15.90±6.57	0.907
Academics	20.39±6.49	19.36±6.92	21.43±5.95	0.184
Manual skills	19.36±8.88	20.00±8.99	18.71±8.86	0.547
Financial obligations	17.28±10.20	17.71±9.73	16.86±10.78	0.730
Patient care	19.43±7.70	18.43±8.49	20.43±6.79	0.280
Personal problems	15.26±5.89	14.90±6.02	15.63±5.82	0.608
Family	16.64±6.63	15.93±6.62	17.36±6.67	0.371

Discussion

Dental education can be a significant source of stress among dental students, and research studies have observed higher levels of stress among dental students than in the general population.[6] A part of the literature that examines stress in students has revealed a significant increase in stress that intensifies with the year of study.[8] Students who experience stress, anxiety, or depression often find it difficult to meet the expectations of the dental curriculum and personal goals for their chosen career. In any profession, transitioning from student to graduation can be challenging.

Students may face psychological stress early in their careers. Initial signs and symptoms of anxiety or depression should be addressed as soon as possible.[9] This is related to the responsibility for the well-being of their patients. Numerous studies have concluded that dentistry students have more stress than any other professional education. In the study conducted on dental professionals by Basudan et al.,[3] 55.9% of respondents had abnormal levels of depression, 66.8% had anxiety, and 54.7% had stress. Severe and extremely severe scores for depression, anxiety, and stress were reported in 20.2%, 34.0%, and 20.2% of respondents.

Committed to the best care for patients, the student may be concerned about professional responsibility and ethical aspects if one part of their treatment plan goes wrong. In a study published in NCBI, a level of depression, anxiety, and stress was observed in 55.9%, 66.8%, and 54.7% of respondents.[10,11]

Alarmingly, severe and extremely severe scores for depression, anxiety, and stress were reported by 20.2%, 34.0%, and 20.2% of respondents.

In a study of fourth-year Greek dental students, most were worried about their professional future and the lack of time off.[12] According to them, the stresses perceived by dental students were related to individual and educational (type of curriculum, competition, and cost of education) factors.[13] In addition, the causes of stress for dental students depend on the length of the studies. It was clarified that there is a lot of anxiety about the exams. [14]

In conclusion, dental students are exposed to various sources of stress. Stress in dental students begins before they

are accepted into the dental program and continues until they graduate. To reduce the stress levels among dental students, it is recommended that dental faculties, their clinical mentors, their peers, and colleagues make an effective plan to reduce stress among their students.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Antimicrobial Resistance

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Detection of Novel *spa* Types in Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* in Iraq

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium that is an opportunistic pathogen, causing infections in hospital settings and communities. MRSA has become a significant and increasing problem in Iraq. The aim of this study was to evaluate the genetic mutations of MRSA strains, especially in the *spa* gene, from patients in Wassit, Iraq.

Methods and Results: Biochemical tests were conducted to identify *S. aureus* isolates and then on MRSA. The MRSA was identified by the Chrome Agar method and confirmed by PCR with genotyping of the *mecA* gene. The disk diffusion method was used to detect antibiotic resistance to three different common antibiotics used at Wassit hospitals. The Vitek-2 compact system was utilized for the detection of the minimum inhibitory concentration (MIC) of vancomycin. All MRSA strains in this study were tested to screen the *mecA* gene, with 21 strains subjected to the molecular typing method for the *spa* gene. Out of 166 samples, 132(79.5%) contained *S. aureus* and 34(20.4%) were identified as MRSA. Genotyping showed that out of 34 MRSA, 31(91.2%) isolates were *mecA*-positive. The *spa* gene was detected in 21(61.8%) isolates out of 34 MRSA samples. The *spa* typing of 21 MRSA samples revealed four different *spa* types, as follows: t386 (3/14.3%), t3576 (1/4.8%), t10002 (1/4.8%), and t10234 (1/4.8%). High polymorphism rates were shown in isolates of *spa* type t386.

Conclusion: Our data represent the first report to detect novel mutations in the *spa* gene in the MRSA clinical isolates from Wassit hospitals, Iraq.**(International Journal of Biomedicine. 2024;14(1):127-133.)**

Keywords: methicillin-resistant *Staphylococcus aureus* • *mecA* • *spa* type

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Abbreviations

MIC, minimum inhibitory concentration; **MRSA**, methicillin-resistant *Staphylococcus aureus*

Introduction

Staphylococcus aureus is the main pathogen that infects humans.[1] Hospital-acquired methicillin-resistant *S. aureus* (MRSA) is accountable for the worldwide spread of MRSA.[2] This pathogen has several virulence factors, such as staphylococcal protein A (spa), with multidrug resistance ability.[3]

However, it should be noted that the detection of resistance via phenotypic procedures remains prevalent, and the amount of available information about the molecular characterization of clinical MRSA strains is relatively limited.[4] MRSA strains resistant to multiple antibiotics are considered a significant challenge to the efficacy of relevant drug therapy. It is imperative to investigate the prevalence of these types of MRSA that are resistant to treatment.[5] Genotypic techniques can quickly and reliably classify the interrelatedness of clinical isolates. These techniques have a promising role in detecting eruptions and monitoring isolates spreading through specific regions.[6]

The mutation in the *spa* gene of this bacteria results from the diversity of the site in chromosome called the x region in the bacteria, and the typing method relies on varying genetic elements at area x in the *spa* gene.[7] The *spa* gene that determines virulence is intimately linked to the mechanism

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and potency of infection. This marker exhibits a high degree of variability and offers valuable insight into strain.[8]

Risks associated with MRSA colonization and infections are no longer restricted to hospitals; therefore, sequence typing and *S. aureus* protein A (spa) detection, which can help track outbreaks, are useful for identifying MRSA strains and colonization sources, and for differentiating between infections in the community and in the hospital. The development of community-associated MRSA has altered MRSA epidemiology. Genetically, hospital infection differs from community infection in that the latter is more sensitive to non-lactam antibiotics.[9] The molecular features of MRSA can exhibit variability across hospitals located in the same nation.[10] A big advantage of molecular methods such as sequencing DNA is that they give simple, fast results for huge amounts of data that can be easily utilized to understand the evolution of these strains.[11] The molecular *spa* typing method is a powerful tool for investigating and revealing genetic diversity, especially in multidrug-resistant MRSA.

The aim of this study was to evaluate the genetic mutations of MRSA bacteria, especially in the *spa* gene responsible for evading the immune system, which makes it difficult to treat patients in Wassit.

Materials and Methods

This study was carried out in Al-Aziziyah Hospital and Al-Suwaira Hospital (Wassit Governorate) in the period between August 2022 and May 2023.

A total of 207 non-duplicated strains of *S. aureus* were isolated from outpatients and patients upon admission to hospitals. Clinical specimens included urine, wounds, blood, abscesses, and sputum. When included in the study, demographic characteristics, diagnosis, and smoking habits were considered.

Collection of the different clinical samples

Samples were instantly transported to the Laboratory of Microbiology, Department of Medical Laboratory Techniques, and Middle Technical University. According to Bailey and Scott's Diagnostic Microbiology, blood agar plates were inoculated with collected samples and incubated for 24 hours at 37°C. A single colony was picked up from blood agar plates and inoculated on Mannitol Salt Agar for 18–24 hours at 37°C. Morphological and biochemical features were then conducted to identify the isolates. The biochemical tests used in this study were catalase and coagulase. Ultimately, *S. aureus* isolates were inoculated with Trypticase soy broth (Himedia, India) with 20% glycerol and kept at -80°C in the freezer until used for the following test.[12]

Diagnosis of MRSA by Chromogenic Modified Agar

To ensure the diagnosis of MRSA bacteria was cultured, the suspected colonies were inoculated on the chromogenic modified agar base (CAMB), including cefoxitin (30 µg) (CandaLab, Spain) as a supplement, and were aerobically incubated for one day at 35±2°C.[13]

Antibiotics Susceptibility Testing

All isolates were tested against vancomycin (30 µg), methicillin (10 µg), and oxacillin (1 µg) (Bioanalysis, Turkey) through the disk diffusion method, according to the CLSI 2010 recommendations.[14] The Vitek-2 system was used to detect the MIC for vancomycin and quality control for *S. aureus* ATCC 29213.

DNA extraction

The DNA was extracted from colonies of MRSA strains by a Wizard® genomic DNA Extraction kit based on the manufacturer's instructions. The purity of extracted DNA was measured by the nanodrop spectrophotometry technique at 260 nm, and the DNA samples were then stored at -18°C to keep samples when needed for use.

Detection of the mecA gene

For genetic identification, a specific primer to detect the *mecA* gene encoding for methicillin resistance (F: 5'ACGAGTAGATGCTCAATATAA3' and R: 5'-CTTAGTTCTTTAGCGATTGC-3') was used to amplify *mecA* loci in MRSA DNA. The amplicons were then run on agarose gel using electrophoresis and visualized by UV light using an ultra-violet transilluminator. The amplification of the *spa* gene for all MRSA isolates from different clinical samples according to the previous study. [15]

Detection of the spa gene

All isolates were subjected to the *spa* gene. Specific primers (*spa* 1 5'-ATCTGGTGGCGTAACACCTG-3' and s*pa* 2 5'-CGCTGCACCTAACGCTAATG-3') (Alpha, Canada) were for a variable region, amplifying a PCR product size of 1500 bp.

PCR reaction and conditions

The final volume is 25 µl, containing 12.5 µl of master mix (Taq-DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers), 5 µl of DNA, 1µ (10 pmol) forward and reverse primer, and 5.5µl of nuclease-free water (Promega, USA). run under the following conditions: initial denaturation at 95°C for 3 minutes for 35 cycles, followed by denaturation at 95°C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 1 minute for 32 cycles, followed by a final extension at 72°C for 1 minute. A total of 32 cycles, and then used the next step at 72°C for five minutes, while Spa used 55°C for 30 seconds in the annealing step. Next run-on agarose 1-1.5% (Sigma-Aldrich, USA) and the band of products visualized by a trans-illuminator.

DNA sequencing

According to the Macrogen Company requirement (Seoul, South Korea), 20 µl of spa gene PCR products of selected 34 MRSA isolates were sent for DNA sequencing for both strands. The primers used for DNA sequencing of the X region of the spa gene were as follows: spa-1113f 5'-TAAAGACGATCCTTCGGTGAGC-3', and spa-1514r 5'-CAGCAGTAGTGCCGTTTGCTT-3' (Oliveira et al. 2001).[16] The ABI 3730xl DNA Sequencer was used.

DNA sequence analysis

The sequences obtained were analyzed and aligned using the Ridom Staph Type program (Ridom, Würzburg, Germany). The *spa* typing and evaluation of *spa* types of *S. aureus* strains were performed using the *spa* database http://www.spaserver.ridom.de and http://spatyper.fortinbras.us/. The *spa* type phylogenetic tree was drawn using the Geneious11.1 Prime software (Auckland, New Zealand). There were evolutionary

relationships between genomic sequences of five local isolates, and they were matched with 12 global isolates of *S. aureus* in NCBI from clinical samples.

Documentation in NCBI

All molecular results of local selective *S. aureus* were sent to the NCBI on May 18, 2023, under accession numbers LC768797, LC768793, LC768796, LC768794, and LC768795 for five isolates of *S. aureus* named Amena, Dimashams, KarBane, Mayar, and Mohammed, respectively. They were then documented as five new allelic variations in the GenBank database (Maryland, USA) and certified on May 23, 2023

Results

Out of 207 participants, only 166 responded to our questions, making an answer rate of 80.2%. The participants were between 20 and 50 years old, with a median of 42. The age group of <40 years was the largest. Among 166 participants, women made up 60.8% and men 39.2%. Analysis of clinical conditions showed that pneumonia and urinary catheters were the most common: 27.1% and 24.1%, respectively (Table 1).

Table 1.

Demographic and clinical characteristics of the study participants

Variable	n	Percentage
Age, years		
< 40	98	59.0%
> 40	68	41.0%
Gender		
Males	65	39.2%
Females	101	60.8%
Smoker		
Yes	28	16.9%
No	138	83.1%
Clinical Characteristics		
Bacteremia	28	16.9%
Pneumonia	45	27.1%
Surgical wound infection	30	18.1%
Urinary catheter	40	24.1%
Meningitis	23	13.8%

Out of 166 samples, 132(79.5%) contained *S. aureus* and 34(20.4%) MRSA. Of the 34(20.4%) MRSA isolates, the maximum number were obtained from sputum samples (15/44.1%), followed by urine samples (9/26.5%), wound samples (7/20.6%), pus from abscesses (2/5.9%), and blood samples (1/2.9%) (Table 2). Also confirmed by the used subculture, isolates on the CMAB included cefoxitin. MRSA was maintained without contamination with other genera of bacteria. For more accuracy in the following procedures of phenotypic and genotypic assays, and based on the manufacturer's instructions, all MRSA isolates showed color rose to mauve of the colony after incubation for 24 hours (Figure 1).

Table 2.

Distribution of MRSA isolates between clinical samples.

Samples	n	*S. aureus*	MRSA
Urine	40	31 (23.5%)	9 (26.5%)
Blood	28	27 (20.4%)	1 (2.9%)
Wound	30	23 (17.4%)	7 (20.6%)
Pus from abscesses	23	21 (15.9%)	2 (5.9%)
Sputum	45	30 (22.7%)	15 (44.1%)
Total	166	132 (79.5%)	34 (20.5%)

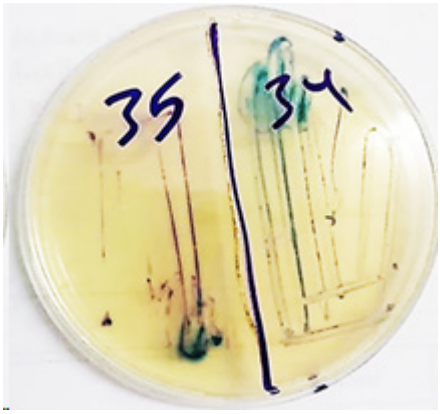

Fig. 1. *Left side: The mauve color shows the growth of MRSA isolates on CMAB media. Right side: The green color shows the growth of Staphylococcus. spp. on CMAB media.*

A phenotypic assay of antimicrobial resistance test for 34 isolates showed high resistance to methicillin and oxacillin in 32 strains of MRSA (94.1%). Genotyping showed that out of 34 MRSA, 31(91.2%) isolates were *mecA*-positive (Table 3, Fig.2).

Table 3.

Phenotypic and genotypic resistance testing of MRSA isolates.

Samples	n	Van	Oxa	Meth	*mecA*
Urine	10	9	10	10	5
Blood	3	3	4	3	3
Wound	5	2	1	1	7
Abscesses	2	4	3	4	4
Sputum	14	13	14	14	12
Total	34	33	32	32	31

Van = vancomycin, Oxa = oxacillin, Meth = methicillin

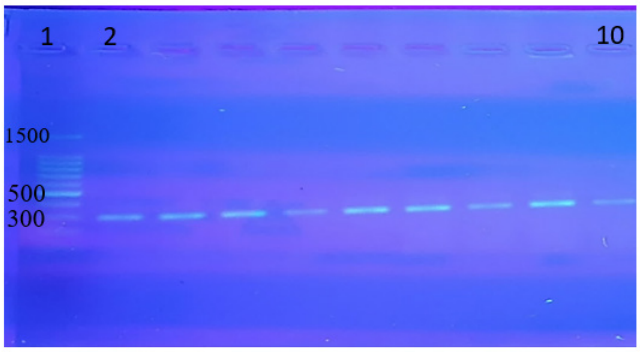

Fig. 2. *PCR products of mecA gene (300 bp) for MRSA isolates (Lanes 2-10); Lane 1 - DNA ladder (1500 bp).*

As for the vancomycin MIC value <0.063-16 µg/ml, at breakpoints ≤2/4–8/≥16 µg/ml, 33(97.1%) strains showed resistance to vancomycin.

The *spa* gene was detected in 21(61.8%) isolates out of 34 MRSA samples (Figure 3). The *spa* typing of 21 MRSA samples revealed four different *spa* types, as follows: t386 (3/14.3%), t3576 (1/4.8%), t10002 (1/4.8%), and t10234 (1/4.8%). High polymorphism rates were shown in isolates of *spa* type t386 (Table 4).

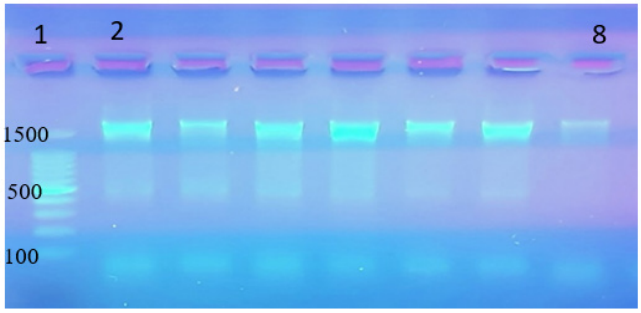

Fig. 3. *PCR products of the spa gene for MRSA isolates (Lanes 2-8); Lane 1 - DNA ladder (1500 bp).*

Table 4.

The spa typing of 21 MRSA samples.

Isolate number of MRSA	*spa*-type (Ridom)	Tandem Repeats
7,10,16	t386	07-23-13
5	t10002	11-10-21-17-34-24
19	t10234	11-10-21-17-34-24-34-22
35	t3576	26-23-17-34-17-20-17-20-17-12-16

The sequences of the *spa* gene for the five MRSA local isolates were analyzed by BLAST with Geneious software. The results showed an alignment feature as described for genomic sequences for linear DNA lengths (1,066 bp, Accession number: LC768794 *S. aureus* Mayar), (728 bp, Accession number: LC768797 *S. aureus* Amena), (961 bp, Accession number: LC768793 *S. aureus* Dimashams), and (932 bp, Accession number: LC768796 *S. aureus* KarBane) with a database of NCBI, except for one in length. Accession number LC768795 *S. aureus* Mohammed had 99% genetic identity with duplicates of the genetic bases for query nucleotide sequence from 839 to 899 bp, and two nitrogen bases (TT) were not found in the query sequence, which indicated deletion mutation at locus 241 bp. In addition, two nitrogen bases (GA) were found in the query sequence but not found in the database, meaning an insertion mutation at locus 298 bp, according to the report of the BLAST result. There are clear reasons for the different identities of the last sequence compared to the first four sequences.

The colored accession number of local MRSA isolates for the spa gene showed variable diversity among bases, compared to control obtained from NCBI in clinical samples. The results data has been split into three monophyletic branches; the first branch contains the control strains NCBI (LT992466, CP077922, CP013616, CP060597, AP019713, CP038270, and CP006630). Most isolates showed diversity in three groups (Figure 4).

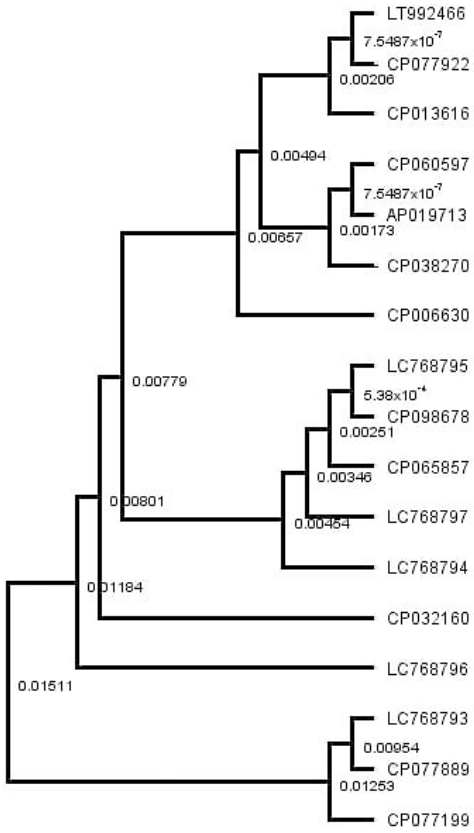

Fig. 4. *Phylogenetic tree to the sequence of the spa gene related to MRSA local isolates from clinical samples.*

Group 1 contains the NCBI control strains (CP098678 *S. aureus* strain TCD12 and the CP065857 *S. aureus* strain CC1153-MRSA). The result revealed that the local isolate

(LC768795 *S. aureus* Mohammed) was similar to the NCBI control strain (CP098678 *S. aureus* TCD12). In contrast, the other local isolates (LC768797 *S. aureus* Amena and LC768794 *S. aureus* Mayar) had distant genetic values of 0.00346 and 0.00454, respectively, with the control CP065857 *S. aureus* strain CC1153-MRSA.

In Group 2, a local isolate (LC768796 *S. aureus* KarBane) had a genetic distance with CP032160 *S. aureus* strain SA G5 at a value of 0.001511.

In Group 3, a local isolate (LC768793 *S. aureus* Dimashams) showed a genetic distance with NCBI control (CP077889 *S. aureus* strain 333) at a value of 0.00954 (Figure 4).

Discussion

Identifying bacterial species typically relies on culture-dependent phenotypic tests. Nevertheless, the reliability of these methods can be limited due to the variable expression of phenotypic characteristics. Additionally, the databases used in these tests are often restricted to a subset of bacterial species, further limiting their overall efficacy.[17] The distinction between *Staphylococcus* species and *Micrococcus* bacteria was made using the catalase and oxidase tests, respectively, with positive outcomes. The bacterial colonies from swabs cultured on an MSA medium were shown as yellow, and under a microscope, they were cocci in form, clustered, and had positive stains based on cultural and morphological criteria. The isolates were reported as positive for coagulase, as revealed by the biochemical analysis. This was in agreement with the study achieved in Jordan.[18]

Detecting accurate species is of immense value to the diagnosis and helps distinguish rare drug-resistant features. A desirable way must possess the potential to distinguish between closely related species with high discriminatory power. It should also be cost-effective, rapid, and reproducible. In this regard, genetic techniques that rely on PCR or sequencing are promising alternatives for identification.[19]

As per our data, the notable spreading of the *mecA* gene in local *S. aureus* isolates of MRSA was at a percentage of 20.4%, which was in agreement with the study achieved in the USA at a percentage of 20% from different clinical samples.[20]

Another study in Eritrea showed a high proportion of MRSA isolates—72%.[21] MRSA prevalence has increased due to various risk factors, including MRSA carriage by healthcare workers and patients, improper use and overuse of antimicrobials, inadequate adherence to the hand-hygiene protocol, extended hospital stays, and a lack of comprehensive bundle approaches.[22]

In Iraq, the augmented frequency of incidence and hospitalization has led to a significant concern regarding MRSA. Given the gravity of the situation, prompt and precise classification of MRSA isolates has become an essential prerequisite for effective screening, epidemiology, surveillance, and infection control. In this context, accurate typing of MRSA isolates is crucial in averting the spread of this perilous pathogen. Chrome MRSA agar employs a chromogenic substrate to differentiate *S. aureus*, especially MRSA, from other pathogens and selectively cultivate MRSA in the presence of antibiotics. Chromogenic agars have been instrumental in reducing the time and cost associated with confirmatory testing, which can be time-consuming as well as costly. CHROMagar Staph aureus has been evaluated in several studies, and a high sensitivity for S. aureus has been reported.[23]

Anand et al.[24] evaluated the efficacy of the cefoxitin disc diffusion test to characterize MRSA and compare it with oxacillin agar screening and detection of the *mecA* gene by PCR. The results of the cefoxitin disc diffusion test were in concordance with the PCR for the *mecA* gene. In our study, 91.2% of MRSA isolates showed positive results for the *mecA* gene, which was almost similar to the outcomes reported in a study conducted by Dhungel et al.,[25] who found 94.1% positivity for the *mecA* gene among the MRSA isolates. The absence of the *mecA* gene in our three MRSA isolates suggests the presence of an alternate pathway for methicillin resistance rather than the conventional *mecA* gene-mediated mechanism.[26]

Vancomycin-resistant S. aureus (VRSA) in the current study was 97.1% from different clinical samples, which agrees with the result of a study by Rehman et al. performed in Pakistan (92.6%).[27] The resistance rate for oxacillin among MRSA isolates was 94.1%, while the rate was higher at 100% in the previous study.[28]

Bacterial resistance to a particular antibiotic may be ascribed to random genetic mutations.[29] This leads to the bacterial microbe acquiring an innate resistance to a greater dosage of an antibiotic due to its frequent usage.[30] Bacteria also can acquire antimicrobial resistance through horizontal gene transfer.[31]

In our study, the *spa* typing of 21 MRSA samples revealed four different *spa* types (t386, t3576, t10002, and t10234) with a higher prevalence of t386 (14.3%) versus other types with a prevalence of 4.8% for each. In contrast, in a study by Mohammed et al.[32] the prevalence of t386 was 5.5%. In a study by Hadyeh et al.[33] performed in Palestine, the *spa* type t386 (CC1) was at a percentage of 12.5%.

The first report from Wassit, which was documented in NCBI, revealed three novel types of staphylococcal protein A. These isolates showed methicillin and vancomycin resistance in samples of sputum and wounds from patients in the Aziziyah and Suwaira Hospitals. The current study analyzed unique *spa* types not previously documented in NCBI. Some *spa* types were also absent in neighboring countries or local regions. This discovery could potentially be attributed to the mobility of patients across borders or migration to and from Iraq, both during and following the Iraq War. These findings contribute significantly to the knowledge of these bacteria in central Iraq.

This study has limitations due to the limited number of MRSA samples studied; therefore, additional research is required with a more significant number of samples in different regions of the country.

In conclusion, the investigation revealed a noteworthy escalation in the frequency of MRSA infections in Wassit. Using *spa* typing, this analysis identified four distinct MRSA *spa* types, with *spa* t386 being the most prevalent. The data

on hospital-acquired MRSA infections may be advantageous in the comprehensive features of these bacteria at Wassit Governorate and in devising an appropriate preventive and therapeutic strategy. It is also imperative that future research endeavors to focus on identifying a variety of MRSA types spreading in Iraqi healthcare facilities. This information will be crucial for developing efficient interventions to restrict the spread of this antibiotic-resistant pathogen in the country. The outcomes of the current study also emphasize the need for enhanced surveillance and control measures to minimize the burden of MRSA infections in Iraq.

Acknowledgments

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Competing Interests

The author declares that there is no conflict of interest.

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ORIGINAL ARTICLE

Antibiotic Prescribing and Use

INTERNATIONAL JOURNAL OF BIOMEDICINE

Prevalence of Antibiotic Prescription in Primary Healthcare Settings in the Municipality of Prishtina, Kosovo

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Abstract

Background: Antibiotics remain among the most prescribed drugs in primary healthcare, contributing to increased antibiotic resistance in the community and prevailing as an emerging global health concern. We aimed to quantify the prevalence and quality of antibiotic prescription in primary healthcare settings in the Municipality of Prishtina to identify targets for quality improvement.

Methods and Results: This study represents a population-based, retrospective cohort, including data from eight randomly selected family medical centers in the Municipality of Prishtina. Each 150th patient on medical records was assessed for demographic data, diagnosis (ICD-10), antibiotic prescription, antibiotic class, and antibiotic form. In total, the study included 1614 cases reviewed. The antibiotic prescription rate was 16%. The health condition for which most of the cases received antibiotics was J18 - Pneumonia, unspecified organism (67%), followed by J03 - Acute tonsillitis (54%), J42 - Unspecified chronic bronchitis (46%), and N39 - Other disorders of the urinary system (43%). Broad-spectrum antibiotics, such as co-amoxiclav (17.7%), amoxicillin (16.5%), and ceftriaxone (12.6%), featured among the most routinely prescribed antibiotics. The antibiotic prescription rate was the highest for cases in the 3-5 age group, of whom 27% received an antibiotic prescription. In 73% of cases, oral antibiotics were prescribed, 69% of which belong to the WHO AWaRe (Access, Watch, Reserve) essential medicines list. Only 18% of antibiotics were prescribed with their generic names.

Conclusion: The prevalence of antibiotic prescription in primary healthcare settings in Prishtina is moderately low. These data cannot be extrapolated to other municipalities in Kosovo or other countries due to different organizational levels. High antibiotic prescription rates for young age groups, prescription of broad-spectrum antibiotics, and high rates of parenteral antibiotics were identified as targets for quality improvement.**(International Journal of Biomedicine. 2024;14(1):134-140.)**

Keywords: antibiotic prescription • primary healthcare • antibiotic resistance • Kosovo

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Introduction

Kosovo, with a population of 1.7 million inhabitants, is located in southeast Europe in the Western Balkans region. To date, Kosovo lags in the establishment of a health insurance system and electronic medical records, which highly influence the real documentation of epidemiological situations or antibiotic prescription rates. Life expectancy at birth is 71 years, and communicable diseases are still among the largest ongoing healthcare problems.[1]

For decades, antibiotics have been crucial in decreasing the morbidity and mortality burden of infectious diseases.[2] There is robust evidence emphasizing that there is little to no clinical benefit from antibiotic treatment for the most common respiratory tract infections, which are usually self-limiting and often caused by viruses.[3] Yet antibiotics remain among

the most prescribed drugs in primary healthcare settings for acute sinusitis, acute pharyngitis, acute bronchitis, nonspecific upper respiratory tract infections, and the common cold.[4]

Prescribing antibiotics to patients increases the risk of side effects, encourages help-seeking behavior, and increases antibiotic resistance in the community.[5] Patients treated with first-line antibiotics can become colonized by resistant bacteria, especially in the first weeks after treatment, which may persist for up to 12 months.[6] Such patients are at a greater risk of infection with resistant bacteria. In recurrent infections, the likelihood of being prescribed second-line antibiotics increases, thereby increasing the population effect of community antibiotic resistance.[7] Furthermore, many doctors do not link antibiotic resistance with their prescribing attitude and consider it a more general issue.[8] Developing effective interventions will require increased knowledge of the mechanisms that underlie these predictors of inappropriate antibiotic prescribing.[9] Although antibiotic prescription in primary care settings has decreased by about a third in most developed countries,[10] the use of broad-spectrum agents has steadily increased.[11] The greatest concern related to excessive broad-spectrum antibiotic prescription in primary healthcare settings remains its continuous contribution to increasing antibiotic resistance globally.[12]

Urinary tract infections are among the most common infections in primary healthcare settings. Countrywide comparative results of the resistance rate among *Escherichia coli* isolates analyzed between 2004 and 2016 showed a significant increase. Resistance to aminopenicillins increased from 40.2% to 75.3%, and ciprofloxacin from 2.6% to 29.4%.[13] Therefore, antimicrobial resistance is one of the major challenges for the healthcare system in Kosovo. The main challenges in this area also remain the inappropriate use of antibiotics, lack of officially approved clinical guidelines and protocols, "over-the-counter sale" of antimicrobials, and strong pressure from the pharmaceutical industry.[14]

The primary healthcare system in Kosovo provides initial diagnoses and patient care for 80%-90 % of the cases, thus acting as gatekeepers for secondary and tertiary healthcare.[15] Primary healthcare services are decentralized to the municipality level, increasing liability and service quality. Yet, primary healthcare is not equally developed and organized in all municipalities.[16] Family medical centers in Kosovo are responsible for diagnosing and curative care, including minor surgery and drug management; immunization; emergency care and stabilization of emergency patients; maternal and child healthcare; reproductive health services, including antenatal and post-natal care, as well as family planning and treatment of sexually transmitted diseases. All primary healthcare facilities in Prishtina offer laboratory services and functional advanced equipment. They also offer public accountability items, guidelines, and information materials for their patients.[17]

Primary healthcare settings of Prishtina have one main Family Medicine Center, which consists of 31 family medicine centers (Table 1). These primary healthcare centers cover Prishtina's entire urban and rural areas, comprising more than 200 thousand inhabitants. During 2017, family medical centers of Prishtina offered over one million medical consultations and 2.4 million other medical services.[18]

Recalling the large number of medical consultations and services offered in primary care settings of the Municipality of Prishtina, with this study we aimed to quantify the percentage of antibiotic prescriptions and quality of antibiotic prescriptions in primary healthcare settings in the Municipality of Prishtina for 2017. Additionally, reducing antibiotic prescriptions at the general practice level was associated with reduced local antibiotic resistance. Therefore, we will use our findings to encourage clinicians to prescribe antibiotics conservatively.[19]

Table 1.

Family medical centres in Municipality of Prishtina.

Primary healthcare settings of Prishtina		
QKMF Prishtinë	QMF Mati1	Mirditë
QMF 1 Prishtinë	QMF Mat	Mramur
QMF 2 Prishtinë	QMF Hajvali	Rimanisht
QMF 3 Prishtinë	QMF Besi	Shahskovcë
QMF 4 Prishtinë	QMF Bardhosh	Shkabaj
QMF 5 Prishtinë	Barilevë	Viti
QMF 6 Prishtinë	Bullaj	Sharban
QMF 7 Prishtinë	Slivovë	Qendra e Studenteve
QMF 8 Prishtinë	Keqekollë	
QMF 9 Prishtinë	Kishnicë	
QMF 10 Prishtinë	Koliq	
QMF 11 Prishtinë	Llukar	

Materials and Methods

Study population

This study represents a population-based, retrospective cohort, including data from eight randomly selected family medical centers in the Municipality of Prishtina. By medical center randomization, we achieved a representative sample and avoided bias. Each 150th patient on medical records was assessed for demographic data, diagnosis, antibiotic prescription, antibiotic class, and antibiotic form (if antibiotics were prescribed). In total, the study included 1614 cases reviewed.

Inclusion Criteria: Every doctor who worked in one of these eight randomly selected primary healthcare centers was included in the study. The data for every 150th patient registered on medical records was analyzed, regardless of whether the patient was prescribed antibiotics or not.

Exclusion Criteria: Topical antibiotics (vaginal pessaries, skin preparations, or nasal, ear, and eye preparations) were not included in this survey.

Data collection

Data were collected retrospectively for 12 months, from January 1 to December 31, 2017. A customized questionnaire

was used to collect data, including the patient's registry number, treatment date, demographic data, patient diagnosis (ICD-10), antibiotic brand and generic name, and pharmacologic form. Antibiotic dose and duration of treatment were not included in the study due to the lack of complete data on protocols. To avoid data collection errors, nurses chosen to collect the data were initially trained. In addition, each questionnaire was signed by the nurse who collected the data, thus internal validation could be performed afterwards.

Data analysis

From the data collected with questionnaires, we created a database further analyzed with the statistical software package SPSS version 20.0 (SPSS Inc, Armonk, NY: IBM Corp). The relation between antibiotic prescription rate and other independent variables, like demographic data and patient diagnosis, were analyzed through correlation and variance tests.

Results

The antibiotic prescription rate was 16%. There were major differences in antibiotic prescription rates among the eight primary healthcare centers included in the study, varying from 6.9% up to 20%. Family Medical Centers #9 and #2 had the highest antibiotic prescription rates, 20% and 19.8%, respectively. Conversely, Family Medical Centre #11 had the lowest antibiotic prescription rate, only 6.9% (Figure 1).

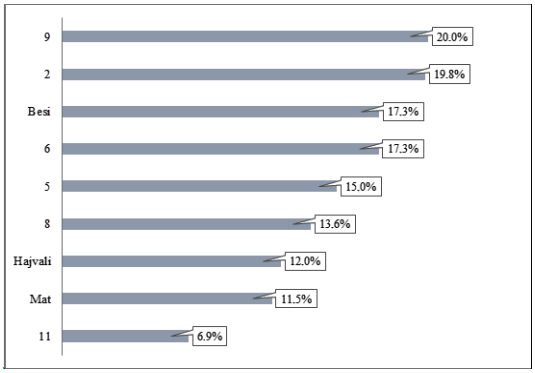


Fig. 1. *Frequency of antibiotic prescription among different primary healthcare centres in Municipality of Prishtina*

The health condition for which most of the cases received antibiotics was J18 - Pneumonia, unspecified organism (67%), followed by J03 - Acute tonsillitis (54%), J42 - Unspecified chronic bronchitis (46%), and N39 - Other disorders of the urinary system (43%) (Figure 2). The "Other" section includes all conditions with five or fewer cases.

Conditions for which antibiotics were not prescribed are depicted below (only conditions with more than 5 cases are included): I10 - Essential hypertension (n=94), J11 - Influenza – unidentified virus (n=23), D50 - Iron deficiency anemia (n=19), E10 - Type 1 diabetes mellitus (n=10), E11 - Type 2 diabetes mellitus (n=20), H10 -Conjunctivitis (n=11), I95 - Hypotension (n=10), M47 - Spondylosis (n=19), M53 - Other and unspecified dorsopathies, not elsewhere classified (n=6), M54 - Dorsalgia (n=28), M79 - Other and unspecified soft tissue disorders, not elsewhere classified (n=8), R11 - Nausea and vomiting (n=22), Z00 - Encounter for general examination without complaint, suspected or reported diagnosis (n=62), Z01 - Encounter for other special examination without complaint, suspected or reported diagnosis (n=60), Z03 - Encounter for medical observation for suspected diseases and conditions ruled out (n=18), Z34 - Encounter for supervision of normal pregnancy (n=18). Overall, Z76- Encounter for the issue of repeat prescription was the most commonly encountered diagnosis, with 329(20.38%) cases.

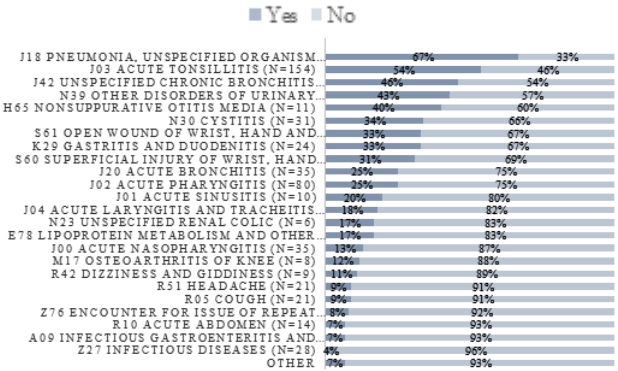


Fig. 2. *Frequency of antibiotic prescription for the most common diagnoses based on ICD-10.*

Broad-spectrum antibiotics, such as co-amoxiclav (17.7%), amoxicillin (16.5%), and ceftriaxone (12.6%), featured among the most routinely prescribed antibiotics. Amoxicillin featured among the most routinely prescribed antibiotics for J02 - Acute pharyngitis, followed by co-trimoxazole for N30 - Cystitis and co-amoxiclav for Z76 - Encounter for issues of repeat prescription. Conditions with 5 cases or below were not included (Figure 3).

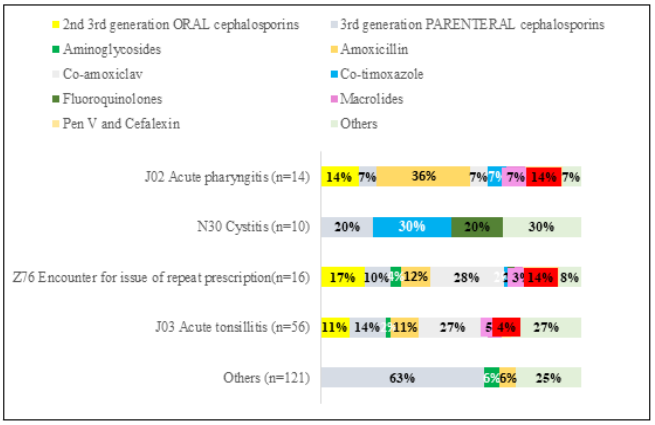


Fig. 3. *The most common prescribed antibiotics in primary healthcare settings in Prishtina by diagnosis.*

The antibiotic prescription rate was the highest for cases in the 3-5 age group, of whom 27% received an antibiotic prescription. This group was followed by the 6-9 age group (21.6%) and the 19-29 age group (21.6%). The 10-14 age group (20%) also was above the general average of 16% (Figure 4a).

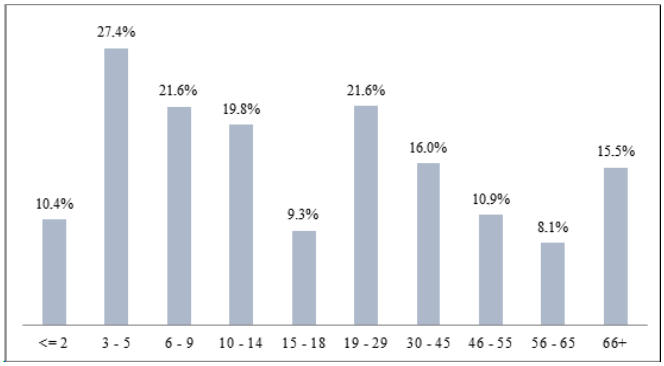


Fig. 4a. *The frequency of antibiotic prescription among different age groups.*

The frequency of groups of antibiotics was assessed for different age groups. While co-amoxiclav is the most frequently prescribed antibiotic for younger age groups, its peak prescription period being for the 15-18 age group, it seems to reduce as individuals get older. The most frequently prescribed antibiotics among older age groups are second- and third-generation oral cephalosporins (Figure 4b).

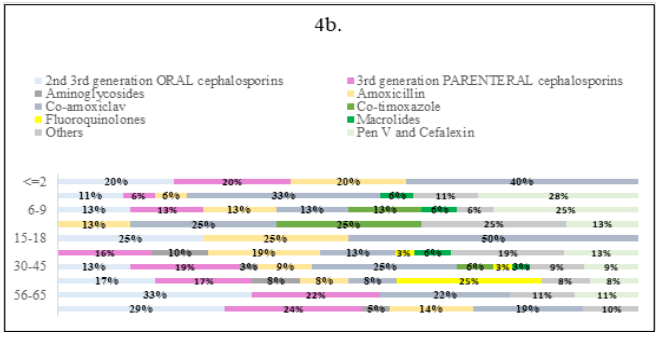


Fig. 4b. *The frequency of antibiotic group prescription among different age groups.*

In addition, we observed the most common diagnoses encountered in different age groups. The most frequently encountered condition among all age groups was J03 - Acute tonsillitis; however, individuals of older age groups (56+) were more likely to be diagnosed with other conditions (Figure 4c).

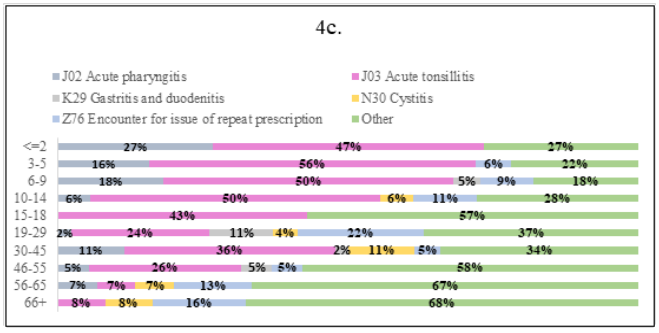
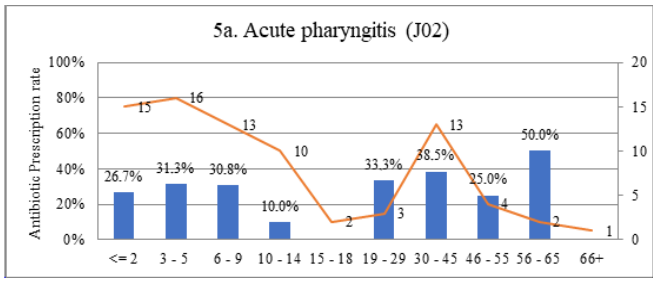
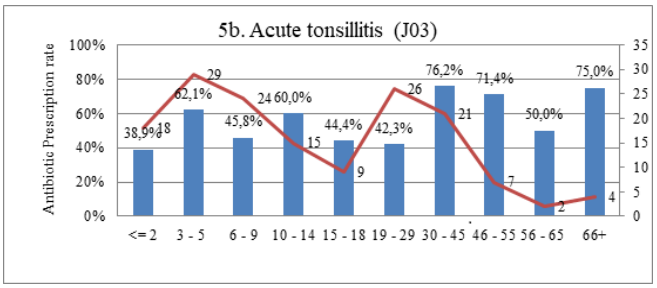


Fig. 4c. *Prevalence of the most common conditions by different age groups.*

Furthermore, we analyzed antibiotic prescription rates among different age groups for the most frequent diagnoses, namely J02 - Acute pharyngitis, J03 - Acute tonsillitis, and Z76 - Encounter for the issue of a repeat prescription (Figure 5a, 5b, 5c). No significant correlation between these variables was observed.





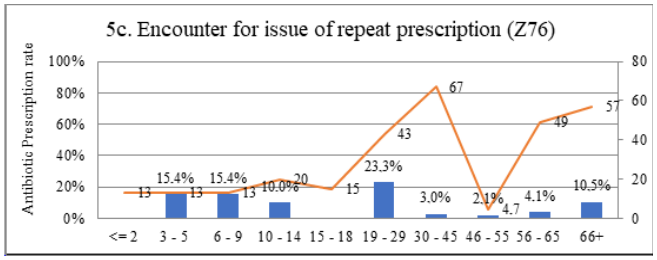


Fig. 5 a.b.c. *The most common conditions by different age groups.*

We further examined whether there was any relation between antibiotic prescription rates for different age groups and genders, mainly focusing on parental pressure for antibiotic prescription to their children or any gender impurities (Figure 6). No significant correlation between antibiotic prescription rates for different age groups and genders were observed.

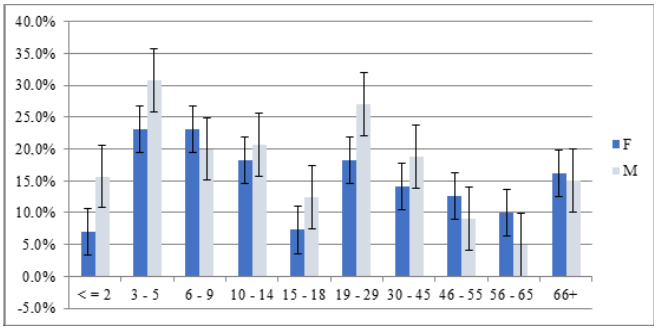


Fig. 6. *Antibiotic prescription rates for different age groups and gender.*

We additionally examined if there was any relation between antibiotic prescription rates through different months or seasons (Figure 7). January (20%), March (20%), and April (21%) had the highest antibiotic prescription rates. In comparison, the antibiotic prescription rate in November was the lowest, only 12%.

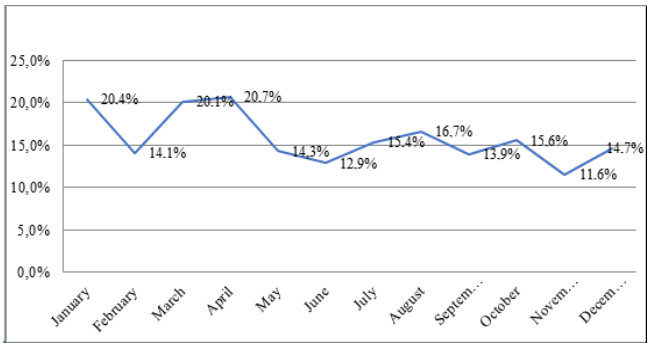


Fig. 7. *The frequency of antibiotic prescription among different months/ seasons.*

In 86% of the cases, primary healthcare physicians prescribed antibiotics for their patients. In only 14% of the cases, antibiotics were encountered for issues of repeated prescription based on a previous prescription from a secondary or tertiary specialist. In 73% of cases, oral antibiotics were prescribed, 69% of which belong to the WHO AWaRe essential medicines list. Seventy-six percent of cephalosporins were prescribed by primary care physicians, and the remaining 24% were a repeated prescription from secondary or tertiary care specialists. Cefalexin (3.5%), cefaclor (4.7%), cefixime (6.3%), and ceftriaxone (12.6%) were the most prescribed cephalosporins. Only 18% of antibiotics were prescribed with their generic names. From the oral forms, amoxicillin, co-amoxiclav, and cefaclor were mainly prescribed with their generic names, while from parenteral drugs, gentamicin was only prescribed with its generic name.

Discussion

In low- and middle-income countries like Kosovo, infectious diseases remain among the main reasons for primary care visits, entailing unnecessary antibiotic prescriptions.[20] Antibiotic prescription rates in the Municipality of Prishtina remain moderately low, varying from 6.9% up to 20% in different family medical centers. This result highlights the major differences in antibiotic prescription attitudes of primary care doctors and the lack of officially approved clinical guidelines and protocols that doctors could rely on.[21]

Our study found a high prevalence of antibiotic prescription among young age groups. Most antibiotics, ranging from 20% to 27.4%, were prescribed to children 3-14 years old for acute tonsillitis. Other studies also reveal that 63%-77% of four-year-old children were prescribed antibiotics for acute otitis media in the U.S.[22] or the Netherlands.[23] Additionally, a German national survey revealed increasing antibiotic prescription rates in children and adolescents, specifically for tonsillitis, bronchitis, otitis media, and acute upper respiratory infections.[24] Our study also revealed that the incidence of antibiotic prescription for the 19-29 age group was 21.6%, mostly for acute tonsillitis (24%), and amoxicillin and second- and third-class cephalosporin (19%) were antibiotics of choice. Recalling that antibiotic prescription in the early days of life is highly related to metabolic diseases and being overweight, these findings are concerning.[25] In addition, different studies found that self-limiting respiratory tract infections, such as pharyngitis, were the main reason for antibiotic prescriptions in up to 60% of patients from all age groups.[26] Nevertheless, prescribing antibiotics for self-limiting respiratory tract infections must be intensively monitored, and educational programs to gradually change physicians' attitudes toward antibiotic prescription should be implemented.[27]

Potentially, the lack of etiologic treatment or empirical management of diseases in primary care centers in Prishtina continuously led to the prescription of broad-spectrum antibiotics such as amoxicillin, co-amoxiclav, and ceftriaxone, as we noticed in our study. In addition, other studies revealed that extensive use of broad-spectrum antibiotics leads to antibiotic resistance developing more rapidly in the community.[28] Moreover, in our study, we noticed that 82% of antibiotics were prescribed with their brand names, thus increasing the cost burden for patients and potentially leading to a lack of treatment adherence. This potentially indicates the pharmaceutical industry influences physicians' daily decisions and emphasizes the immediate need for drug- price equalization in the Kosovo market.[29]

Besides, antibiotic prescription rates in outpatient care settings, inappropriate antibiotic selection, dosing, and duration of treatment, as well as increasing antibiotic resistance, remain emerging topics of public health and a national priority in many countries.[30] Of the total antibiotics consumed in the outpatient settings, at least 30% are unnecessary, and 50% of prescriptions are inappropriate concerning drug selection, dosing, and/or duration.[31]

Our study has a few minor limitations due to incomplete protocol data. We could not quantify the antibiotic doses and duration of treatment. We also could not include the number of over-the-counter sales of antibiotics in Prishtina due to the lack of electronic medical records and public health insurance systems. These could be of great interest to investigate in other studies soon.

Surveillance of antibiotic consumption was one of the success stories during the implementation of the first National Strategy and Action Plan (2011-2015) in Kosovo.[32] Data for antibiotic consumption were collected in three levels of health care in Kosovo: wholesale data, data from all hospitals, and data at the primary care level. Wholesale data on antibacterial use in Kosovo was 26.3 DID in 2012.[33] However, the latest WHO publication on antibiotic consumption in Europe, published in December 2018, showed that Kosovo has marked a significant decrease in antibiotic consumption by almost 25%.[34] The main factors influencing this decline in consumption are the increased awareness of the population

and healthcare workers about antimicrobial resistance, media pressure, and governmental commitment to address antimicrobial resistance.

Ceftriaxone was the most prescribed antibiotic in hospitals and at the primary healthcare level. Prescription with generic names was noted only in 31% of cases in primary care. [35]

To address the challenge of antimicrobial resistance, the Ministry of Health initially completed the National Strategy and Action Plan to Combat Antimicrobial Resistance 2011-2015, where 80% of planned activities were successfully implemented. In December 2018, the Minister of Health signed a new action plan for antimicrobial resistance for three years. The new action plan has five strategic objectives and 47 activities. The cornerstone of this action plan will be antimicrobial stewardship. Other action areas will be strengthening the "One Health" approach, improving the surveillance of antimicrobial resistance and antimicrobial consumption in humans, animals, and the environment; prudent use of antimicrobials in clinical practice and the veterinary sector; infection prevention and control in healthcare settings and community; and promotion of research and international cooperation.[36]

To successfully implement national strategies for proper antibiotic use and to rightly address the threat posed by antimicrobial resistance, physicians and patients must have a profound knowledge of the topic. Public health campaigns and treatment guidelines for specific infectious diseases and target groups proved unsuccessful in reducing inappropriate antibiotic prescriptions through the years.[37] Nevertheless, multifaceted interventions—considering infectious diseases in all age groups, vaccination, changing physicians' prescribing behavior, blended learning, and physician-patient shared decision-making—are recently considered the most effective tools to lower antibiotic prescription rates.[38]

Antibiotic optimization may be limited due to social, value-based, and ethical dilemmas, and reducing antibiotic prescription in primary healthcare settings should not imply increased patient revisits or admission to the hospital, thus leading to increased healthcare expenses and overall costs.[39]

Conclusion

We can conclude that antibiotic prescription rates in primary care in Prishtina remain satisfying. Yet these numbers might not reflect the real situation influenced by the lack of electronic medical records and public health insurance systems. Healthcare professionals in Kosovo should adopt antibiotic stewardship recommendations for quality improvement by prescribing antibiotics only when there is a clear clinical benefit, by using simple generic antibiotics and reducing broad-spectrum antibiotic prescription in young age groups, as well avoiding unnecessary antibiotic prescriptions for self-limiting conditions or parenteral forms, avoiding widespread use of topical antibiotics, and finally trying to narrow down and control over-the-counter sale of antibiotics. Finally, developing and monitoring the implementation of clinical guidelines and protocols for antibiotic prescriptions in physicians' daily practice and continuously educating patients on appropriate antibiotic use would further help reduce antibiotic prescription rates and antibiotic resistance in the community.

Competing Interests

The authors declare that they have no competing interests.

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Palatal Rugae Pattern in Adolescents of Southeastern Kosovo with Class I, II, III Malocclusions According to Angle's Classification

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Abstract

Background: Palatal rugae, or plicae palatinae, are uniquely designed structures, well-formed, asymmetric, irregular mucosal folds suited in the anterior part of the hard palate. Palatoscopy, or the analysis of the palatal rugae pattern, is a simple, low-cost, non-invasive, innovative, and highly sensitive technique that can be successfully used in stomatology. This study aimed to analyze the palatal rugae pattern among an adolescent sample of the Albanian population in Kosovo, to determine the most prevalent palatal rugae pattern in both genders in association with Class I, II, and III malocclusions according to Angle's classification.

Materials and Methods: In this cross-sectional study, a total of 100 adolescents (50 males and 50 females) aged from 12 to 18 were randomly selected from schools in southeastern Kosovo. All subjects were divided into classes of malocclusion according to Angle's classification (Class I, Class II, and Class III). The rugae patterns were classified based on shape, unification, and length according to the Thomas and Kotze classification. In the present study, the palatal rugae pattern in Class I, II and III malocclusions show no significant difference between female and male subjects. In Class I malocclusion, the straight pattern was dominant in female subjects, and the wavy pattern was dominant in male subjects. The straight pattern was dominant in males and females with Class II malocclusion. In Class III malocclusion, the wavy pattern was dominant in female subjects, and the curved pattern was dominant in male subjects. The study showed that male subjects were at slightly higher risk for having Class I malocclusion and slightly lower risk for having Class II and Class III malocclusions than female subjects.

Conclusion: This study provides essential information regarding the dominant palatal rugae pattern among Albanian adolescents of southeastern Kosovo with Class I, II, and III malocclusions according to Angle's classification.**(International Journal of Biomedicine. 2024;14(1):141-147.)**

Keywords: palatoscopy • palatal rugae • malocclusions • Albanian population

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Introduction

Palatal rugae (PR) are unique mucosal elevations seen on the anterior part of the hard palate and are the most stable characteristics in the oral cavity. PR develop in the third month in utero from connective tissue. They are genetically determined, well-formed, asymmetrical, irregular mucosal called "plicae palatinae." [(1)] Physiologically, the relief of the palate helps oral swallowing, speech, tasting food, and sucking of the finger in children.[(2)]

Palatoscopy, or the analysis of the PR pattern, is a simple, low-cost, non-invasive, innovative, and highly sensitive technique that can be successfully used in stomatology. The PR patterns of an individual may be considered to be a useful tool for sex determination and a person's identity.[(3)] Like fingerprints, the PR pattern does not change during life. The PR are protected from traumas, high temperatures, and injuries because of their internal position in the mouth. The Thomas and Kotze classification is used most in identifying palatal patterns, including shape, length, and number of rugae.[(4)]

During orthodontic treatment, some changes occur in the rugae, but the primary morphology of PR remains stable throughout life. Also, events such as finger sucking in childhood or constant pressure from dentures may affect the PR pattern. Due to their location, PR in the molar area are the most stable.[5] After maxillary expansion in orthodontic treatment, the separation distance of the palate could be measured using the distance between the left and right rugae.[6]

Malocclusions fundamentally impact a patient's psychological health; hence, early treatment and prevention of malocclusion may provide an advantage in the duration of treatment outcomes. Since PR are very stable and unique structures, they may serve as an excellent diagnostic appurtenance for predicting Angle's classes of malocclusion early in life to mitigate future dental–skeletal aberrations.[7,8]

Since both malocclusion and PR display hereditary predisposition, many studies have been done to approve the application of the PR pattern as an auxiliary diagnostic method of malocclusion.[9]

According to a study by Alshahrani et al.,[10] wavy and complex rugae are good predictors for class I and III malocclusions. Also, a significant number of wavy rugae in childhood could be a strong predictor for class I malocclusion according to Angle's classification; on the contrary, a study conducted by Ekrem et al.[11] found that there is no significant correlation between rugae pattern and malocclusions among classes I, II, and III. Sudhakar et al.[12] found a correlation between PR and forthcoming growth patterns. A wavy type of rugae was observed in both vertical and horizontal growth patterns. Curved and diverging PR patterns were mainly observed in vertical growth patterns. Thus, the PR pattern may be beneficial in determining the malocclusion early during the growing stage.

Palatoscopy is a simple, low-cost, non-invasive, innovative, and highly sensitive technique that can be successfully used in stomatology. The purpose of this study was to analyze the PR pattern among an adolescent sample of the Albanian population in Kosovo, to determine the most prevalent PR pattern in both genders in association with Class I, II, III malocclusions according to Angle's classification.

Materials and Methods

Study design

The participants for the study were recruited from January 2020 to May 2022 among schools in southeastern Kosovo. In this cross-sectional study, 180 adolescents aged from 12 to 18 were selected. Of 180 subjects, 69 did not fulfill the full inclusion criteria, and 11 plaster models were not poured well. The final group comprised 100 subjects, 50 males and 50 females.

All subjects were divided into classes of malocclusion according to Angle's classification (Class I, Class II, and Class III) without considering the etiology of malocclusion. Malocclusions were evaluated only clinically. Data distribution according to malocclusion group of 50 females: Class I -19, Class II - 24, Class III - 7. Data distribution according to malocclusion group of 50 males: Class I - 22, Class II - 22, and Class III - 6.

The study has compiled a study card with general data on the subjects with dental and medical anamnesis. Palatal impressions were taken with elastomers (C-silicone), and models of the upper jaw were poured into a dental cast of stone.

Selection criteria

Participants without previous orthognathic surgeries, without any congenital abnormalities, without previous orthodontic treatment, and inflammation, trauma, or malformation.

Materials for data collection

- C-silicone impression material (Zhermack)
- Graphite pencil (AIHAO 0.7mm)

Palatal impressions were taken with elastomers (C-silicone), and models of the upper jaw were poured into a dental cast of stone. The shape of the rugae is traced with a graphite pencil under adequate light (Image 1).

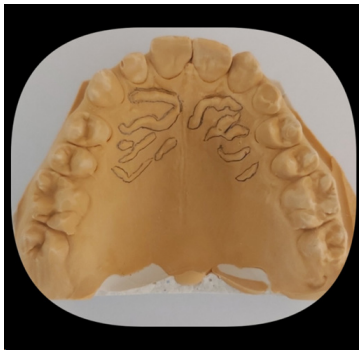


Image 1. *The pattern of palatal rugae.*

The rugae patterns were classified based on shape, unification, and length according to the Thomas and Kotze classification.[4]. The rugae were divided into 4 types based on their shape:

1.Wavy – The wavy rugae were serpentine (snake-like) in shape.

2. Straight - The rugae run directly from their origin to termination

3. Circular – Rugae that forms continuous ring

4. Curved - Crescent and curved gently

Unification was defined as when two rugae are joined at their origin or termination. Unification was classified into two categories:

Diverging: Rugae were considered to be diverging if two rugae had the same origin but immediately branched.

Converging: Rugae were considered to be converging if two rugae with different origins joined on their lateral portions.

The rugae were also classified based on their length as:

Primary rugae (>5 mm); Secondary (3-5 mm); Fragmentary (<3 mm).

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Differences in attributive series between the patient groups

were tested using Pearson Chi-square / Monte Carlo Sig. (2-sided), Fisher's Exact Test / Monte Carlo Sig (2-sided). Binary logistic regression analysis was performed to predict malocclusion. In all cases, a probability value of $P<0.05$ was considered statistically significant.

Ethical approval for this study was obtained from the Ethical Committee of the University of Pristina (protocol N #02-150115) and the Ethical Committee of the Kosovo Dental Chamber (N #19). All participants provided written informed consent.

Results

Palatal Rugae Patterns in Malocclusions according to Angle's Classification

Class I malocclusion

The results presented in Graph 1 refer to the rugae patterns in the Class I malocclusion group concerning the gender of the subjects. The straight pattern was dominant in female subjects, and the wavy pattern was dominant in male subjects (Table 1). There was no significant difference in the rugae patterns between male and female subjects (Fisher's exact test = 20.223, P=0.215 / Monte Carlo Sig. (2-sided) / 0.204 – 0.225 /). The enter method was used to determine the gender predictive value for Class I malocclusion. The global accuracy of this model in predicting Class I malocclusion was 56.0%. The sensitivity was 0.0%, and the specificity was 100.0% (Table 2). Male subjects were at a slightly higher risk from Class I malocclusion than female subjects (Table 3).

Class II malocclusion

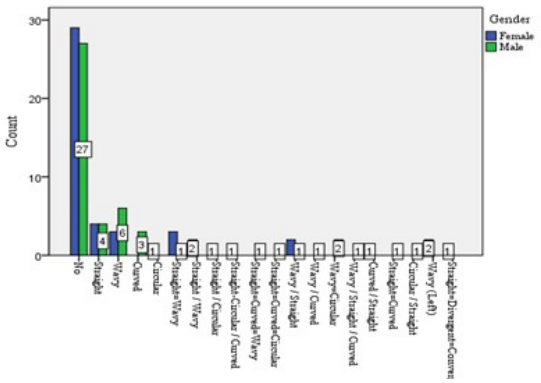
The results shown in Graph 2 refer to the rugae patterns in the Class II malocclusion group concerning the gender of the subjects. The straight pattern was dominant in males and females (Table 4). There was no significant difference in the rugae patterns between male and female subjects (Fisher's exact test = 24.973, P=0.236 / Monte Carlo Sig. (2-sided) / 0.225 - 0.247 /). The enter method was used to determine the gender predictive value for Class II malocclusion. The global accuracy of this model in predicting Class II malocclusion was 54.0%. The sensitivity was 54.0%, and the specificity was 54.0% (Table 5). Male subjects were at a slightly lower risk from Class II malocclusion than female subjects (Table 6).

Class III malocclusion

The results shown in Graph 3 refer to the rugae patterns in the Class III malocclusion group concerning the gender of the subjects. The wavy pattern was dominant in female subjects, and the curved pattern was dominant in male subjects (Table 7). There was no significant difference in the rugae patterns between male and female subjects (Fisher's exact test = 9.529, P=0.610 / Monte Carlo Sig. (2-sided) / 0.598 – 0.623 /). The enter method was used to determine the gender predictive value for Class III malocclusion. The global accuracy of this model in predicting Class III malocclusion was 87.0%. The sensitivity was 0.0%, and the specificity was 100.0% (Table 8). Male subjects were at a slightly lower risk from Class III malocclusion than female subjects (Table 9).

Table 1.
Rugae patterns in the Class I malocclusion group according to Angle's classification / Gender

		Gender		Total
		Female	Male	
No	Count	29	27	56
	%	58.0%	54.0%	56.0%
Straight	Count	4	4	8
	%	8.0%	8.0%	8.0%
Wavy	Count	3	6	9
	%	6.0%	12.0%	9.0%
Curved	Count	0	3	3
	%	0.0%	6.0%	3.0%
Circular	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight=Wavy	Count	3	1	4
	%	6.0%	2.0%	4.0%
Straight / Wavy	Count	2	0	2
	%	4.0%	0.0%	2.0%
Straight / Circular	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight-Circular / Curved	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight=Curved=Wavy	Count	0	1	1
	%	0.0%	2.0%	1.0%
Straight=Curved=Circular	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy / Straight	Count	2	1	3
	%	4.0%	2.0%	3.0%
Wavy / Curved	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy=Circular	Count	0	2	2
	%	0.0%	4.0%	2.0%
Wavy / Straight / Curved	Count	0	1	1
	%	0.0%	2.0%	1.0%
Curved / Straight	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight=Curved	Count	0	1	1
	%	0.0%	2.0%	1.0%
Circular / Straight	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy (Left)	Count	2	0	2
	%	4.0%	0.0%	2.0%
Straight=Divergent= Convergent (Left)	Count	1	0	1
	%	2.0%	0.0%	1.0%
Total	Count	50	50	100
	%	100.0%	100.0%	100.0%



Graph 1. Class I malocclusion

Table 2.

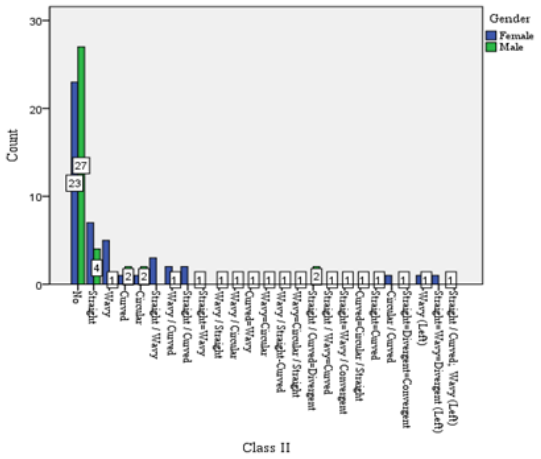
Gender predictive value for Class I malocclusion / Model discrimination

Observed			Predicted		
			Class I		Percentage Correct
			No	Yes	
Step 1	Class I	No	56	0	100.0
		Yes	44	0	.0
	Overall Percentage				56.0
The cut value is .500					

Table 3.

Binary logistic regression analysis for prediction of Class I malocclusion / Gender

		B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)	
								Lower	Upper
Step 1[a]	Gender (1)	.162	.403	.162	1	.687	1.176	.534	2.593
	Constant	.323	.287	1.269	1	.260	.724		
a. Variable entered on step 1: Gender.									



Graph 2. Class II malocclusion

Table 4.

Rugae patterns in the Class II malocclusion group according to Angle's classification / Gender

		Gender		Total
		Female	Male	
No	Count	23	27	50
	%	46.0%	54.0%	50.0%
Straight	Count	7	4	11
	%	14.0%	8.0%	11.0%
Wavy	Count	5	1	6
	%	10.0%	2.0%	6.0%
Curved	Count	1	2	3
	%	2.0%	4.0%	3.0%
Circular	Count	1	2	3
	%	2.0%	4.0%	3.0%
Straight / Wavy	Count	3	0	3
	%	6.0%	0.0%	3.0%
Wavy / Curved	Count	2	1	3
	%	4.0%	2.0%	3.0%
Straight / Curved	Count	2	0	2
	%	4.0%	0.0%	2.0%
Straight=Wavy	Count	1	0	1
	%	2.0%	0.0%	1.0%
Wavy / Straight	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy / Circular	Count	0	1	1
	%	0.0%	2.0%	1.0%
Curved=Wavy	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy=Circular	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy / Straight-Curved	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy=Circular / Straight	Count	0	1	1
	%	0.0%	2.0%	1.0%
Straight / Curved=Divergent	Count	0	2	2
	%	0.0%	4.0%	2.0%
Straight / Wavy=Curved	Count	0	1	1
	%	0.0%	2.0%	1.0%
Straight=Wavy / Convergent	Count	0	1	1
	%	0.0%	2.0%	1.0%
Curved=Circular / Straight	Count	0	1	1
	%	0.0%	2.0%	1.0%
Straight=Curved	Count	0	1	1
	%	0.0%	2.0%	1.0%
Circular / Curved	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight=Divergent= Convergent	Count	1	0	1
	%	2.0%	0.0%	1.0%
Wavy (Left)	Count	1	1	2
	%	2.0%	2.0%	2.0%
Straight=Wavy= Divergent (Left)	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight / Curved; Wavy (Left)	Count	1	0	1
	%	2.0%	0.0%	1.0%
Total	Count	50	50	100
	%	100.0%	100.0%	100%

Table 5.

Gender predictive value for Class II malocclusion / Model discrimination

Observed			Predicted		
			Class II		Percentage Correct
			No	Yes	
Step 1	Class II	No	27	23	54.0
		Yes	23	27	54.0
	Overall Percentage				54.0

The cut value is .500

Table 6.

Binary logistic regression analysis for prediction of Class II malocclusion / Gender

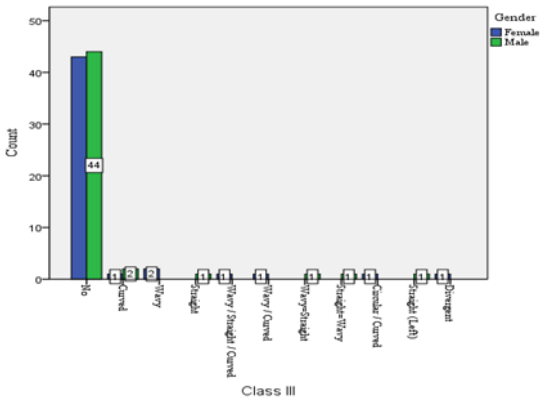
		B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)	
								Lower	Upper
Step 1[a]	Gender (1)	.321	.401	.639	1	.424	.726	.330	1.593
	Constant	.160	.284	.319	1	.572	1.174		

a. Variable entered on step 1: Gender.

Table 7.

Rugae patterns in the Class III malocclusion group according to Angle's classification / Gender

		Gender		Total
		Female	Male	
No	Count	43	44	87
	%	86.0%	88.0%	87.0%
Curved	Count	1	2	3
	%	2.0%	4.0%	3.0%
Wavy	Count	2	0	2
	%	4.0%	0.0%	2.0%
Straight	Count	0	1	1
	%	0.0%	2.0%	1.0%
Wavy / Straight / Curved	Count	1	0	1
	%	2.0%	0.0%	1.0%
Wavy / Curved	Count	1	0	1
	%	2.0%	0.0%	1.0%
Wavy=Straight	Count	0	1	1
	%	0.0%	2.0%	1.0%
Straight=Wavy	Count	0	1	1
	%	0.0%	2.0%	1.0%
Circular / Curved	Count	1	0	1
	%	2.0%	0.0%	1.0%
Straight (Left)	Count	0	1	1
	%	0.0%	2.0%	1.0%
Divergent	Count	1	0	1
	%	2.0%	0.0%	1.0%
Total	Count	50	50	100
	%	100.0%	100.0%	100%



Graph 3. Class III malocclusion

Table 8.

Gender predictive value for Class III malocclusion / Model discrimination

Observed			Predicted		
			Class III		Percentage Correct
			No	Yes	
Step 1	Class III	No	87	0	100.0
		Yes	13	0	.0
	Overall Percentage				87.0

The cut value is .500

Table 9.

Binary logistic regression analysis for prediction of Class III malocclusion / Gender

		B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)	
								Lower	Upper
Step 1[a]	Gender (1)	.177	.596	.088	1	.766	.838	.260	2.695
	Constant	1.815	.408	19.838	1	.000	.163		

a. Variable entered on step 1: Gender.

Discussion

Palatal rugae are considered as the most stable anatomic landmark in the oral cavity. Palatal rugae have unique phenotype characteristics for each person, including their number, shape, and size.[13,14] The palatine rugae have clinical importance as a reference landmark for certain types of dental treatment,[15,16] identification of submucous cleft palate,[17, 18] for forensic identification.[19,20] Studies have demonstrated that no two individual's rugae patterns are alike in their arrangement, and the characteristic rugae pattern of the palate does not change as a result of growth.[20] Differences between genders using the rugae pattern have been studied without any conclusions till now.[21]

A study by Shrestha et al.[22] aimed to find the association between gender and palatal rugae patterns. The study included 100 maxillary plaster casts. The authors concluded that females had more rugae than males, but it was not statistically significant. The prevalent rugae among the young adults of Nepal were wavy shapes followed by curved shapes.

Few studies have been performed to probe the relation of palatal rugae patterns with early diagnostic of malocclusions.[23] During orthodontic movements, Kulkarni and Gore[24] revealed that palatal rugae remain stable, but Deepak V et al.[25] found minimal changes in rugae length.

Shukla et al.[5] concluded that some changes occur during orthodontic treatment, but the morphology of palatal rugae does not change. In addition, the authors concluded that the most reliable points that remain stable over a person's life are the medial and lateral third rugae points. These could be used as reference points to evaluate dental movements.

Shailaja et al., in their study "Assessment of palatal rugae pattern and its significance in orthodontics and forensic odontology,"[6] aimed to compare the shape of rugae and its positional changes before and after rapid maxillary expansion. During the study, the shape of the rugae and the distance between the median points and lateral points of the first and the last two rugae on either side of the mid-palatal raphe were noted and marked. It was found a statistically significant difference in the distance between the medial and lateral points of the first two and last two rugae. The authors concluded that during maxillary expansion, there is stability of palatal rugae with respect to its shape and number but not with respect to its position. Barbieri et al.[19] also found that in the presence of intra-oral changes owing to the use of palatal expanders, the palatine rugae retained the biological and technical requirements for the human identification process.

Juvva et al.,[26] studying 105 subjects, concluded that the most common pattern in three classes of malocclusion was a wavy shape followed by a straight pattern. They found no statistical significance between the palatal rugae patterns and malocclusions.

A study by Qadeer et al.[27] aimed to find the association between malocclusions and palatal rugae patterns. They concluded that palatal rugae, including shape, number, and orientation of rugae, do not have any statistical significance in predicting malocclusions and do not show any significant correlation between palatal rugae and dental arch form. Similar results were obtained by Ekrem et al.,[11] who evaluated the morphological structure of palatal rugae in Turkish orthodontic subjects with different sagittal skeletal malocclusions. Wavy and curved types were the most common types of rugae pattern in all groups (Class I, Class II, Class III malocclusions According to Angle's classification). The number of primary and secondary rugae on the left and right sides was not statistically different among subjects with different skeletal malocclusions. All rugae patterns were unique for each individual.

The present study provides essential information regarding the dominant palatal rugae patterns among Albanian adolescents of southeastern Kosovo with Class I, II, and III malocclusions according to Angle's classification.

Conclusion

In our study, the palatal rugae patterns in Class I, II and III malocclusions show no significant difference between female and male subjects. In Class I malocclusion, the straight pattern was dominant in female subjects, and the wavy pattern was dominant in male subjects. The straight pattern was dominant in males and females with Class II malocclusion. In Class III malocclusion, the wavy pattern was dominant in female subjects, and the curved pattern was dominant in male subjects. The study showed that male subjects are at slightly higher risk for having Class I malocclusion and slightly lower risk for having Class II and Class III malocclusions than female subjects.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Variations of the Plaque Index in Four Timelines during 12 Months in Patients with Two Models of Fixed Retainers after Orthodontic Treatment is Finished

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Abstract

Background: Fixed retainers are retention tools bonded on the lingual side of the frontal teeth to prevent relapse after orthodontic treatment is finished. While stability remains the biggest concern, periodontal response remains the subject of discussion. This study aimed to compare plaque index (PI) levels on the lingual side of the lower dental arch in the inter-canine region after bonding two different models of fixed retainers.

Methods and Results: The study included 60 subjects aged 16-25 who finished orthodontic treatment. Thirty subjects got flat fixed retainer (FFR), and 30 other subjects got round fixed retainer (RFR) bonded in the lower six frontal teeth on the lingual side. Adapted PI was recorded and photographed at four time points (3, 6, 9, and 12 months). Three months after the intervention, there were no significant differences between the FFR and RFR related to the PI value (P=0.363). PI was significantly lower in the FFR group than in the RFR group 6, 9, and 12 months after the intervention (P<0.004, P=0.004 and P=0.001, respectively).

Conclusion: Bonded fixed retainers, in general, cause increased plaque formation and make oral hygiene routines more difficult.**(International Journal of Biomedicine. 2024;14(1):148-152.)**

Keywords: fixed retainers • flat retainer • round retainer • plaque index

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Abbreviations

FFR, flat fixed retainer; **IQR**, interquartile range; **Me**, median; **PI**, plaque index; **RFR**, round fixed retainer

Introduction

Orthodontic treatment aims to move teeth to correct malocclusion. After the treatment is finished, there is often a tendency for teeth to relapse; therefore, to maintain final results, orthodontists use various retention tools such as fixed retainers in order to maintain achieved outcomes.[1]

Despite suggestions that a precise diagnosis and treatment planning, followed by comprehensive stability of the final outcomes, would diminish the relevance of retention, relapse tendencies persist in a considerable fraction of treated cases.[2]

Through the years, there have been presented and documented various types of methods and tools that have been used to retain post-treatment tooth position. Various removable retainers have been advocated, but most often, the use of bonded fixed retainers has been suggested. These fixed retainers are bonded in both jaws' lingual side of anterior teeth but mostly in mandibular incisors and canines.[3-5] In most

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studies related to fixed retainers, orthodontists believe that the only way to maintain the ideal alignment after orthodontic treatment is a form of permanent retention. This can be an FR bonded in the lingual area of anterior teeth, left in the mouth for an extended period of time.[1]

Research has found that this technique doesn't require strong compliance from the patients, and it has served as a reliable solution for long-term stability.[6] While for some researchers, the main concern was stability, many studies focused on changes and periodontal response during the retention phase. Studies suggest that after tooth movement, significant residual forces persist in the periodontal tissue.[7] When using bonded fixed retainers for a long time, the main worry is that they might make it more difficult to maintain oral health and harm periodontal health.[8,9]

However, no consensus is found on this topic when the literature is evaluated. Many studies have shown that bonded fixed retainers increase plaque and calculus formation and induce gingival irritation, while many others have found no detrimental consequences. Therefore, this study aimed to compare, every 3 months during a year, PI levels from the lingual side of the lower dental arch in the inter-canine region after bonding two different models of fixed retainers.

Our study focused on whether the different shapes and sizes of retainers influences plaque accumulation during one year of the post-treatment phase.

Materials and Methods

The study included 60 subjects aged 16-25 who finished orthodontic treatment in the Orthodontic Department (Dental Faculty, UBT College, Prishtina, Kosovo). Thirty subjects got flat fixed retainer (FFR), and 30 other subjects got round fixed retainer (RFR) bonded in the lower six frontal teeth on the lingual side (Figure 1). All the fixed retainers were bonded by the same experienced orthodontist with the same bonding technique and the same bonding tools. The subjects got an informational letter with all the details about the retention phase, and they signed a consent letter to participate in the study. The subjects were called for obligatory follow-up visits in 3, 6, 9, and 12 months.

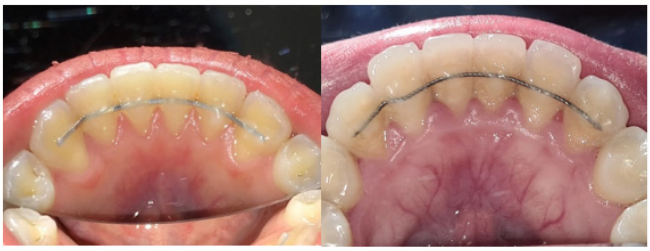


Fig. 1 *(a) FFR- Flat fixed retainer, (b) RFR- round fixed retainer*

Inclusion criteria were no caries, restorations, crowns, or bridges presented and no plaque at the time of bonding the retainer. Exclusion criteria were the participants that failed to be present at requested follow-up periods, those that had any kind of prosthetic restoration during the follow-up period, smokers, pregnant subjects, subjects with syndromes, subjects with general conditions like diabetes, and those had used any kind of hormonal and other medications.

Adapted plaque index (PI) was recorded and photographed at four time points (3, 6, 9, and 12 months). Measurements were performed by one experienced periodontologist and calculated. PI, according to Turesky plaque scoring, was adapted for six frontal teeth of the mandibula on the lingual side where the fixed retainer was bonded, and measured on three surfaces: mesial, distal, and lingual. Premeasurement was applied with the same plaque indicator solution and rinsed according to the instructions.

The measure used to determine plaque score:

(1) No plaque

(2) A thin continuous band of plaque (≤1 mm) at the cervical margin of the tooth

(3) A band of plaque >1mm but covering less than 1/3 of the crown of the tooth

(4) Plaque covering at least one-third but less than two-thirds of the crown of the tooth

(5) Plaque covering two-thirds or more of the crown of the tooth

The value was given to each tooth separately, multiplied, then divided to 6 (teeth) and divided to 3 (surfaces).

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of the distribution of continuous variables was tested by the Shapiro-Wilk W test. The Mann-Whitney U Test was used to compare the differences between the two independent groups. The Wilcoxon criterion was used to compare the differences between two paired samples. The Friedman test was used to test the differences between 3 and more dependent samples. A probability value of $P<0.05$ was considered statistically significant.

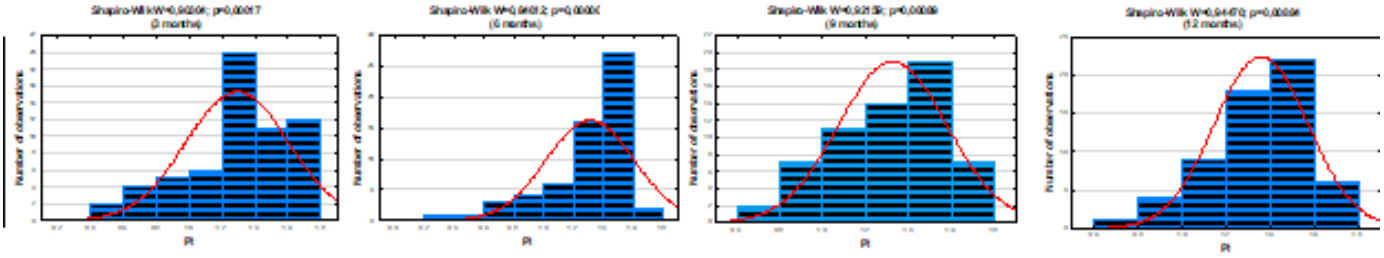
Results

The subjects from both groups (FFR/RFR) were analyzed in relation to PI at four time points. PI was determined for each respondent individually.

The analysis of the distribution of the obtained PI values indicated a non-normal distribution of the frequencies in all four measurement times (Graph 1), and non-parametric tests were applied in the further analysis. The analysis covered intra-group comparison of FFR and RFR, as well as intergroup comparison of FFR/RFR in four time points.

Intra-group comparison of PI

In each of the two groups, the PI level was compared between the four time points after the intervention (Table 1, Graphs 2-3). In the FFR group, 3 months after the treatment, 50% of the patients had PI<1.28 mm. In the period 3-9 months, there was a general decrease in PI, followed by no change between 9 and 12 months. In 50% of the patients, the PI value was <1.27 mm after 6 months, <1.22 mm after 9 months, and <1.22 mm after 12 months. In the FFR group, there were no significant differences between the four time points related to PI ($P=0.714$) (Table 1, Graph 2).

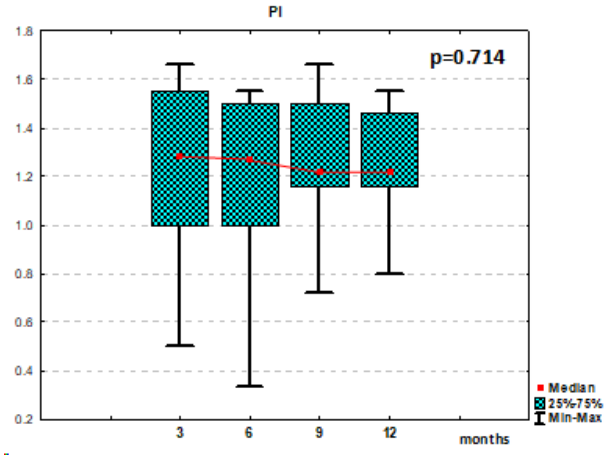


Graph 1. *Distribution of PI in four time points.*

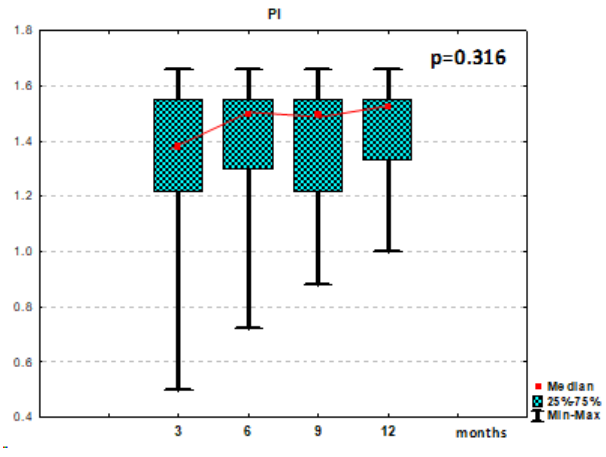
Table 1.

Intra-group comparison of PI in four time points.

Intra-group comparison	Plaque index – PI					Friedman Test
	N	Mean± SD	Min/ Max	Me (IQR)	Mean Rank	
FFR						
3 months	30	1.25±0.34	0.50/1.65	1.28 (0.98-1.55)	2.72	χ^2 (3) = 1.366 *P*=0.714
6 months	30	1.21±0.33	0.33/1.55	1.27 (1.00-1.50)	2.50	
9 months	30	1.23±0.26	0.72/1.66	1.22 (1.10-1.50)	2.40	
12 months	30	1.27±0.20	0.80/1.56	1.22 (1.15-1.47)	2.38	
RFR						
3 months	30	1.34±0.28	0.50/1.66	1.38 (1.22-1.55)	2.17	χ^2 (3 = 3.535 *P*=0.316
6 months	30	1.42±0.21	0.72/1.68	1.50 (1.29-1.55)	2.50	
9 months	30	1.42±0.22	0.88/1.66	1.50 (1.22-1.55)	2.63	
12 months	30	1.45±0.18	1.00/1.66	1.52 (1.33-1.56)	2.70	



Graph 2. *Intra-group comparison of PI in four time points in the FFR group.*



Graph 3. *Intra-group comparison of PI in four time points in the RFR group.*

In the RFR group, after 3 months of intervention, in 50% of the patients the value of PI was <1.38 mm. In the period of 3-12 months, a general increase in PI was observed. In 50% of patients, the PI value was <1.50 mm after 6 months, <1.50 mm after 9 months, and <1.52 mm after 12 months. In the RFR group, no significant difference was found between the four measurement time points related to PI value (*P*=0.316) (Table 1 and Graph 3).

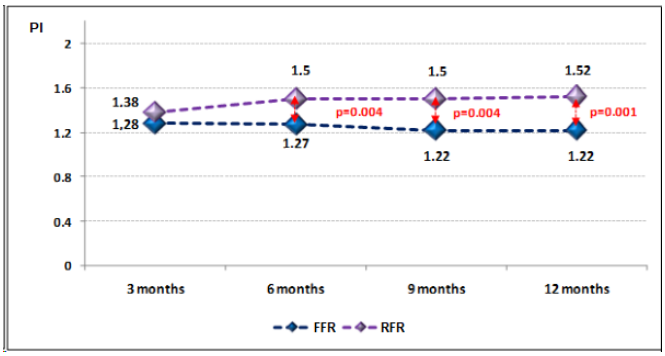
Intergroup comparison of PI

Intergroup comparison of PI between the FFR and RFR groups was made at four time points after the intervention (Table 2 and Graph 4). Three months after the intervention, there were no significant differences between the FFR and RFR related to the PI value (*P*=0.363). PI was significantly lower in the FFR group than in the RFR group 6, 9, and 12 months after the intervention (*P*<0.004, *P*=0.004 and *P*=0.001, respectively).

Table 2.

Intergroup comparison of PI in four time points.

Intergroup comparison	Plaque index – PI						
	N	Mean± SD	Min/ Max	Percentiles			Mann-Whitney U Test
				25th	50th (Me)	75th	
3 months							
FR	30	1.25±0.34	0.50/1.65	0.98	1.28	1.55	Z=-0.909; *P*=0.363
RR	30	1.34±0.28	0.50/1.66	1.22	1.38	1.55	
6 months							
FR	30	1.21±0.33	0.33/1.55	1.00	1.27	1.50	Z=-2.868; *P*=0.004
RR	30	1.42±0.21	0.72/1.68	1.29	1.50	1.55	
9 months							
FR	30	1.23±0.26	0.72/1.66	1.10	1.22	1.50	Z=-2.867; *P*=0.004
RR	30	1.42±0.22	0.88/1.66	1.22	1.50	1.55	
12 months							
FR	30	1.27±0.20	0.80/1.56	1.15	1.22	1.47	Z=-3.363; *P*=0.001
RR	30	1.45±0.18	1.00/1.66	1.33	1.52	1.56	



Graph 4. *Comparison of PI Me by groups (FFR/FRR) in four time points.*

Discussion

In our study, at the measurements in the 3-month follow-up period, we found no significant difference between the FFR group and the RFR group. But in 6, 9, and 12 months of follow-up, FFR subjects had significantly lower PI than RFR subjects. These findings agree with the study by Torkan et al.[10] and Dietrich et al.[11] They reported increased plaque accumulation in patients with round bonded retainers in short- and long-term follow-up periods during retention. In contrast, a study by Antun et al.[12] showed a decrease in the presence of plaque at 3 years in retention with various types of retainers. Still, FFRs were not included in the study, only several RFRs. However, the findings on the retention period and periodontal health are mixed. Some periodontal characteristics improve quickly after debonding, whereas others remain unchanged or worsen over time.[9,13,14]

According to a study by Levin et al.,[15] different types of fixed retainers were linked to higher plaque accumulation but with low clinical significance. However, the dimensions of the retainers were uncertain. In agreement with our study are also several studies where subjects with round, multistrained fixed retainers showed higher plaque accumulation over a 24-month follow-up period despite receiving frequent oral hygiene education.[16,17]

This research contradicts the findings of Artun et al.,[12] who determined that there were no changes in plaque or calculus accumulation between round spiral wire and plain wire retainers.

A study by Shirasu et al.[18] agrees with our results and explains the effect of RFRs with the fact that the nature of the wire in a twisted shape, and also if the bends are close to the gingival papilla, could promote some retentive sites that would make tooth brushing and biofilm disorganization harder. Like our study, it states that multistranded RFRs tend to acquire more plaque and gingival inflammation than FFRs.[19] Other studies also mention the fact that bonding retainers to all anterior teeth retains more plaque than bonding exclusively to canines.[18,20] Shirasu et al.[18] looked at gingival parameters after using two different types of fixed retainers. The results showed that RFRs in the proximal and lingual surfaces had greater PI and Gingival Index, which agrees with our study, too. Also, they stated that FRRs were associated with the best hygiene and comfort for the patients. In the study of Buzzata et al.,[21] it was acknowledged that the small amount of research comparing the two types of retainers must be considered. The variability of wires and bonding types, as well as the small sample sizes and short follow-up timeframes, were significant drawbacks. On the other hand, the majority of trials comparing smooth plain or flat retainers to multistranded wires showed no difference between the methods.[10,22]

Regarding periodontal health in general, a literature review found no consensus on this topic. Studies have revealed that bonded fixed retainers, in general, cause increased plaque and calculus formation, as well as gingival inflammation. Other studies, on the other hand, have found no harmful impact. While

the long-term periodontal implications of using fixed retainers are unknown, it is widely agreed that fixed retainers make oral hygiene routines more difficult.[23] Patients must be trained in how to care for their bonded retainers, which involves using some type of interdental cleaning agent. Furthermore, the cleaning process affects oral hygiene, implying that a patient's motivation should be considered when determining whether or not to use a fixed retainer.[24] This study reveals that bonded fixed retainers, in general, cause increased plaque formation, as well as gingival inflammation. While the long-term periodontal implications of using fixed retainers are unknown, this study found that fixed retainers make oral hygiene routines more difficult.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

This prospective study was approved by the Ethics Committee of the UBT college (Protocol N # 05-PA-30-XV-3/2021) and the Ethical Committee of the Dental Chamber in Kosovo (N # 23/2021).

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ORIGINAL ARTICLE

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Comparison of Immunohistochemical Expression of Calretinin, Map2, S-100 and Glut1 in Rectal Biopsies from Suspected Hirschsprung's Disease

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Abstract

Background: This study aimed to compare the immunohistochemical expression of calretinin, Map2, S-100, and Glut1 in rectal biopsy samples from patients suspected of Hirschsprung's disease (HD).

Methods and Results: Rectal biopsy samples from 40 patients with suspected HD were analyzed using hematoxylin and eosin (H&E) and immunohistochemistry (IHC). Immunohistochemical stains were assessed after previous routine histology interpretation, which was classified as "Positive or in favor for HD" and "Equivocal or negative for HD." The staining patterns for calretinin, Map2, S-100, and Glut1 were analyzed regarding the following structures: lamina propria small nerve fibrils, submucosal small nerve fibrils, submucosal nerve fibers, and submucosal ganglia. The IHC stains for calretinin and Map2 were score-ranked as 0 – negative and 1 – positive. The IHC stain for S-100 was score-ranked as 0 – normal and 1 – hypertrophic. The IHC stain for Glut1 was ranked as 0 – normal perineural and 1 – conspicuous perineural accentuation

Calretinin had 92% accuracy, the highest sensitivity (80%) and specificity (92.00%). Map2 also had the same accuracy as calretinin but lower sensitivity (46.67%). Regarding S-100 and Glut1, these two markers did not support a conclusive diagnosis. The accuracy for S-100 and Glut1 was 66.7% and 60%, respectively.

Conclusion: Calretinin remains the currently most valuable single IHC marker in diagnosing difficult cases of HD.**(International Journal of Biomedicine. 2024;14(1):153-158.)**

Keywords: Hirschsprung's disease • diagnosis • biopsy • immunohistochemistry

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Abbreviations

AChE, acetylcholinesterase; **GC**, ganglion cells; **Glut1**, glucose transporter 1; **H&E**, hematoxylin and eosin; **HD**, Hirschsprung's disease; **IHC**, immunohistochemistry; **MAP-2**, microtubule-associated protein-2.

Introduction

Hirschsprung's disease (HD) is a congenital disease of the enteric neural system characterized by the absence of GC in submucosal and myenteric plexuses of the distal digestive tract due to failure to migrate of the neural crest cells during embryonic development. Usually, this occurs around the 12th week of embryogenesis. Migration and differentiation occur from the proximal to the distal segment. As GC are responsible for normal peristalsis, their absence results in functional

obstruction of the aganglionic segment, followed by bowel dilatation proximally from the affected zone. In approximately 80% of cases, aganglionosis involves the recto-sigmoidal segment. The disease-related mortality in the first year of life has dropped from 25%-30% to 1% due to early diagnosis and successful treatment methods.[1-4]

The gold standard for diagnosis of HD remains the histological examination of biopsy samples obtained from the affected narrow segment. These tissue samples exhibit a lack of GC with hypertrophy and disorganization of the nerve fibers in the submucosal and muscular plexus.[5] Adequate and well-oriented tissue samples, coupled with the experience of the pathologist, are the most important elements in the diagnosis of HD.[6] H&E staining in formalin-fixed and paraffin-embedded tissue is generally used for histological diagnosis. The acetylcholinesterase (AChE) stain in frozen samples obtained by aspiration rectal biopsy is also a well-established method.[7] However, histochemistry with AChE is not universally used due to difficulties related to technical aspects and interpretation.[5,8] In recent decades, various markers have been tested using immunohistochemistry (IHC) to increase diagnostic accuracy in HD.[9-12] According to studies, calretinin and Map2 showed sensitivity in identifying GC even in insufficient or non-optimal samples.[13] Additionally, Glut1 and S-100 were shown to be valuable in detecting nerve fibers.[13] Such an immunohistochemical diagnostic panel provides dual accuracy in terms of conclusion for the absence of GC and the evaluation of nerve fibers and their thickness and distribution in tissue samples.

This study aimed to compare the immunohistochemical expression of calretinin, Map2, S-100, and Glut1 in rectal biopsy samples from patients suspected of HD.

Materials and Methods

This study was conducted at the Pediatric Surgery Clinic, Institute of Pathology at the University Clinical Center of Kosovo, and NUCLEUS Pathology Diagnostics & Research Laboratory. Rectal biopsy samples from 40 patients with suspected HD were analyzed using H&E and IHC, respectively, from 2017 to 2023. Patients had been previously evaluated clinically and with imaging studies. Twenty-three cases were prospectively analyzed, and 17 cases were obtained from our archive and medical records. Two independent general pathologists examined biopsy samples. Since the study was morphological, partly retrospective, and unrelated to any additional clinical interventions, patient consent was not explicitly obtained.

Routine Histology

After optimal fixation for 24 hours in neutral buffered formalin, tissue samples were processed in a tissue processor (Leica TP 1020), where they underwent an additional fixation procedure in 10% neutral buffered formalin (NBF), gradual dehydration in 70%, 80%, 96% and absolute ethanol, xylene cleansing and immersion in liquid paraffin at 60°C. Subsequently, the labeled specimens were molded into paraffin blocks, sectioned at 3–4-micron-thick sections, and applied on microscope glass slides. After deparaffinization and gradual rehydration in decreasing solutions of ethanol and distilled water, tissue sections were stained with hematoxylin and eosin (H&E), covered by the mounting medium, and coverslipped. The H&E-stained sections were analyzed to evaluate the sample's diagnostic adequacy and the presence, distribution, and morphological features of the neural ganglia and nerve fibers in the submucosal layer. Immunohistochemical stains were assessed after previous routine histology interpretation, which was classified as "Positive or in favor for HD" and "Equivocal or negative for HD."

Immunohistochemistry

IHC analysis was carried out for calretinin, Map2, S-100, and Glut1. Antigens were retrieved by placing the slides in a target retrieval solution for 45 minutes at 95-98°C. After the peroxidase block, the slides were incubated with the primary antibody against calretinin, Map2, S-100, and Glut1 for 30 minutes. In the next step, dextran polymer conjugated with peroxidase and a secondary antibody (EnVision+, DAKO, K534011) were applied for another 30 minutes. The visualization was carried out with DAB and chromogen. The vendor, clone, and dilution of the antibodies are presented in Table 1. The slides were counterstained by Harris's hematoxylin, washed, covered by the mounting medium, and coverslipped.

Table 1.

Antibody, Vendor, Clone, and Dilution of the Employed Immunohistochemical Markers

Antibody	Vendor	Clone	Dilution
Calretinin	DAKO	DAK-Calret1	RTU
Map2	Milipore	Polyclonal	1:1000
S-100	DAKO	Polyclonal	RTU
Glut1	Milipore	Polyclonal	1:300

The staining patterns for calretinin, Map2, S-100, and Glut1 were analyzed regarding the following structures: lamina propria small nerve fibrils, submucosal small nerve fibrils, submucosal nerve fibers, and submucosal ganglia. The IHC stains for calretinin and Map2 were score-ranked as 0 – negative and 1 – positive. The IHC stain for S-100 was score-ranked as 0 – normal and 1 – hypertrophic. The IHC stain for Glut1 was ranked as 0 – normal perineural and 1 – conspicuous perineural accentuation (Fig. 1).

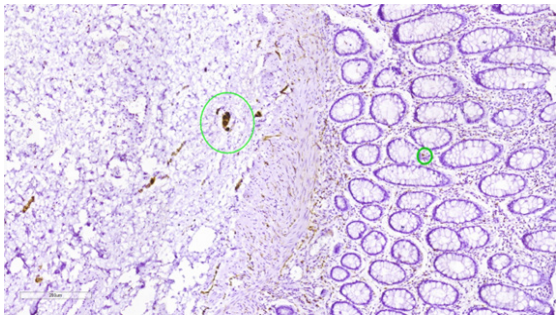

Fig. 1a. *Calretinin stain in a normal subject highlights the neural ganglion (big circle) and small nerve fibers of submucosa and lamina propria. Cross reaction with mast cells (small circle) serves as an internal "built-in" positive control.*

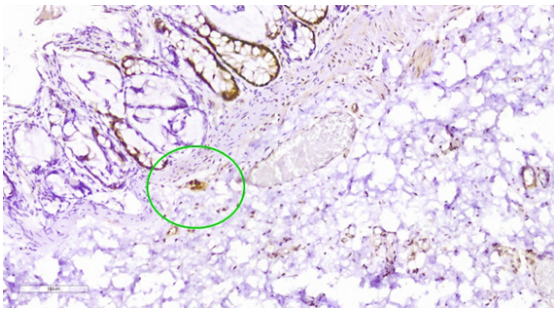

Fig. 1b. *Map2 stain in a normal subject highlights the neural ganglion (circle). Cross-reaction with nuclei of mucosal epithelial cells and lymphatic tissue (not shown) was consistently observed in our stains.*

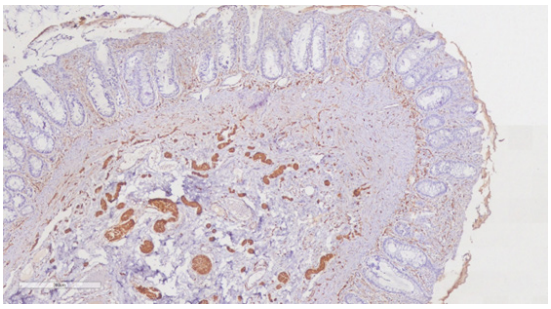

Fig. 1c. *S-100 stain in HD marks the hypertrophic and disorganized nerve fibers in the submucosal layer.*

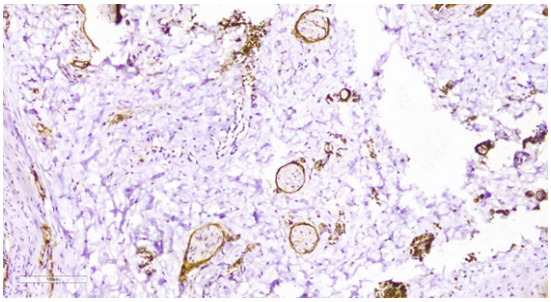

Fig. 1d. *Glut1 stain in HD shows increased, hypertrophic, and disorganized nerve fibers with characteristic perineural accentuation in the submucosal layer. Cross-reaction of Glut1 with red blood cells was observed (not shown).*

Statistical Analysis

Statistical analysis examined the relationship between H&E and immunohistochemistry groups. Descriptive statistics were calculated using the chi-square test or Fisher's exact test. Furthermore, each immunohistochemistry group's accuracy, sensitivity, and specificity were calculated. Descriptive statistics were employed to summarize the main characteristics of the data. The chi-square or Fisher's exact tests are used for categorical data analysis. These tests determine whether there is a significant association between two categorical variables. These tests were applied to explore the relationship between different staining methods (H&E and immunohistochemistry groups). Immunohistochemistry tests are used in pathology to detect the presence, abundance, and localization of specific proteins within tissues. This study also assessed the accuracy, sensitivity, and specificity of immunohistochemistry tests. Accuracy represents the proportion of true results (both true positives and true negatives) among the total number of cases examined. It provides an overall measure of how well the immunohistochemistry tests identify specific proteins. Sensitivity, also known as the true positive rate, measures the proportion of actual positives correctly identified by the test. It is a crucial metric in medical diagnostics, indicating the test's ability to correctly identify individuals with the condition (true positives). Specificity, also known as the true negative rate, measures the proportion of actual negatives correctly identified by the test. It signifies the test's ability to correctly identify individuals without the condition (true negatives). A P value of <0.05 was chosen as the threshold for statistical significance. This indicates that if the P value obtained from the statistical tests is less than 0.05, the results are considered statistically significant, suggesting that the relationship observed between HE and immunohistochemistry groups is unlikely to have occurred by chance. IBM SPSS Statistics® version 22 was used to perform descriptive analysis, and MedCalc® was used to perform a detailed analysis of the relationship between different staining methods.

Results

Statistical significance between histology interpretations and corresponding IHC results was observed when routine histology interpretations were clustered in Group 1 (n=25 – Positive or in favor of HD) and Group 2 (n=15 – Equivocal or negative for HD). However, during the accuracy calculation, calretinin was shown to be the most useful test for the two histological subgroups. Regarding Group 1, all four markers confirmed the presence or absence of HD by their positive or negative reaction, respectively. However, in Group 2, calretinin proved to have the highest accuracy. Out of 25 patients with an HE interpretation of HD, 23(92%) were confirmed by calretinin staining (Table 2).

Table 2.

Comparison between Routine Histology Interpretation and Immunohistochemical Expression of Calretinin, Map2, S-100, and Glut1

		H&E Interpretation		*P*-value
		Positive or in favor for HD [n (%)]	Equivocal or negative for HD [n (%)]	
Calretinin	Positive	2 (8)	12 (80,0)	<0.01[a]
	Negative	23 (92.0)	3 (20,0)	
Map2	Positive	2 (8)	7 (46.7)	<0.01[b]
	Negative	23 (92)	8 (53.3)	
S-100*	Positive	22 (88)	5 (33.3)	<0.01[b]
	Negative	3 (12)	10 (66.7)	
Glut1	Positive	22 (88)	6 (40)	<0.01[b]
	Negative	3 (12)	9 (60)	

**S-100 hypertrophy. The chi-square test or Fisher's exact test was appropriately used to explore the relationship between H&E and immunohistochemistry groups (IBM SPSS Statistics® version 22). The immunohistochemistry tests' accuracy, sensitivity, and specificity were calculated using MedCalc® statistical software.*

Also, out of 15 patients in Group 2, 12(80%) were confirmed as negative. Calretinin had 92% accuracy (Table 3). Calretinin staining also showed the highest sensitivity (80%) and specificity (92%). This was not the case with the other three markers. Map2 also had the same accuracy as calretinin but lower sensitivity (Table 3). Regarding S-100 and Glut1, in Group 2, these two markers did not support a conclusive diagnosis (Table 2). The accuracy for S-100 and Glut1 was 66.7% and 60%, respectively (Table 3).

Table 3.

Sensitivity, Specificity, and Accuracy of Calretinin, Map2, S-100, and Glut1 Stains in the Study Groups.

	Statistics	Value (%)	95% CI
Calretinin	Sensitivity	80.00	51.91 - 95.67
	Specificity	92.00	73.97 - 99.02
	Accuracy	92.00	78.94 - 98.20
Map2	Sensitivity	46.67	21.27 - 73.41
	Specificity	92.00	73.97 - 99.02
	Accuracy	92.00	78.94 - 98.20
S-100 (*)	Sensitivity	88.00	68.78 - 97.45
	Specificity	66.67	38.38 - 88.18
	Accuracy	66.67	50.02 - 80.75
Glut1	Sensitivity	88.00	68.78 - 97.45
	Specificity	60.00	32.29 - 83.66
	Accuracy	60.00	43.33 - 75.14

Discussion

IHC for calretinin remains the most valuable single IHC marker in diagnosing difficult cases of HD. Our study had a statistical accuracy of 92% and a high sensitivity and specificity of 80% and 92%, respectively. In contrast, S-100, Glut1, and Map2 did not support a conclusive diagnosis. The diagnosis of HD is complex and requires multidisciplinary management involving neonatologists, pediatric gastroenterologists, pediatric surgeons, radiologists, and pathologists. The gold standard for diagnosing this disease is histological examination of tissue samples obtained by rectal biopsy.[14,15] Histological diagnosis consists of determining the absence of GC and hypertrophy, as well as disorganization of nerve fibers, in the submucosal layer of the biopsy sample.[16] The muscular layer is usually not present in the biopsy samples. Therefore, the submucosal layer should be thoroughly evaluated. In practical terms, obtaining a sufficient sample with a representative submucosal layer very much depends on the expertise and experience of the pediatric surgeon. Hence, small samples with limited amounts of submucosal layer and consequent need for a repeat biopsy are common in our practice. In these situations, IHC as a valuable diagnostic tool may overcome the need for repeat biopsy and avoid unnecessary interventional complications for the patient. Historically, hypertrophic and disorganized nerve fibers in the submucosal plexus were identified through histochemical staining of frozen tissue samples with AChE.[7,17] However, in practical terms, assessing nerve fibers with AChE may be difficult due to cross-interaction with red blood cells and smooth muscle. Also, identifying small nerve fibers extending into the lamina propria is subjective and depends on the pathologist's experience.[18] Recently, choline transporter IHC has been introduced as an alternative to AChE histochemistry with a similar reaction pattern but a much simpler interpretation.[19] Surgical treatment for HD in recent decades has evolved from 3-stage surgery to a single operation. By this method, a significant number of patients undergo surgery in the neonatal period. Thus, the preoperative diagnosis should be rendered at neonatal age despite the difficulties in identifying GC and nerve fibers by H&E and AChE stains in this period.[20-23] In recent decades, various immunohistochemical markers have been tested to increase diagnostic sensitivity and specificity for HD. These markers have shown great potential in accuracy and ease of applicability, given that they are suited for formalin-fixed, paraffin-embedded tissue. Hence, there is no need to depend on difficult-to-perform and difficult-to-interpret frozen techniques.

Barshack et al.[24] evaluated the applicability of calretinin in identifying intrinsic nerve fibers and GC in samples from patients suspected of having HD. Also, Kapur et al.[21] demonstrated the importance of combining calretinin with H&E and AChE in cases of total colonic aganglionosis and ultra-short HD. Yang et al.[25] concluded that calretinin and Map2 are useful markers in identifying HD in aspiration rectal biopsies. The presence of GC in the submucosal layer by calretinin stain excludes HD. Still, caution should be exercised in cases with ultra-short HD where biopsy specimens may show slight positivity for calretinin in the transition zone.[24,26] Guinard-Samuel et al.[26] found that calretinin visualized specimens in the "black and white" model and had excellent consistency between experienced and inexperienced pathologists, avoiding the need for repeat biopsies.[26]

In the study by Musa et al.,[27] samples with HD showed an absence of expression for calretinin in lamina propria of mucosa and submucosa. In contrast, all samples negative for HD showed positive expression by ganglia as well as nerve fibers in these layers. This was also observed in our study. Calretinin stained the nucleus and cytoplasm of GC and the cytoplasm of fine nerve fibrils and nerve fibers of the lamina propria and submucosa. In our study, the interpretation of calretinin IHC was straightforward. Cross-reaction with mast cells was used as a "built-in" internal control for negative cases. In the study by Burtelow and Longacre,[28] Map2 was successfully applied to identify GC. Map2 successfully marks GC without staining other neural elements of the neuroenteric system.[28] In our study, Map2 stained a smaller number of GC than did calretinin. However, the difference between the groups was significant. Map2 showed the same accuracy as calretinin. In contrast to calretinin, it showed a lower statistical sensitivity. We believe that in a larger study group, higher sensitivity may be observed.

Monforte-Muñoz et al.[17] investigated the efficacy of S-100 in highlighting nerve fibers and found that about 90% of samples from HD had hypertrophic fibers of over 40 microns

in diameter. Our study also observed this even though no measurements have been taken. Lim et al.[29] recorded two false negative results from 27 patients with HD. In our study, S-100 identified nerve fiber hypertrophy in cases with HD but was less reliable in the equivocal or negative study group. Hence, the accuracy and specificity of S-100 were lower than calretinin and Map2. Glut1, like S-100 and AChE, identifies hypertrophic fibers in HD patients.[30]

In our study, Glut1 stained the hypertrophic nerve fibers and the perineurium in the submucosa in HD but was less sensitive than the S-100 stain. Like S-100, this marker was less reliable in the equivocal or negative study group and had lower statistical accuracy and specificity than calretinin and Map2.

The study's main strengths are the robust methodology, including the staining techniques and interpretation criteria, which are well-detailed. The study incorporates multiple IHC markers, providing a comprehensive analysis. This approach increases the reliability of the findings and allows for a more nuanced understanding of the disease. The study also combines retrospective and prospective analyses, enhancing the robustness of the results by considering a diverse set of cases over several years. The study employs appropriate statistical tests to analyze the relationship between different staining methods, providing a quantitative basis for the conclusions. The study compares IHC results with routine histology interpretations, allowing for validation of the IHC markers against established diagnostic methods.

Limitations of the Study

The main limitation of the study is a relatively small sample size. While the results are significant within this sample, a more extensive and more diverse sample could enhance the generalizability of the findings. Furthermore, the study is conducted in a single center, potentially limiting the diversity of cases and diagnostic challenges encountered. Multi-center studies might provide a broader perspective on the applicability of these markers. The study does not provide information on the long-term outcomes of patients diagnosed using IHC markers. Long-term follow-up data could validate the accuracy of the diagnoses made based on these markers. Finally, the interpretation of IHC stains involves a certain level of subjectivity, which could introduce observer bias.

Conclusion

Calretinin remains the currently most valuable single IHC marker in diagnosing difficult cases of HD. The pediatric surgeon's or gastroenterologist's role in assessing the clinical features and biopsy site cannot be overstated.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Intrabulbar, Intraorbital and Intracranial Perforating Eye Injury with Foreign Body: A Case Report

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Abstract

Ocular trauma is a significant cause of preventable blindness and visual impairment worldwide. Eye globe perforation can happen due to work with sharp tools or different particles that are spread during work with metals, wood, and other solid substances. We present the case of a 14-year-old male patient admitted as an urgent case at the Ophthalmology Department in 2014 due to a perforating eye injury with a foreign body – a 7 cm rusty metallic nail in the right eye. The X-ray of the orbit revealed that the foreign body had penetrated the eyelid, the eye eyeball, and through the orbit and its posterior wall, penetrated the skull and 2 cm in the brain. Under general anesthesia, we performed the anterior chamber lavage, excision of the prolapsed iris, and pupilloplasty. The anterior chamber was reconstructed using a saline solution (0.9% NaCl) and air. The patient was treated conservatively with antibiotics and steroids for 10 days; there were no signs of wound filtration, hypotonia, or endophthalmitis. Due to the limited resources for the posterior segment surgery, the patient was referred to another center for pars plana vitrectomy (PPV) after 10 days of hospitalization in the Department of Ophthalmology at the University Clinical Center of Kosovo. After PPV with silicon oil and cataract surgery, the patient could see the light and its projections (BCVA = L+P+). We followed up on the patient until September 2022, there were no signs of bulbus atrophy, and the visual acuity remained the same, BCVA = L+P+.

The correct diagnosis and treatment at the right time play a leading role in achieving one of the main goals of surgical treatment of perforating eye injuries, which is the preservation of the anatomical structure and the physiology of the eye.**(International Journal of Biomedicine. 2024;14(1):159-161.)**

Keywords: perforating eye injuries • foreign body • surgical treatment

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Introduction

Ocular trauma is a significant cause of preventable blindness and visual impairment worldwide, with approximately 1.6 million people being blind annually due to the condition. Despite being a mostly avoidable condition, it continues to pose a significant burden, especially in developing countries. Increasing socioeconomic burden, inadequate safety measures, lack of optimal treatment facilities, and poor education are some of the factors that contribute to the high incidence of ocular trauma in these regions. [(1)] Industrialization and urbanization may alter or modify prevalent aetiological factors and the presentation of ocular trauma.[(2)]

Eye globe (bulbus oculi) perforation can happen due to work with sharp tools or different particles that are spread during work with metals, wood, and other solid substances. Penetrating/ perforating injury could lead to lacerations of the eyelids, cornea, or sclera, which may be associated with intraocular hemorrhage, retained foreign bodies, or tractional retinal detachment.[(3,4)] We present a case of a young male patient with a perforating eye injury with a metallic foreign body.

Case Presentation and Discussion

A 14-year-old male patient was admitted as an urgent case at the Ophthalmology Department in 2014 due to a perforating eye injury with a foreign body – a metallic nail

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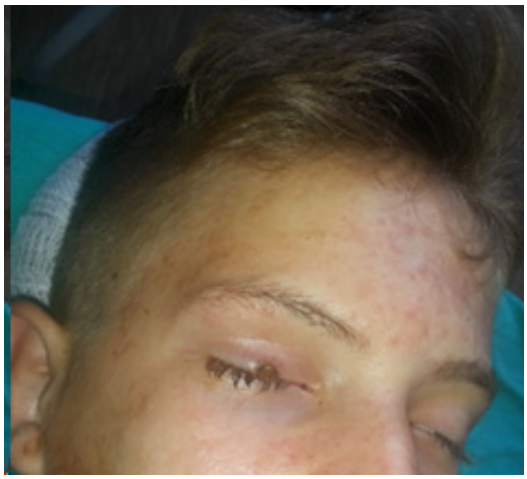
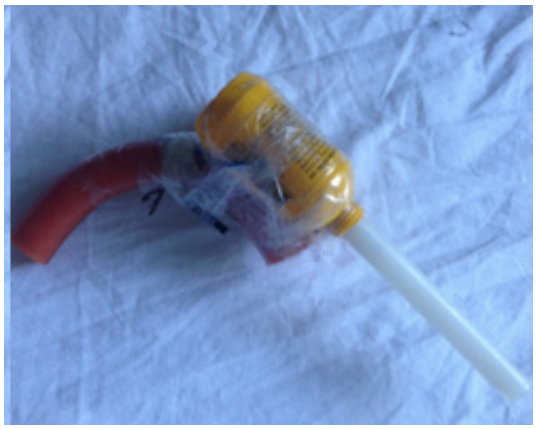
in the right eye (Picture 1). Firstly, a detailed patient history was taken from the parents, where the circumstances of the injury were explained. The perforating injury of the eye was caused by a metal nail. The injury happened while the patient tried experimenting with a "toy" he had built himself. This equipment comprised two plastic containers of 100 mL; the patient filled them with gas and tied the lighter switch to the containers. Afterward, he filled plastic tubes with metal nails and fixed all these parts together. While trying to see them through the tubes, he accidentally pushed the switch; the gas was lit, pushing the nail out of the tube with a very high pressure (Picture 2).



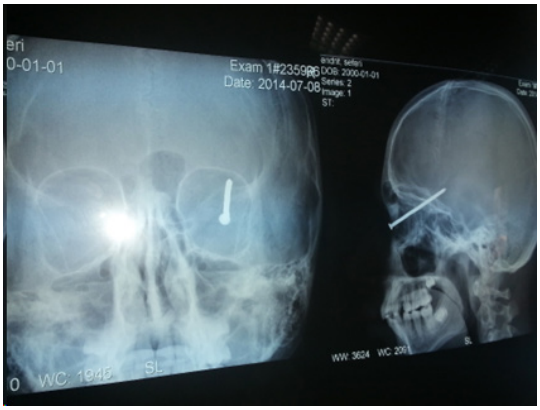
Pic. 1. *The presence of the metallic foreign body in the right eye.*



Pic. 2. *The tool that caused the injury.*

At the time of admission, the patient was conscious. The X-ray of the orbit revealed that the foreign body had penetrated the eyelid, the eye eyeball, and through the orbit and its posterior wall, penetrated the skull and 2 cm in the brain (Picture 3).

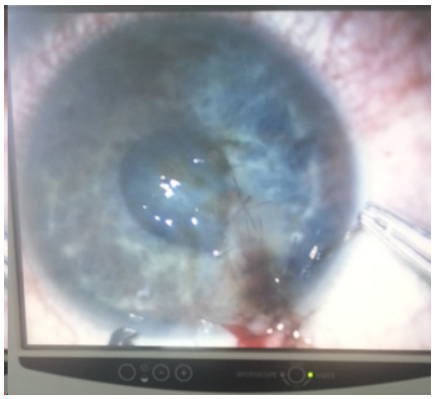
After receiving the complete laboratory results, anti-tetanic protection was administered, and surgery under general anesthesia was planned. Based on the data we obtained from the radiological finding, we identified our case as a duplicate perforating injury with a foreign body. After having a clear idea of the penetration depth of the nail, we proceeded with the surgery. The first step was the removal of the foreign body, where a 7 cm rusty metallic nail was removed from the eye (Picture 4).



Pic. 4. *After the foreign body was removed from the eye.*

When we gained access to the eye, we managed to open the eyelid and an irregular wound with constant bleeding was present. With the examination in the operating room, we evaluated that there was a prolapse of the iris and corpus vitreous, and the anterior chamber of the eye was destructed and filled with blood.

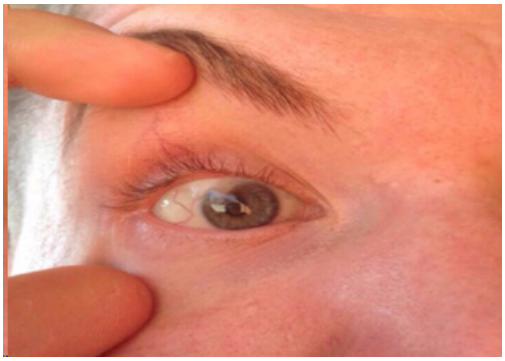
As the first step of the surgical treatment, the anterior chamber was lavaged with a saline solution (0.9% NaCl) to remove the blood and gain a clearer view. Afterward, we performed an excision of the prolapsed iris and reconstructed the pupil by performing the pupilloplasty. Being a challenging case, our primary purpose was to reconstruct the destroyed parts of the eye to maintain its anatomical structure. After performing the pupilloplasty, we constructed the anterior chamber by using a saline solution (0.9% NaCl) and air and sutured the cornea using 10.0 Nylon sutures (Picture 5).



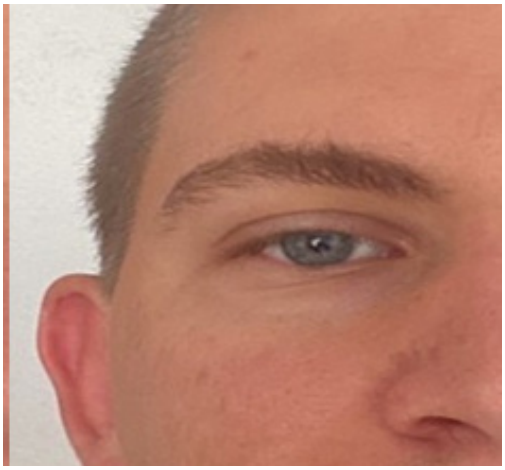
Pic. 5. *Pupilloplasty.*

Conservative treatment was started immediately after the surgery, and the patient remained under surveillance. We treated the patient with intravenous antibiotics (Cefazolin 2g every 12h), topical antibiotics (Cefazolin-Vancomycin

eyedrops every 1h), and topical and oral steroids. The patient was examined daily to see the recovery process; there were no signs of wound filtration, hypotonia, or endophthalmitis. Due to the limited resources for the posterior segment surgery, the patient was referred to another center for pars plana vitrectomy (PPV) after 10 days of hospitalization in the Department of Ophthalmology at the University Clinical Center of Kosovo. After PPV with silicon oil and cataract surgery, the patient could see the light and its projections (BCVA = L+P+). We followed up on the patient until September 2022, there were no signs of bulbus atrophy, and the visual acuity remained the same, BCVA = L+P+ (Pictures 6 and 7).



Pic. 6. *The patient's eye in 2017.*



Pic. 7. *The patient's eye in 2022.*

We presented a very rare case of a 14-year-old male patient admitted as an urgent case at the Ophthalmology Department in 2014 due to a perforating eye injury with a foreign body – a metallic nail in the right eye.

The treatment of intraorbital foreign bodies depends on a variety of factors. Using broad-spectrum antibiotics with or without anaerobic and antifungal coverage is recommended, as well as the timely vaccination of the patient against tetanus.

The most important procedure, and the first one in open ocular trauma, is to restore the structural integrity as soon as possible.[6,7] Guven et al.,[7] using multivariate logistic regression analysis, found that lens damage is also an essential factor affecting the final visual acuity.

Less severe injuries tend to require less surgery, whereas more severe injuries, with more subsequent complications, would require more surgery and carry a worse prognosis.[8-10]

In conclusion, the correct diagnosis and treatment at the right time play a leading role in achieving one of the main goals of surgical treatment of perforating eye injuries, which is the preservation of the anatomical structure and the physiology of the eye.

Ethical Considerations

Publication of the report was approved by the Ethics Committee at the University Clinical Centre of Kosovo. The patient's legal guardians gave informed consent for publishing the case report, including images and other clinical information, except individual details identifying the patient.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Complex Odontoma Associated with Impacted Teeth and Supernumerary Tooth

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Abstract

This study aimed to present the case of supernumerary teeth and complex odontoma. It highlights the debate about odontomas, the most common benign tumors of odontogenic origin. Due to their hamartomatous characteristics, they are usually asymptomatic but can cause impaction of one or more teeth. Microscopically, they comprise all the tissue types found in a developed tooth. Here, we report a case of complex odontoma associated with impacted teeth and a supernumerary tooth, followed by bony expansion and failure of eruption in a 15-year-old boy.**(International Journal of Biomedicine. 2024;14(1):162-164.)**

Keywords: supernumerary tooth • odontoma • tooth retention

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Introduction

The term odontoma was first introduced by Paul Broca in 1867 to describe the odontogenic tumor formed by the overgrowth of transitory or complete dental tissues. Although WHO classifies odontomas as benign odontogenic tumors,[1] odontomas are clinically considered to be tumor-like formations (hamartomas of dental tissues) or developmental anomalies rather than true odontogenic neoplasms.[2] The etiology of odontomas is not known, but there are several theories, such as local trauma during the time of primary teeth, inflammatory and infectious processes, hereditary anomalies, and changes in the genetic components responsible for the roots of the teeth.

Two main types of odontoma have been described: (a) complex odontoma, an amorphous and disorderly pattern of calcified dental tissues, and (b) compound odontoma, multiple miniature or rudimentary teeth.[3-7] The compound odontoma has a predisposition toward the anterior maxilla (61%), whereas only 34% of complex odontomas occur in this area; the complex type shows a preference for the posterior jaws (59%) and lastly, the premolar area (7%). Both variants are made of all dental tissues, such as enamel, dentin, cementum, and pulp.[7,8] It is worth mentioning, and interesting, that both types of odontomas occur more often on the right side of the jaw than on the left.

The reported cases of odontoma are mainly during the second and third decades of life.[9-12] Sometimes, odontoma can cause disturbances in the eruption of teeth, such as impaction, delay in eruption, or retention of primary teeth. In general, odontomas appear more often in permanent dentition, and are rarely associated with primary teeth.[13,14]

At X-ray evaluation, compound odontomas appear as well-delimited lesions with a radiotransparent halo containing radiodense zones, representing small denticles separated by fibrous septae. In contrast, in the complex types, the radiodense elements appear as irregular and disorderly masses with no similarity to dental structures.[2,15] These lesions are often associated with impacted permanent teeth.[16,17] A complex odontoma may be confused radiographically with an osteoma or other highly calcified bone lesion.[18]

Conventional radiography cannot always demonstrate the details of difference. Histopathologic evaluation confirms the diagnosis, especially in cases of complex odontoma, which may be confused with an osteoma or another highly

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calcified bone lesion on radiographs.[19,20] A differential diagnosis is usually made by comparing the degree of morpho-differentiation and histodifferentiation of the dental hard tissue. A complex odontoma is presented by all dental tissues in an organized form or disorderly pattern, by the formation of calcified enamel and dentin in an abnormal arrangement because of a lack of morphodifferentiation. In a compound odontoma, all dental tissues are represented in a more orderly pattern so that the lesion consists of many tooth-like structures or denticles, anomalous miniature teeth, composed of enamel, dentin, cementum, and pulp.

In all cases, surgical removal represents the best therapeutic option, and the prognosis after treatment is very favorable, with very low incidence of recurrence.[21-24]

Case Presentation

This case report presents a 15-year-old male who came to the Oral Surgery Clinic in 2019 with an unerupted right maxillary canine and first maxillary premolar, followed by swelling in that region and persistent first primary premolar (Figure 1). His medical anamnesis was clear. There was no history of trauma to the orofacial region. There was no family history of unerupted teeth or hypodontia. Panoramic radiography of the upper canine region showed irregular radiopaque mass near the crown of an unerupted canine and supernumerary tooth (Figure.2). The first diagnostic hypothesis was a complex odontoma, and the patient was scheduled for surgical removal of the lesion.

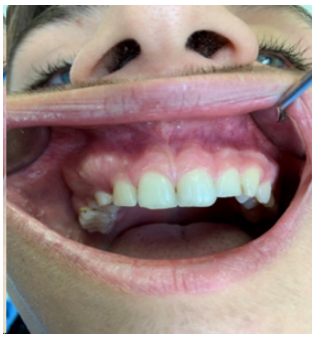

Fig. 1. *Intraoral examination: Frontal view.*

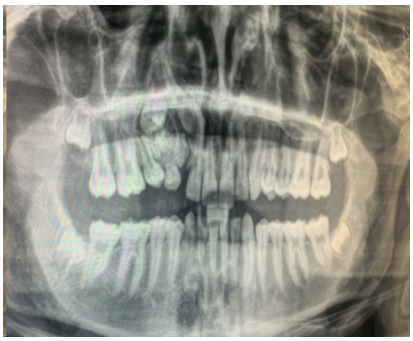

Fig. 2. *Panoramic X-ray.*

The operation was performed under local anesthesia. The buccal mucoperiosteal flap was raised in the upper canine region. The thin overlying bone was removed with a bur, and then the odontoma was removed, as well as a supernumerary tooth (Figures 3 and 4). The surgical wound was closed primarily with 3/0 Vicryl sutures (Figure 5). The right primary canine and first premolar were unerupted. The chance of re-eruption of the impacted primary canine was auspicious. The postoperative period was uneventful.

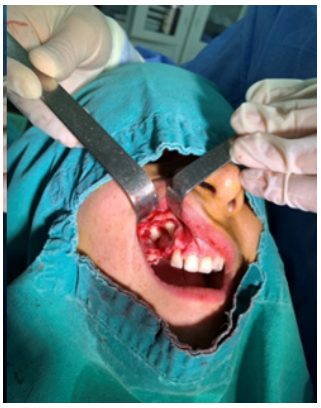

Fig. 3. *Mucoperiosteal flap reflection and bone removal.*

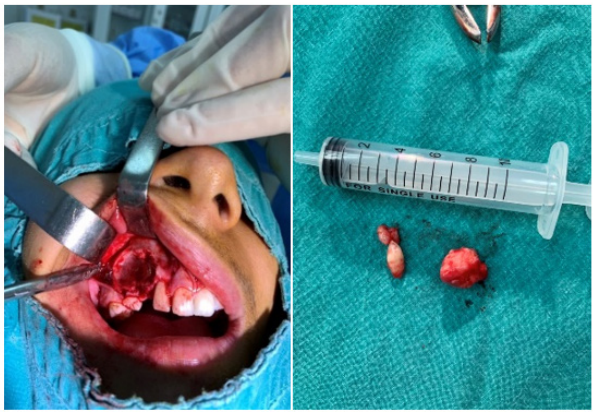

Fig. 4. *Surgical removal of complex odontoma a supernumerary tooth.*

Histologic sections revealed dental tissues, consisting of immature dentin, enamel, enamel matrix, and cementum, intermingled with pulp-like tissues in a few areas. These structures were haphazardly arranged. Histopathologic examination confirmed the diagnosis of complex odontoma.

The patient was followed up regularly to see the state of eruption. At the end of the 2-year follow-up visit, the canine primary tooth and first premolar were close to the dental arch (Figure 6).

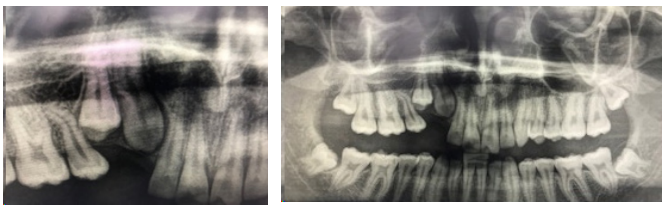

Fig. 6. *The canine primary tooth and first premolar are close to the dental arch.*

Discussion

Odontomas are considered relatively common odontogenic lesions. These lesions are asymptomatic and rarely diagnosed before the second decade of life. Their diagnosis is usually made during the routine check-up, where we notice the absence of a tooth in the dental arch, and radiographs reveal an odontoma with or without an impacted permanent tooth.[18] They frequently cause the impaction or lead to a delayed eruption of teeth. Some hereditary anomalies can also show odontomas, such as Gardner syndrome and Hermann's syndrome.[25] If a portion of dental lamina persists during the developmental stages, it results in the formation of a compound or complex odontoma.[25] If odontomas are removed at an early stage without damaging the underlying tooth germ, the eruption of impacted teeth can be expected spontaneously or after orthodontic traction.[11,23,24] In this case, the chance of eruption of the impacted primary canine was auspicious.

In conclusion, odontomas are treated with conservative surgical removal. Diagnosis of odontoma at an early age and its surgical excision can prevent eruption disorders and the formation of malocclusion.

Ethical Considerations

Publication of the report was approved by the Ethics Committee at the University of Prishtina. The patient's legal guardians gave informed consent for publishing the case report, including images and other clinical information, except individual details identifying the patient.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Mutation Detection in *MYO15A* Gene in an Iranian Family with Non-Syndromic Hearing Loss

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Abstract

Hearing loss, recognized as one of the most prevalent sensory disorders, encompasses both syndromic and non-syndromic manifestations, with the identification of 87 genes and over 100 genetic loci in autosomal recessive non-syndromic hearing loss marking significant progress in understanding its genetic basis. In this case report, we showcase a non-syndromic hearing loss scenario involving a 21-year-old man experiencing progressive hearing loss. Through whole-exome sequencing, we unveiled a previously unreported homozygous mutation, c.1178_1179delAC; p.Tyr393Serfs*38, located in exon 2 (NM_016239.4) of the *MYO15A* gene in the proband. The newly identified mutation, causing a new reading frame (p.Tyr393Serfs*38), results in an early encounter with a stop codon, leading to the formation of a shortened protein. These findings advance our understanding of the molecular mechanisms involved in autosomal recessive non-syndromic hearing loss, contributing to broader scientific knowledge and potential breakthroughs in hearing loss research.**(International Journal of Biomedicine. 2024;14(1):165-169.)**

Keywords: hearing loss • *MYO15A* gene • mutation

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Abbreviations

AD, autosomal dominant: **AR**, autosomal recessive; **ARNSHL**, autosomal recessive non-syndromic hearing loss; **HL**, hearing loss; **mt**, mitochondrial; **NSHL**, non-syndromic hearing loss; **NGS**, next-generation sequencing; **WES**, whole-exome sequencing; **XL**, X-linked; **YL**, Y-linked.

Introduction

Hearing loss (HL) stands as one of the most pervasive sensory disorders, exerting a substantial impact on an individual's quality of life. This condition manifests in diverse forms, both syndromic and non-syndromic, exhibiting an array of inheritance patterns that contribute to its complexity. The inheritance patterns include autosomal dominant (AD), autosomal recessive (AR), Y-linked (YL), X-linked (XL), and mitochondrial (mt), each presenting unique challenges and considerations in understanding and addressing the underlying causes of hearing impairment.[1–3] Autosomal recessive non-syndromic hearing loss (ARNSHL) is particularly common and constitutes approximately 80% of NSHL cases.[4] To date,

87 genes and over 100 genetic loci associated with ARNSHL have been documented, as detailed in the hereditary HL database (http://hereditaryhearingloss.org/).

ARNSHL is frequently associated with mutations in key genes that play a pivotal role in auditory function. Among these genes, several stand out as particularly significant contributors to ARNSHL, each encoding crucial proteins involved in the intricate mechanisms of hearing. The genes commonly implicated in ARNSHL include myosin XVA (*MYO15A*, MIM#602666), cadherin-related 23 (*CDH23*, MIM#605516), solute carrier family 26 member 4 (*SLC26A4*, MIM#605646), transmembrane channel-like 1 (*TMC1*, MIM#606706), gap junction protein beta 2 (*GJB2*, MIM#121011), and otoferlin (*OTOF*, MIM#603681). Remarkably, each of these genes has exhibited a remarkable diversity of mutations, with more than 20 different variants identified. Intriguingly, the majority of these mutations have been identified in consanguineous families, suggesting a complex interplay of genetic factors within closely related individuals.[5]

The prevalence of numerous genes implicated in hearing impairment contributes to the heterogeneity of HL, highlighting the need for more efficient approaches in routine genetic testing and comprehensive genetic analysis. Whole-exome sequencing (WES), a technique based on next-generation sequencing (NGS) platforms, has revolutionized the discovery of causative genes and diagnoses for heterogeneous inherited disorders.[1, 6–9] WES stands as a valuable method, offering a thorough and efficient approach to pinpoint causative mutations in individuals with genetic disorders. This advanced technique allows for the targeted sequencing of the coding regions of genes, enabling rapid, accurate, and cost-effective identification of mutations underlying single-gene disorders.

In societies marked by a high rate of consanguinity, such as Iran with approximately 40%, Hereditary HL holds special significance, providing a pathway for rare pathogenic mutations to manifest. HL emerges as the second most prevalent disability within the Iranian population, representing a significant health concern. The prevalence of HL in Iran underscores the need for a comprehensive understanding of its etiology and the factors contributing to its occurrence. One notable aspect contributing to the dynamics of HL in Iran is the prevalence of consanguineous marriages within the population. Consanguinity, or marriage between close relatives, is a cultural practice that has been historically prevalent in Iran. It is anticipated that within the Iranian HL population, the rate of consanguineous marriages could reach as high as 65%.[10]

Based on this evidence, our objective was to identify defective genes associated with NSHL in an Iranian family using WES. This study reveals a novel chr17-18023291 TAC>T mutation in the *MYO15A* gene, demonstrating a pathogenic effect that could potentially explain the NSHL phenotype observed in this specific Iranian family.

Case Presentation

We present the case of a 21-year-old male, the only son offspring of an Iranian consanguineous couple, as illustrated in Figure 1. The patient's medical history is noteworthy for a diagnosis of congenital HL, with no apparent dysmorphic features detected upon clinical examination. The familial context is particularly significant, as there is no substantial history of HL within the family unit. Both parents share a consanguineous relationship, which adds a layer of complexity to the genetic considerations in this case.

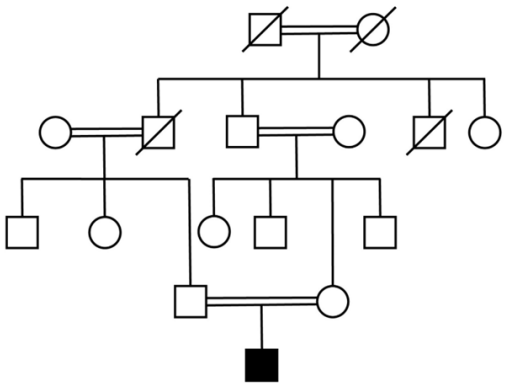

Fig. 1. *Pedigree of the studied family. Symbols marked by a slash indicated that the subject was deceased. Males were indicated by squares; females were indicated by circles. The blackened symbol represents the proband.*

In addition to the absence of a familial HL history, the patient's medical background is marked by the lack of any documented systemic diseases. This absence of systemic health issues further focuses the investigation on the genetic underpinnings of the congenital HL observed in the patient. Given the genetic implications associated with consanguinity, our objective is to explore and characterize the molecular basis of congenital HL in this unique case. To achieve this, we plan to employ advanced genetic testing methodologies, including WES, to unravel the potential genetic mutations or variants contributing to the patient's hearing impairment.

As we delve into the intricacies of this case, the aim is to not only provide a comprehensive understanding of the patient's condition but also to contribute valuable insights into the broader genetic landscape of congenital HL within consanguineous families, particularly within the Iranian population. Through this exploration, we aspire to enhance our knowledge of the genetic factors influencing HL, paving the way for improved diagnostic strategies and potential interventions in similar cases.

Isolation of genomic DNA from blood leukocyte samples was performed using an established salting-out procedure. The concentration of DNA samples was assessed using a NanoDrop 1000 spectrophotometer. Subsequently, the isolated DNA was either stored at -20ºC for future use or subjected to immediate amplification.

Subsequently, DNA served as the instrumental medium for the creation of libraries and the implementation of targeted sequencing in our research endeavors. A bespoke Human capture array was meticulously designed to selectively capture the complete coding regions and intron/exon boundaries of

the specified genes linked to the pathogenesis of NSHL. The sequencing procedures were executed by the utilization of the WES method (Macrogen, Seoul, South Korea).

Alignment of sequence reads was conducted using the reference human genome build hg19 from UCSC, and subsequent annotation was performed utilizing datasets and tools. To refine the analysis, previously identified common variants (frequency >1%) and synonymous substitutions were filtered out, leveraging public databases such as the 1000 Genome Project (https://www.internationalgenome.org/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and the gnomAD browser (https://gnomad.broadinstitute.org/).

To verify the accuracy of the novel variant identified by WES, Sanger sequencing was conducted on the patient and other family members. The PCR products underwent direct sequencing using the automated genetic analyzer (ABI 3100; Applied Biosystems). Subsequently, Sanger sequencing was conducted to confirm the segregation of the candidate variant within the family.

The analysis of DNA sequences within genes implicated in NSHL pathogenesis revealed a novel mutation in the *MYO15A* gene, which co-segregated among healthy family members (Figure 2). This mutation, identified as a novel deletion (c.1178_1179delAC) in exon 2 (NM_016239.4) of the *MYO15A* gene, induces a frameshift mutation. Specifically, it results in the substitution of Tyrosine to Serine at codon 393, potentially leading to a truncated MYO15A protein (p.Tyr393Serfs*38).

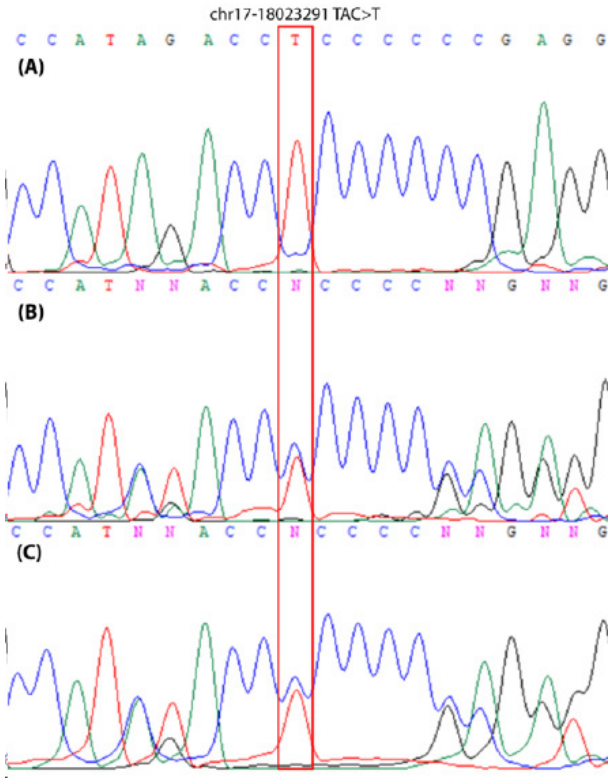

Fig. 2. *The genetic analysis results for the proband and his parents are depicted. Sanger sequencing for the proband (A) unveiled a novel homozygous c.1178_1179delAC mutation in exon 2 of the MYO15A gene. Notably, the identified mutation is found in a heterozygous state in his parents (B, C).*

This novel c.1178_1179delAC; p.Tyr393Serfs*38 deletion mutation in the *MYO15A* gene holds significant implications for ARNSHL. Thus, The deletion-induced frameshift disrupts the protein's structural integrity, potentially causing the loss of critical domains necessary for its proper function, and the dysfunctional MYO15A protein is unable to carry out its essential role in the development and maintenance of hair cells within the inner ear. This impairment leads to compromised auditory function, contributing to ARNSHL. Moreover, due to the premature termination, a truncated and likely non-functional MYO15A protein is produced.

Discussion

Our investigation into an Iranian family grappling with deafness has yielded a pivotal discovery by WES: the identification of a novel mutation in the *MYO15A* gene as the primary cause of the observed impairment within the family. This finding significantly advances our understanding of the genetic basis of deafness, especially within the Iranian population. The *MYO15A* gene's association with auditory function is well-established, and our study underscores its significance in familial cases of deafness. The detection of a novel mutation within this gene not only contributes to the expanding spectrum of genetic variants linked to hearing impairment but also highlights the intricate genetic diversity that may underpin such conditions, particularly within distinct ethnic groups like the Iranian population.

MYO15A's intricate structure with 66 exons and its coding protein, myosin XVa, play a pivotal role in the formation of stereocilia within the cochlea's hair cells.[11] Myosin XVa within the organ of Corti is specifically concentrated at the extremities of stereocilia. It operates as an actin-activated ATPase, utilizing ATP hydrolysis to traverse along actin filaments. The apex of a stereocilium is suggested as a potential location for mechano-electrical transduction, as well as the site for stereocilia expansion.[12] The proper functioning and formation of the mechanotransduction machinery depend on the presence of Myosin XVa. Myosins typically consist of one or two heavy chains along with several light chains. The tails of myosins are believed to bind to membranous compartments, allowing them to be displaced in relation to actin filaments.[13]

In a study by Xia H. et al.,[14] the authors presented compelling evidence of a causative frameshift mutation in the *MYO15A* gene within a Chinese family experiencing NSHL. This finding underscores the genetic complexity underlying NSHL and contributes to our understanding of the role of *MYO15A* mutations in the manifestation of this auditory disorder within specific populations. Moreover, earlier investigations have scrutinized families with NSHL to assess pathogenic genomic defects. These studies identified forty-three mutations in the *MYO15A* gene and determined that these modifications were responsible for ARNSHL.[15–17] Subsequently, Zhang F. et al.[18] reported three pathogenic mutations in the *MYO15A* gene c.3971C>A; p.A1324D, c.4011insA; p.Q1337Qfs*22, and c.9690+1G>A within a Chinese family affected by ARNSHL. Consistent with these discoveries, Asgharzade S. et al.[19] conducted an

assessment of mutations associated with NSHL within the Arab population in Southwest Iran, employing WES. Their study revealed a novel homozygous mutation, c.1047C>A; p.Y349*, in one of the twenty-five families. This mutation resulted in a premature stop codon, further emphasizing the genetic diversity underlying NSHL in this specific geographic and ethnic context. The identification of such novel mutations adds to the understanding of the genetic landscape of NSHL and highlights the importance of population-specific genetic studies in unraveling the intricacies of hereditary hearing disorders. These findings contribute valuable insights that may have implications for genetic counseling and diagnostic approaches in the affected population.[19]

This is the first report of c.1178_1179delAC mutation of the *MYO15A* gene in a patient affected by NSHL. The following evidence proves that this mutation can lead to NSHL: 1) WES exclusively identified this variant as the underlying cause of NSHL in the proband. 2) As depicted in Figure 2, Sanger sequencing validated the variant in both the patient and the unaffected family members, and, based on a recognized heterozygous mutation in the parents, indicates an AR inheritance pattern for *MYO15A*. 3) Furthermore, variant chr17-18023291 TAC>T located in exon 2 of the *MYO15A* gene within the ferm domain and tail region of the Myosin protein has been identified. This variant induces a novel reading frame (p.Tyr393Serfs*38), triggering premature encounter with a stop codon. Consequently, this event results in the synthesis of a truncated protein, which significantly disrupts the normal functioning of the protein, leading to impaired auditory function and contributing to the development of ARNSHL.

Our findings emphasize the substantial utility of employing WES in the context of consanguineous parents. This investigative approach proves to be a valuable and effective tool for uncovering both potential and novel mutations associated with ARNSHL. The comprehensive coverage of the exome allows for the detection of genetic variations that might otherwise go unnoticed, providing crucial insights into the genetic basis of ARNSHL in consanguineous family structures. This underscores the importance of adopting advanced genetic techniques, such as WES, to enhance our understanding of hereditary HL and contribute to the identification of causative mutations in affected individuals.

Conclusion

This study marks a significant milestone as it represents the first confirmed case of HL in an Iranian family by comprehensive genetic analysis, revealing a novel c.1178_1179delAC; p.Tyr393Serfs*38 mutation in the *MYO15A* gene. The identification of this unique mutation underscores the importance of genetic investigations in elucidating the diverse molecular underpinnings of hereditary hearing disorders within specific populations.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the family members for this publication.

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CASE REPORT

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Association of Autism Spectrum Disorder in an Iranian Pedigree with a Novel Hereditary Mutation in *SETD5*

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Abstract

Autism spectrum disorder has evolved from a rare childhood-onset disorder to a widely acknowledged, extensively researched, and heterogeneous lifelong condition. This study focuses on an Iranian pedigree affected by autism spectrum disorder. By employing whole-exome sequencing, we detected a novel heterozygous (c.3694T>A: p.Tyr1232Asn) in exon 22 (NM_001080517.3) of the *SETD5* gene. The presence of this mutation was consistent with observed clinical features, confirming the genetic basis of autism spectrum disorder in the patient and his father. In contrast, the mother, with a normal genotype, did not exhibit the identified mutation. Genetic counseling implications are underscored by the shared heterozygous mutation in both, emphasizing the importance of incorporating genetic insights into psychological counseling. This integration can empower families with informed strategies to navigate the challenges associated with autism spectrum disorder, fostering resilience and tailored support. **(International Journal of Biomedicine. 2024;14(1):170-174.)**

Keywords: Autism Spectrum Disorder • *SETD5* gene • mutation

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Introduction

Over the past fifty years, autism spectrum disorder (ASD) has experienced a profound transformation. Initially perceived as a rare childhood-onset disorder, it has evolved into a widely acknowledged and extensively studied lifelong condition. This shift reflects not only increased awareness but also a growing understanding of the remarkable diversity within the spectrum. ASD, now recognized as fairly common, is marked by enduring core features, including social communication deficits and repetitive, unconventional sensory-motor behaviors.[1] Autism is presently conceptualized as a spectrum, encompassing a spectrum of severity from mild to severe. Despite this diversity, it is important to recognize that a considerable number (though not all) of individuals with ASD necessitate some form of lifelong support.

Individuals with ASD exhibit a diverse range of characteristics, yet the disorder is primarily defined by core features in two key domains: social communication and restricted, repetitive sensory-motor behaviors. These fundamental traits persist consistently across various backgrounds, regardless of cultural, racial, ethnic, or socioeconomic factors.[2] The origins of ASD are rooted in early disruptions in brain development, leading to subsequent neural reorganization.[3,4] However, the absence of reliable biomarkers necessitates reliance on observable behavior for diagnosis. In an effort to improve diagnostic precision, the American Psychiatric Association introduced the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria in 2013.[5] This revision aimed to streamline ASD diagnosis by creating a unified spectrum based on the two core

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domains: social communication and restricted, repetitive, or unusual sensory-motor behaviors. Notably, subtypes like Asperger's disorder and pervasive developmental disorder not otherwise specified, previously inconsistently utilized by clinicians, were amalgamated into the consolidated diagnosis of ASD. Additionally, the DSM-5 explicitly recognizes that ASD can co-occur with other conditions, including genetic disorders like fragile X syndrome and psychiatric conditions such as attention-deficit hyperactivity disorder (ADHD). This acknowledgment demonstrates a more comprehensive understanding of the intricacies and comorbidities associated with ASD.

SETD5, a widely expressed protein, belongs to the SET domain-containing protein family. While SET domains in most proteins catalyze protein lysine methylation, SETD5 and its paralog MLL5 diverge due to amino acid substitutions at critical positions, rendering them devoid of methyltransferase activity. Recent studies have unveiled that SETD5 interacts with two chromatin-regulating complexes—the polymerase-associated factor 1 (PAF1) and histone deacetylase 3 (HDAC3) complexes. This association underscores SETD5's role in epigenetic regulation and control of gene expression. Crucially, heterozygous loss-of-function mutations in genes encoding components of the HDAC3 complex have been identified in individuals with ASD or intellectual disability (ID). This observation suggests a functional link between *SETD5* and the HDAC3 complex in the pathogenesis of ASD and ID. Despite this insight, the precise mechanisms by which SETD5 regulates gene expression related to ASD and ID have remained elusive. Further exploration of SETD5's interactions and its impact on gene expression holds promise for advancing our understanding of the molecular underpinnings of ASD and ID.[6]

Whole-exome sequencing (WES), emerges as a highly valuable methodology, providing a comprehensive and efficient approach to identify causative mutations in individuals grappling with genetic disorders. Distinguished by its advanced capabilities, this technique facilitates the focused sequencing of the coding regions of genes, thereby ensuring a swift, precise, and economically feasible identification of mutations that underlie single-gene disorders.[7-9] Based on this evidence, we employed WES technique to discern the causative genetic defect in a nonconsanguineous Iranian family affected by ASD. Our primary diagnosis of ASD was initially established through comprehensive psychological assessments. Through these efforts, we identified a novel heterozygous mutation within the *SETD5* gene, shedding light on a potential genetic basis for the ASD phenotype observed within this family.

Case Presentation

A 9.5-year-old boy patient was referred to Noor-Gene Genetic Laboratory in Ahvaz, Iran, based on developmental concerns noted by the parents (Figure 1). Born full-term without complications, the individual experienced delayed developmental milestones, particularly in language and social interaction. Parents reported challenges in communication, repetitive behaviors, and forming peer relationships. A comprehensive psychological evaluation, inclusive of standardized assessments, resulted in a provisional diagnosis of ASD.

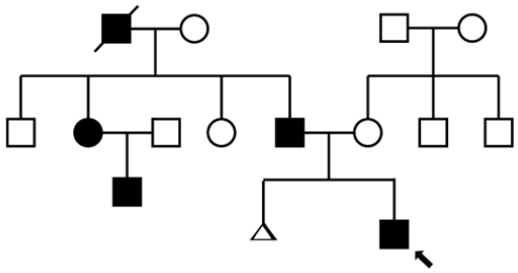

Fig. 1. *The pedigree of the studied family. Squares denote males, circles represent females, triangles signify spontaneous abortions, and slashes indicate deceased individuals, while enclosed symbols mark affected members, with the proband identified by an arrow.*

In a parallel context, the patient's father underwent psychological assessment due to a history of developmental challenges during childhood, marked by language delays and difficulties in forming social connections. Despite achieving academic milestones, persistent challenges in social interactions and communication were observed throughout adulthood. Conversely, the patient's mother has no reported history of neurodevelopmental challenges, achieving typical developmental milestones during childhood and exhibiting normal social communication and cognitive abilities. Given the notable resemblance in clinical characteristics between the patient and his father, we suspected a potential genetic basis for the observed neurodevelopmental phenotype. To validate this hypothesis and complement the psychological diagnosis, we employed WES, with confirmation from his father's statement that the individuals represented by the blackened symbol in the pedigree shown in Figure 1 have been diagnosed with ASD. This also indicates that they are affected by a hereditary condition such as ASD, emphasizing the genetic basis of the disease.

Blood samples were obtained with informed consent from his parents. Following established protocols, DNA extraction from the buffy coat was conducted using the FAVORGEN kit (Biotech Corp, Cat. No.: FABGK 001, Taiwan).

A comprehensive WES analysis, with a specific focus on genes associated with ASD, was performed by Macrogen in Seoul, South Korea. The intentional emphasis on ASD-related genes aimed to identify potential genetic variations and mutations contributing to the manifestation of this neurodevelopmental disorder. The analysis revealed a novel single heterozygous mutation in the affected son, specifically identified as a novel missense mutation in the *SETD5* gene (c.3694T>A: p.Tyr1232Asn) located in exon 22 (NM_001080517.3) of chromosome 3p. Sanger sequencing of coding exons confirmed the presence of this new Y1232N mutation, predicting a consequential alteration in codon

translation, leading to the conversion of tyrosine to asparagine. This transformation was identified as heterozygous in both the patient and his father, confirming the disease as they exhibited similar clinical manifestations along with the heterozygous mutation. The mother, on the other hand, exhibited a normal genotype (Figure 2). This missense mutation substitutes Tyrosine with Asparagine (TAC>AAC) at the 1232-position of the SETD5 protein (Figure 2D). Bioinformatic tools, such as PolyPhen-2, SIFT, FATHMM-MKL, LIST-S2, and MutationTaster (Table 1), collectively indicate the identified mutation as a probable pathogenic variant. Furthermore, mutations reported in the *SETD5* gene are compiled and summarized in Table 2 using data from the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php).

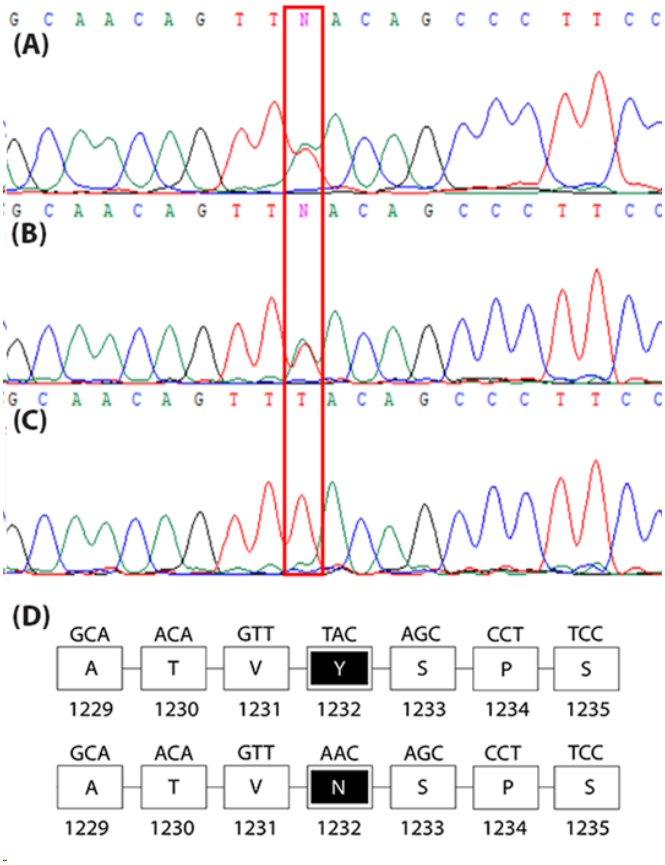

Fig. 2. *Sequence chromatograms portray a novel heterozygous SETD5 mutation (c.3694T>A) in the affected son (A) and his father (B), while the mother (C) exhibits a normal genotype in the sequence analysis. (D) the consequential amino acid modification arising from the substitution of T with A at position 3694 within the SETD5 gene. This genetic alteration results in the replacement of Y by N at position 1232 in the corresponding protein.*

Table 1.

Pathogenicity assessment of the new variant identified by WES.

Gene	Variant	Polyphen-2 HDIV score	SIFT score	FATHMM-MKL score	LIST-S2 score	Mutation Taster
SETD5	Y1232N	0.998 (Probably damaging)	0 (Pathogenic supporting)	0.9902 (Pathogenic supporting)	0.9902 (Pathogenic supporting)	Disease -causing

Table 2.

Reported mutations in SETD5 gene.

Pathogenic variant	Protein effect	Type of mutation	Phenotype
c.179C>T	p.Thr60Met	Missense	Autism spectrum disorder
c.509A>G	p.Lys170Arg	Missense	Autism spectrum disorder
c.922C>T	p.Arg308Ter	Nonsense	Autism spectrum disorder
c.1405G>A	p.Val469Ile	Missense	Autism spectrum disorder
c.2005G>A	p.Gly669Arg	Missense	Autism spectrum disorder
c.3773G>C	p.Ser1258Thr	Missense	Autism spectrum disorder
c.4029T>G	p.Ser1343Arg	Missense	Autism spectrum disorder
IVS14 as -2 A>T	-	Splicing	Autism spectrum disorder

Discussion

ASD is divided into syndromic and nonsyndromic types. Syndromic cases often present additional phenotypes, including ID, ADHD, epilepsy, and craniofacial dysmorphology. Nonsyndromic ASD is believed to have a predominantly polygenic etiology, while syndromic cases are often associated with chromosomal abnormalities, copy number variations, or single-gene mutations. Investigating molecular mechanisms in syndromic ASD, using targeted gene modification in cellular and animal models, sheds light on the disorder's pathophysiology. ASD-related mutations commonly disrupt cellular processes like neurogenesis, neurite growth, and synaptic plasticity. Evidence suggests that ASD-related gene mutations recurrently affect three major cellular activities: protein translation, WNT signaling, and synaptic signaling.[10-12]

At the forefront of this study, the identified *SETD5* gene mutation adds to the growing body of evidence linking this gene to ID and developmental disorders, particularly ASD. The familial context emphasizes the role of genetics in the manifestation of neurodevelopmental disorders. Beyond its genetic implications, this study underscores the potential benefits of genetic findings in psychosocial counseling for affected families.

Numerous studies have investigated the varied manifestations of ASD, emphasizing the heterogeneity of symptoms and the importance of recognizing early indicators. According to Jones et al.,[13] delayed language development is a prevalent characteristic in children later diagnosed with ASD. Social communication difficulties, such as challenges in understanding and responding to social cues, have also been extensively documented.[14] Repetitive behaviors are considered a core feature of ASD, as highlighted in studies by Leekam et al.[15] Challenges in communication, particularly nonverbal communication, have been linked to social interaction deficits in ASD.[16] The impact on forming peer relationships is a consistent theme in the literature, with studies suggesting that these difficulties often persist into adolescence

and adulthood.[17] In our case, the individual exhibited delayed language and social milestones, consistent with findings by Jones et al.[13] The challenges in communication, nonverbal cues, and forming peer relationships align with broader patterns observed in the literature.[14,16,17] Additionally, the presence of repetitive behaviors in our case mirrors the core characteristics outlined by Leekam et al.[15]

In an animal model study, Setd5$^{+/-}$ mice underwent a comprehensive behavioral analysis, demonstrating phenotypes analogous to those observed in individuals with ASD and ID. The transcriptomics analysis of the Setd5$^{+/-}$ mouse brain unveiled disruptions in the expression of both rDNA and ribosomal protein genes. Examining the regulation of rDNA expression by *SETD5* in neuroblastoma cell lines, the study revealed that *SETD5* binds to the rDNA promoter, recruiting HDAC3. This recruitment leads to a reduction in the acetylated form of histone 4 at Lys16 (H4K16ac), subsequently inducing the dissociation of TIP5 and facilitating rDNA expression. Furthermore, the study observed a depletion of rRNA due to *SETD5* deficiency, resulting in a diminished cell proliferation in cultured neuroblastoma cells. This reduction in cell proliferation extended to Setd5$^{+/-}$ mouse embryos in vivo and adult neural stem cells in vitro. Additionally, the translation of cyclin D1 mRNA was specifically down-regulated in *SETD5*-deficient cells. Altogether, these findings emphasize the crucial role of *SETD5* in regulating neural cell proliferation through epigenetic control of rDNA expression.[6]

In a comprehensive study, the researchers conducted a systematic literature review and analyzed public databanks to identify all mutations within the *SETD5* gene. The outcomes revealed a noteworthy association between these mutations and a diverse spectrum of ID and ASD-like symptoms. Notably, the observed high penetrance in males indicated the pathogenic nature of these mutations. Interestingly, within the female population, the majority of reported cases exhibited a similarly elevated penetrance. However, the study brought to light two instances of female carriers with normal phenotypes, yet their offspring, specifically male children, manifested symptoms of ID and ASD after inheriting the mutation.[13] This intriguing finding adds a layer of complexity to our understanding of *SETD5* mutations, highlighting their potential as causative factors in the development of ID and ASD, particularly in males. Also, the results of our study have contributed significantly to the existing body of knowledge by identifying a novel missense heterozygous *SETD5* mutation. This newfound genetic variation is a noteworthy addition to the current understanding of the genetic landscape associated with ASD. The clinical manifestations of ASD were observed in both the patient and the father, both of whom carry the identified heterozygous mutation. Intriguingly, however, the mother exhibited a normal genotype, suggesting a potential link between the identified genetic mutation and the manifestation of ASD symptoms.

This particular heterozygous *SETD5*: c.3694T>A: p.Tyr1232Asn mutation, previously unreported in mutation databases, represents the first documented instance of a mutation in the SETD gene in a patient affected by ASD. Several lines of evidence substantiate the assertion that this mutation is causally linked to ASD: 1- WES exclusively identified this mutation as the primary factor underlying ASD in the patient. 2- Figure 2 illustrates that direct Sanger sequencing verified the presence of the mutation in both the proband and the affected father within the family, but his healthy mother has a normal genotype. The recognized heterozygote mutation in the father suggests an autosomal dominant pattern of inheritance for the *SETD5* gene. 3- Bioinformatics tools, including PolyPhen-2, SIFT, FATHMM-MKL, LIST-S2, and MutationTaster, collectively suggest that these genetic variants are likely to be deleterious and associated with disease development. 4. The new c.3694T>A: p.Tyr1232Asn mutation in exon 22 of the *SET5* gene results in a missense alteration, replacing tyrosine with asparagine at position 1232 in the protein. This amino acid substitution has the potential to impact the three-dimensional structure of the SET5 protein, affecting its folding, stability, and interactions. 5- Significantly, this alteration was absent in the healthy mother, reinforcing the hypothesis that it may contribute to the phenotype observed in these patients. Consequently, the identified mutation in the *SETD5* gene is deemed pathogenic in our patient with ASD.

Conclusion

The discovery of the novel heterozygous *SETD5* mutation (c.3694T>A: p.Tyr1232Asn) in an Iranian pedigree affected by ASD using WES sheds light on the genetic basis of ASD in this population. The identification of a heterozygous mutation shared by the patient and his father, with a normal genotype in the mother, underscores the importance of genetic counseling. Beyond its genetic implications, this information holds significant value for psychological counseling, offering a deeper understanding of the condition. Incorporating genetic insights into psychological counseling can empower families, providing them with informed strategies to navigate the emotional and practical challenges associated with ASD, fostering resilience and tailored support.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the family members for this publication.

Acknowledgments

We express our gratitude to the family members for their valuable cooperation.

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CASE REPORT

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Frantz Tumor, a Rare Indolent Pancreatic Neoplasm Entity: A Case Report and Brief Review

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Abstract

Solid pseudopapillary neoplasm (SPN) of the pancreas, also known as Frantz tumor, is an uncommon tumor with indolent clinical features that primarily affects young women. We are presenting a 27-year-old Caucasian female with an accidental finding with pancreatic SPN who underwent complete resection of the tumor using a distal pancreatectomy and splenectomy procedure. Immunohistochemistry revealed low-grade pancreatic SPN. Despite the rare appearance, it should be considered in the differential diagnosis of a young female with a large pancreatic mass.**(International Journal of Biomedicine. 2024;14(1):175-178.)**

Keywords: Frantz tumor • solid pseudopapillary neoplasm • pancreas • distal pancreatectomy

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Abbreviation

CECT, contrast-enhanced computed tomography; **EUS**, endoscopic ultrasound; **FNA**, fine needle aspiration; **MDCT,** multiphasic multidetector-row CT; **MRI**, magnet resonance imaging; **SPN**, solid pseudopapillary neoplasm.

Introduction

Solid pseudopapillary neoplasm (SPN) of the pancreas is a low-grade malignant tumor composed of poorly cohesive epithelial cells, forming solid and pseudopapillary structures and lacking a specific line of pancreatic epithelial differentiation.(1) These entities were first described by Virginia Kneeland Frantz in 1959 as pancreatic papillary-cystic tumors.(2)

The WHO classified them as solid pseudopapillary tumors in 1996 and reclassified them as SPNs in 2010. The WHO classification describes SPNs as low-grade malignant neoplasms composed of loosely cohesive, monomorphic epithelial cells forming solid and pseudopapillary structures.(3)

Case Presentation

We are presenting a 27-year-old Caucasian female with an accidental neoplasm found from a routine check-up by a gastroenterologist. An MRI revealed a pancreatic tail mass, symptomless, adjacent to the spleen hilus (Figure 1).

Using general anesthesia, we performed laparotomy surgical treatment, and intraoperatively discovered a solid

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tumor in the distal pancreas adjacent to the spleen hilus. We performed a distal pancreatectomy with splenectomy and saved the discovered accessory spleen (Figure 2).

Postoperative recovery went well. Macro findings: encapsulated, gray-colored nodular tumor located in the distal pancreas, 6.5cm in diameter, soft consistency, which was built with stromal hyalin, myxoid vascular and hemorrhagic foci, and degenerative changes. Resection margins were microscopically free of tumor, R0 (Figure 3a).

Immunohistochemistry: SPN of the pancreas, low grade, pT3pNx (UICC 8th Edition), B-catenin (+) (Figure 3b), Vimentin (+) (Figure 3c), E-cadherin (-). The proliferation cell index measured with Ki-67 was low, about 7%. The patient has been followed up for 10 months and is in good condition.

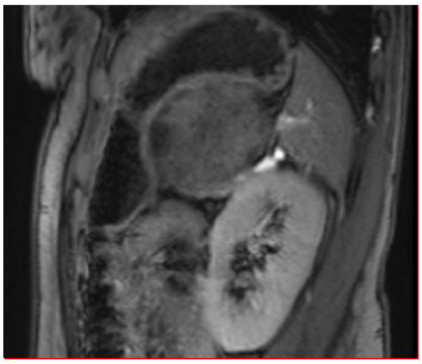

Fig.1. *MRI: A pancreatic tail mass, adjacent to the spleen hilum.*

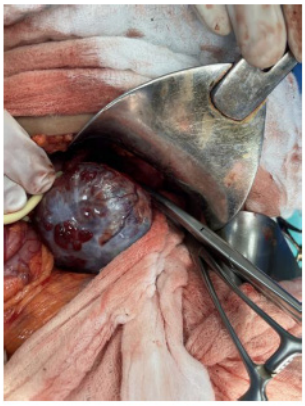

Fig.2a. *Intraoperative findings, pancreatic tail with SPN.*

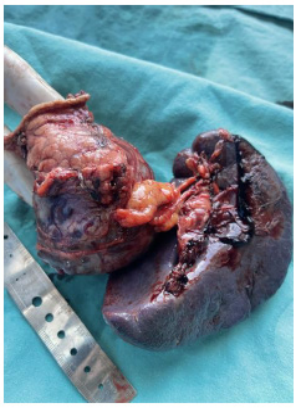

Fig.2b. *Specimen of resected distal pancreas with SPN and spleen.*

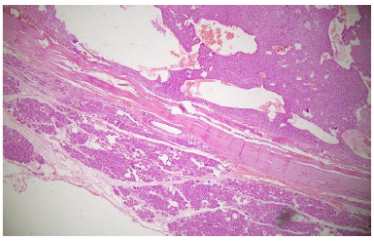

Fig.3a. *SPN and pancreas, H&E staining.*

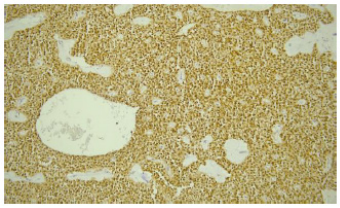

Fig.3b. *Immunohistochemistry: Beta-catenin, strong diffuse positive.*

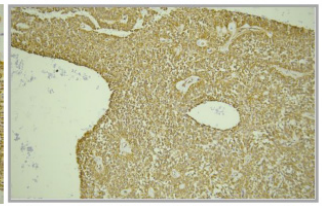

Fig.3c. *Immunohistochemistry: Vimentin positive.*

Discussion

Since the first description by Frantz as "papillary tumor of the pancreas, benign or malignant" in 1959, various names have been used to describe this rare tumor, such as a solid-cystic tumor, solid-cystic acinar tumor, papillary-cystic tumor, solid-papillary epithelial neoplasm, and Frantz tumor.[1,2] SPNs are rare, comprising approximately 0.17%-2.7% of all pancreatic tumors and only 5% of cystic neoplasms.[3]

An SPN primarily affects young women in their second and third decades. Although the hypotheses of its origin include endocrine, ductal, acinar, neurosecretory, and totipotent primordial cells, the histogenesis and pathogenesis remain unclear, which still motivates discussions.[4] Neoplasms morphologically identical to pancreatic SPNs arise in retro pancreatic tissue, ovaries, and testes.[1]

Two large retrospective reviews of 340 patients with SPN from the National Cancer Database showed that 82% of patients were female, the median age was 39 years,[5] and the mean age of the 553 SPN patients included in a review by Yu et al.[6] was 27.2 years, 88% were female. Similarly, in a study by Sun et al.[7] with a total of 118 patients, the mean age was 30.8 years, and the majority were female (n=95, 80.5%).

The exact reason for female predilection is unclear, but the literature suggests that sex hormones may be part of the pathogenesis of SPN.[8] According to studies by Lanke et al.[9] and Kurokawa et al.,[10] SPNs are considered hormone-sensitive because they express progesterone receptors, and female hormones influence the growth of SPNs. Unlike this study, Omiyale et al.[11] consider that "there is no association with functional endocrine syndromes."

The clinical symptoms are non-specific. Many patients are asymptomatic (38.1%); however, most patients are symptomatic, presenting with abdominal pain or discomfort. Other symptoms include abdominal mass, weight loss, jaundice, anorexia, fever, fatigue, abdominal discomfort, nausea, and vomiting.

Our patient was asymptomatic and we incidentally found SPN through imaging. SPN can occur in all parts of the pancreas. Most tumors (59%) were in the tail.[12] Outside the pancreas, they can occur in the retroperitoneum, liver, stomach, mesentery, duodenum, omentum, ovary, or lung. SPN can also be found in regional lymph nodes, the portal vein, the colon, the spleen, and blood vessels.[9]

The diagnosis may be difficult because of indolent clinical features. Studies have shown that tumor markers such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic

antigen (CEA) were usually within normal ranges in patients with this disease.[13]

SPT is often diagnosed during complementary imaging investigations, such as ultrasound or CT scan of the abdomen, usually showing a well-encapsulated complex mass with solid and cystic components. MRI is better than CT in detecting the cystic or solid components of the tumor.[6]

Typically, SPNs are large, well-encapsulated masses that demonstrate variable degrees of internal hemorrhage and cystic degeneration and are often associated with calcifications. When these features are encountered in a young female patient, this neoplasm should be a strong diagnostic consideration with multiphasic multidetector-row CT (MDCT).[14]

Hanada et al.[15] compared pathologic and image findings at cystic component, with MRI: specificity was 80%, accuracy 68%. On delayed phase contrast-enhanced CT (CECT), pathologically aggressive SPNs may show greater enhancement than non-aggressive SPNs.[16]

It is recommended to request an MRI, besides the abdomen CT, of all patients suspected of SPN of the pancreas to avoid possible diagnostic mistakes. [17] MRI shows a well-defined mass with heterogeneous signal intensity on T1- and T2- T2-weighted images indicative of the tumor's variably solid and cystic nature.[18] EUS-FNA significantly increased the pre-operative diagnostic yield of SPN to 82.4%.[19]

Radical surgical resection is established as the standard treatment protocol for the disease; it is also recommended to perform metastasectomy, vascular resections, and/or resections of other compromised organs to ensure therapeutic success in 95% of the cases.[17,20,21] Different surgical procedures are available depending on the tumor localization, such as the Whipple procedure, central pancreatectomy, distal pancreatectomy with or without splenectomy, enucleation, etc. The outcomes are excellent if complete resection is achieved. Similarly, with an open approach, considerations should be made for a minimally invasive approach in patients with SPN.[4,8]

In appropriate indications, the enucleation of SPNs can be considered a safe and effective surgical procedure for pediatric patients.[22] The need for lymphadenectomy has been discussed due to the description of ganglion metastases in approximately 15% of cases.[23]

There were no significant clinical factors, such as age, sex, tumor size, tumor location, elevated carcinoembryonic antigen levels, and elevated carbohydrate antigen 19-9 levels, suggesting malignant potential.[21]

Patients with SPN who undergo resection have an excellent survival at 5 years, from 95%-97.7%.[6,7,24]) A reported 10-year disease-specific survival rate of 96%.[25]

SPNs have an excellent prognosis with minimal recurrence after resection. There have been reports from 1.8%-4.5% of patients with evidence of recurrence at the last follow-up. [7,12]

Adjuvant therapy is used only in a small number of patients because of the high resectability of SPN. The role of chemotherapy or chemoradiotherapy in treating SPN is also unclear. In some studies, adjuvant or neoadjuvant chemotherapy, radiotherapy, or chemoradiotherapy are reported in some unresectable cases with good results. [26,27]

In conclusion, pancreatic SPN is a rare entity that primarily affects young women. With indolent clinical features affecting young age, this distinctive neoplasm creates diagnostic difficulties. Surgery with R0 resection is the curative treatment of choice. Mandatory follow-up for early local recurrence or distant metastasis diagnosis is important. We should consider an asymptomatic young female who presents with a large pancreatic mass, accidentally revealed by diagnostic tools, as a possibly pancreatic SPN patient.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

Publication of the report was approved by the Ethics Committee at the National Institute of Public Health (Prishtina, Kosovo). Informed written consent was obtained from the patient to publish this case report and any accompanying medical images.

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CASE REPORT

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Unintended Consequences: Exploring Iatrogenic Injuries in Cesarean Section Deliveries

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Abstract

Cesarean delivery (CD), or C-section, which prevents injury and death in mothers and babies at higher risk of complicated deliveries, like any surgery, does carry a risk of complications. By reviewing the medical literature and analyzing documented CD cases, we examined the spectrum of iatrogenic injuries, including unintentional injuries, affecting both maternal and neonatal outcomes. This case report describes iatrogenic bladder damage after CD in a 31-year-old woman who had a previous emergency CD two years ago. This case calls for a comprehensive approach to minimize iatrogenic risks and optimize maternal and neonatal well-being during repeat CD.**(International Journal of Biomedicine. 2024;14(1):179-181.)**

Keywords: Cesarean delivery • iatrogenic injuries • bladder injury

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Introduction

Cesarean delivery (CD) has become a routine and often life-saving intervention in obstetric care, providing a safe means to deliver infants when vaginal delivery poses risks to the mother or the baby. However, like any surgery, a C-section does carry a risk of complications. Among the array of potential adverse events, iatrogenic injuries, particularly those involving the bladder, represent a significant concern in the realm of maternal health.

Bladder injuries during C-section are considered iatrogenic when they result from accidental damage caused by medical intervention rather than underlying pathological conditions. The proximity of the bladder to the lower uterine segment, the area commonly incised during CD, puts it at risk of injury. Although advances in surgical techniques and an improved understanding of anatomy have reduced the incidence of iatrogenic BI, they remain a notable complication.

Iatrogenic bladder injury is a rare complication during C-section, with an event rate ranging from 0.08% to 0.94%. It was noted that the frequency of bladder injury is higher in women with repeated CD (58.5%) than in women with primary C-sections (41.2%).[1]

Iatrogenic bladder injuries during CD can manifest in various forms, ranging from minor tears to more severe complications, such as bladder perforations.[2] The consequences of such injuries can be significant, impacting both short-term recovery and long-term pelvic health. Immediate complications may include urinary tract infections, hematuria, and impaired bladder function, while long-term effects may involve chronic pain, incontinence, or the formation of fistulas.

Several factors contribute to the risk of iatrogenic bladder injuries during C-section.[3] These include variations in pelvic anatomy, surgical experience, emergencies requiring rapid interventions, and cases involving conditions like placenta previa or extensive adhesions. Understanding these factors is crucial for developing strategies to prevent or minimize such injuries.

Case Presentation

A 31-year-old woman, after CD, was admitted to the ward of the Clinic of Obstetrics and Gynecology for monitoring. The patient had a previous emergency CD two

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years ago. Postoperatively, the ureteric catheter was pulled out by the end of the first day in the intensive recovery room. Immediately after the catheter was pulled out, the patient complained of abdominal pain and abdominal distension. Routine ultrasonography showed moderate abdominal free fluid (Figure 1).

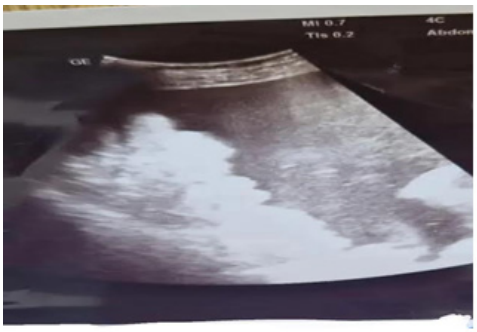

Fig. 1. *Routine ultrasonography: Moderate abdominal free fluid.*

Based on laboratory results (Blood test: WBC - 10.5×10^3/μL, RBC - 3.11×10^6/μL, Hb - 8.4 g/dl, HCT - 25.3 %, platelets - 266 $\times10^3$/μL, C-reactive protein -189.8 mg/L, blood urea nitrogen - 6.67 mmol/l, serum creatinine - 155.0 μmol/L) and symptoms, the patient was advised of the need for immediate catheterization. The unit continued postoperative recovery interventions, including thrombosis and bleeding prevention, maternal monitoring, and wound care (monitoring C-section incision for any infection).

An urgent abdominal and pelvic CT scan with contrast was performed to reveal the origin of the free abdominal fluid. It showed no organ pathology except for only moderate free fluid in all parts of the abdominal cavity. After consultation at the urologist's request, renal ultrasonography was used and confirmed that there was no hydronephrosis and that the urinary bladder was empty, with the presence of a catheter.

The adnominal surgeon confirmed abdominal distension without acute abdomen and realized a punch biopsy of free abdominal fluid follow-up results with urea of 10.9 mmol/l and creatinine of 594.4 μmol/l; urinary urea 45.5 mmol/l, creatinine - 1244.4 mmol/l. After repeated consultation with a urologist, the diagnosis was made: "Iatrogenic bladder damage."

After four days of bladder catheterization, intravenous antibiotics, antianemic medications, a healthy diet, and hydration, the patient's condition improved significantly. Conventional ultrasound did not reveal free fluid in the abdominal cavity. Blood test: WBC - 9.1×10^3/μL, RBC - 4.02×10^6/μL, Hb - 10.6 g/dl, HCT - 34.3%, platelets - 385 $\times10^3$/μL, C-reactive protein - 52 mg/L, blood urea nitrogen - 3.9 mmol/l, serum creatinine: 70 μmol/L. The urethral catheter was maintained for 10 days of observation and treatment in the hospital. Blood test before discharge: C-reactive protein - 30.4 mg/L, blood urea nitrogen - 3.32 mmol/l, serum creatinine - 61.7 μmol/L Upon discharge from the hospital, an abdominal ultrasound revealed no free fluid in the abdominal cavity (Figure 2).

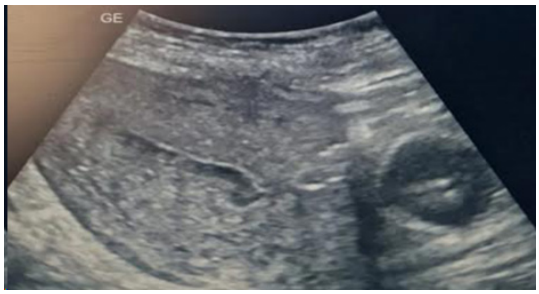

Fig. 2. *Abdominal ultrasonography upon discharge from the hospital: No free fluid in the abdominal cavity.*

Discussion

Bladder injuries following CD, though relatively uncommon, can have significant clinical implications. The incidence varies, with most injuries being minor and manageable, but severe cases can lead to substantial morbidity. Understanding the frequency and severity of such injuries is crucial for informing clinical practice and improving patient outcomes.[4] Several factors contribute to the occurrence of bladder injuries during CD. Anatomical variations, adhesions from previous surgeries, emergencies, and the surgeon's experience all play roles. Identifying these factors is essential for risk stratification and developing preventive strategies. For instance, preoperative imaging or thorough evaluation of a patient's surgical history may aid in anticipating potential challenges.[5] Immediate consequences of bladder injuries include urinary tract infections, hematuria, and impaired bladder function. These complications, if promptly identified and managed, can mitigate long-term consequences. However, more severe injuries may lead to chronic pelvic pain, urinary incontinence, or the formation of vesicovaginal fistulas, significantly impacting a woman's quality of life. Recognizing and addressing these issues promptly is crucial for minimizing the long-term impact on patients.[6,7] Preventing bladder injuries during CD requires a multi-faceted approach. Surgeons' awareness and meticulous surgical techniques are paramount.

Intraoperative cystoscopy, though not universally adopted, has been proposed as a valuable tool for real-time visualization of the bladder, aiding in the prevention and early detection of injuries. Additionally, improved preoperative planning, especially in complex cases, can contribute to minimizing the risk of bladder injuries.[8] Advancements in surgical techniques, such as lower-segment transverse incisions and careful dissection, aim to reduce the risk of bladder injuries. Utilizing minimally invasive approaches, such as laparoscopic or robotic-assisted C-section, may also offer advantages in terms of visualization and precision. However, these techniques require specialized skills and may not be suitable for all cases.[9] Postoperative vigilance is essential for early detection of bladder injuries. Monitoring for signs of urinary tract infection, persistent hematuria, or altered bladder function is critical. In cases where injuries are identified, prompt management, possibly involving consultation with urological specialists, is crucial to minimizing complications and ensuring optimal recovery.[10] Given the potential for bladder

injuries during CD, comprehensive patient counseling and informed consent are paramount. Expectant mothers should be educated about the possibility of such complications, the signs and symptoms, and the planned interventions to address them. This communication ensures that patients actively participate in their care and can make informed decisions.[11] Continued research into refining surgical techniques, adopting innovative technologies, and enhancing preoperative risk assessment is essential. Collaborative efforts between obstetricians and urologists can further contribute to comprehensive care strategies and improve outcomes for patients undergoing CD.[12]

Conclusion

Bladder injuries following CD are complex and multifactorial. Through a combination of improved surgical techniques, preventive measures, and vigilant postoperative care, surgeons can strive to minimize the incidence and impact of bladder injuries, ultimately ensuring the safety and well-being of mothers undergoing C-sections. Early diagnosis and therapy may prevent complications and loss of organs that may lead to morbidity and mortality. Also, it is crucial to inform patients correctly; pregnant women with secondary or emergent cesarean deliveries should be advised about the significant risk of inadvertent surgical complications.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Harlequin Ichthyosis – Genetic and Dermatological Challenges: A Case Report and Literature Review

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Abstract

Harlequin ichthyosis (HI) is an extremely rare and severe genetic skin disorder characterized by thick, diamond-shaped scales covering the body, often giving the appearance of a harlequin costume. This paper provides an overview of the genetic and dermatological aspects of HI, delving into its etiology, clinical manifestations, and management. The genetic underpinnings of HI involve mutations in the *ABCA12* gene, leading to impaired skin barrier function and abnormal keratinization. Understanding the molecular basis of the disorder is crucial for accurate diagnosis and potential therapeutic interventions.

Clinically, HI presents challenges related to skin integrity, thermoregulation, and potential complications, such as infections. The management of HI requires a multidisciplinary approach involving dermatologists, geneticists, and other healthcare professionals. Supportive care, including emollients, careful bathing, and prevention of infections, is essential to improve the quality of life for individuals affected by this condition.

Despite its rarity and severity, advancements in medical research and genetic therapies offer hope for improved treatments and interventions. This paper aims to contribute to the collective understanding of HI, fostering ongoing research and compassionate care for those living with this unique and challenging dermatological condition. We presented a premature eutrophic harlequin baby, born at 32+ weeks of gestation via emergency C-section. A clinical diagnosis was established minutes after birth, based on the typical features of HI, from scaly skin, marked fissures, and limbs in flexion contractures to prominent eclabium and bilateral ectropion.**(International Journal of Biomedicine. 2024;14(1):182-186.)**

Keywords: Harlequin ichthyosis • ectropion • scaly skin • consanguinity • *ABCA12* gene

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Introduction

Ichthyosis encompasses a large group of skin disorders that share an abnormal stratum corneum proliferation and shedding.[1-4] They are characterized by dry, rough, scaly skin from a mild to severe extent.[2,4] The word ichthyosis comes from the Greek word ichthyos – resembling fish, reflecting the cutaneous scaling pathognomonic for this disorder.[3] Despite many attempts to establish a classification through genotype-phenotype correlation and molecular basis, different terminologies for ichthyosis remain in practice worldwide.[1]

The epidermal barrier function is maintained by a regular pattern of stratum corneum differentiation, which is composed of keratinocytes (known as hydrophilic bricks) and inter-keratinocytes lipids serving as a barrier to water loss (the mortar). Specific mutations in "barrier" proteins and enzymes disrupt the lipid bilayer, hence barrier integrity, resulting in ichthyosis.[4-7]

Congenital ichthyosis manifests in a broad clinical heterogeneity and genetic profile, from autosomal recessive to dominant and recessive X-linked ichthyosis. Autosomal recessive congenital ichthyosis (ARCI) is a modified term that covers mostly non-syndrome ichthyosis: HI, lamellar ichthyosis, and congenital ichthyosiform erythroderma.[1] Newborns are

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encased in a collodion membrane, with a different extension of hyperkeratosis and scales.[1,2,5]

HI, also known as keratosis diffusa fetalis, ichthyosis fetalis, or harlequin fetus, is a rare, yet the most severe and escalated, type of ichthyosis.[3,5,7-9]

The pattern of inheritance is autosomal recessive. This pattern is supported by consanguinity in some families. The recurrence rate in subsequent pregnancies is 25%.[6,7,9] The *ABCA12* gene located on chromosome 2q34 encodes the ATP binding cassette transporter 12.[8,9,10-12] Mutations in this gene lead to defective lipid transport via lamellar granules in the keratinocytes, resulting in malformation of the epidermal lipid barrier and ichthyosis phenotypes. [8,9,13]

Harlequin fetuses are often born prematurely.[7,14] A newborn's skin typically presents with hard, thickened scales and yellowish-grey, diamond-shaped plaques covering most of their bodies. Plaques are separated by deep polygonal erythematous fissures that are formed prenatally. Although they transverse the whole body, scalp fissures are usually more prominent. The presence of hard plaques and fissures gives the appearance of a cracked armor coat that causes limb flexion contractures and restrictive lung movement, sometimes followed by compartment syndrome, with indistinguishable toes in some cases.[1,3,4,8,9,11-13,15]

Other clinical features associated with HI are flattened to the absent nose, dysplastic ears to varying degrees, alopecia, dysfunctional sweat glands, eclabium, and bilateral ectropion. The latter ones are caused by tightness and skin tension that forces lips and eyelids to turn inside out, exposing the mucosal lining. The O-shaped mouth of these newborns also resembles a fish's mouth.[8,12,14-16]

Newborns with HI have an extremely high mortality rate during the neonatal period. The high mortality rate explains fulminant complications associated with hyperkeratosis, such as sepsis in 75% of cases and respiratory failure in 25%, or both.[1,6,8,11,13,15] There have been reported cases of developmental delays, growth retardation, and microcephaly.[12,16,17]

A severely impaired skin barrier makes these newborns prone to excessive water loss and electrolyte imbalances with hypernatremic dehydration and hypothermia.[12,16] On the other hand, ectropion increases the risk of developing exposure to keratitis and restrictive mouth movements, inevitably requiring nasogastric tube feeding.[13]

Neonates who survive the perinatal period under intensive therapy, after a few weeks, shed the armor-like coat but, over time, develop severe ichthyosiform erythroderma.[8,11,18] In addition, extracutaneous manifestations, keratoderma, and skin infections remain for life.[15] Usually, a diagnosis is made during delivery because of the characteristic features.[13] A sonographic prenatal diagnosis is often made during the early third trimester.[14,18]

Features suggestive of HI during 3/4-D ultrasonography are bulging eyes, large open mouth, flattened nose, abnormal limbs in general, or micromelia. Other features include snowflake signs suggesting dense floating particles/plaques in amniotic fluid, poly/oligohydramnios, and intrauterine growth restriction.[9,14,18]

Molecular genetics methods via prenatal fetal DNA testing currently play an irreplaceable role in diagnoses of ichthyoses. Amniocentesis and chorionic villus sampling have replaced fetal skin biopsy as less invasive procedures.[8,9,13]

Newborns with HI require intensive multidisciplinary care. Apart from the complications caused by the infant's immature growth, actions should be taken to prevent electrolyte imbalance, respiratory distress, malnutrition, and infection, such as incubation with added humidity, respiratory support, nasogastric feeding, regular serum electrolytes, topical antibiotics, fissure cultures, daily bathing, and applying only bland emollients. Also, systemic retinoids have been shown to increase survival rates.[3,7,13,15]

Case Presentation

A 28-year-old primigravida during triage was admitted to our Clinic with obstetric pain in preterm labor at 32w+4d based on the first day of her last menstrual period. During the consultation, 7 previous ultrasound reports were evaluated. No abnormal findings were found. She had a negative consanguinity history of marriage. A non-invasive prenatal test was performed during the 10th week of gestation. The test was performed to evaluate risk for certain aneuploid and deletional syndromes, and the test results were interpreted as low risk.

On admission ultrasound, reduced amniotic fluid was noted, the baby's lie was breech, and fetal heart rate was normal. On obstetrical exam, the cervix showed 40% effacement with 4cm dilation. Membranes were intact, and soft, small tissues were palpated as the presenting part. Because of progressed dilation, one dose of 12 mg of dexamethasone was administered IM during CTG monitoring; no variable and late decelerations were noted.

Because of oligohydramnios and preterm labor in progress with the fetus in a breech position, an emergency cesarean section was performed to deliver the baby. A male, 2000 g weight, 39 cm of height, and 34 cm head circumference was born. First, the baby didn't show any signs of life, which made the specialists think of a macerated baby. The baby was in a state of suspended animation, where just about half a minute later, the baby started crying and moving. In the first minute of life, evaluated well-being by the neonatal specialists was 5 points, after the 5 minutes of life - 6 points. An immediate clinical diagnosis based on the clinical features of the newborn was made (Images 1-3). During the clinical exam, the skin was covered with thick yellow plaques in a diamond shape, separated by deep fissures that were marked in the scalp and trunk. Scalp fissures demarcated only parts covered with membrane. The hair-covered scalp was not separated by fissures in between. Flat fontanels were noted. Eyelid ectropion was more prominent than eclabium, broad nose and underdeveloped external ears attached to the scalp and covered with membrane. Limbs were fixed in flexion contractures. Fewer cracks were noted on the limbs. Toes were incurved, and palms were swollen and claw-like, with well-developed digits. Despite prematurity, the newborn was eutrophic.

The newborn was isolated in a humidified incubator, and feeding was maintained with a nasogastric tube. The first

few hours, body temp was 35°C, respiratory rate - 53, heart rate - 155 bpm, and SpO2 - 96%. The abdomen sonography showed no abnormalities during examination, and the heart echocardiogram showed neither. Despite the vigorous multidisciplinary management after the first 3 hours, clinical deterioration with costal retractions was noted. The newborn expired after 12 hours, with the cause of death being acute respiratory distress syndrome.

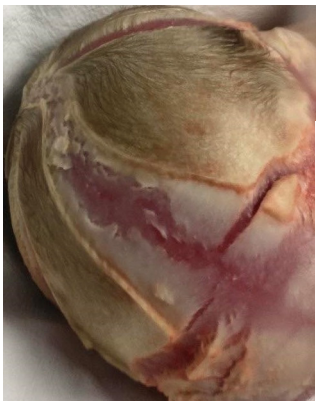

Image 1. *Marked fissures on the scalp.*

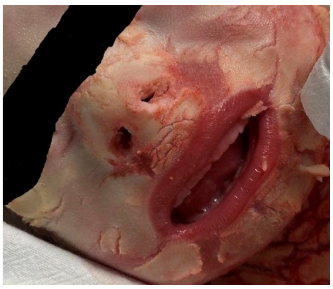

Image 2. *Flattened broad nose, prominent ectropion, and eclabium.*

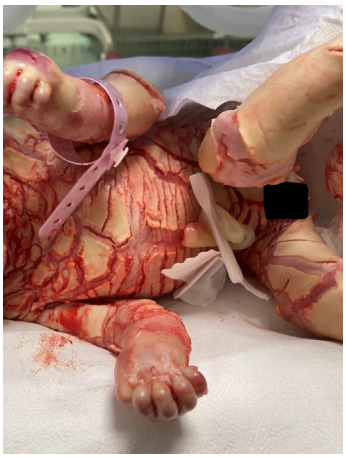

Image 3. *Severe extension of diamond-shaped hard plaques separated by deep fissures, prominent in the upper trunk region.*

Discussion

Ichthyosis represents a wide heterogeneous group of inherited and acquired skin disorders. The most severe form of congenital ichthyosis is HI, a Mendelian disorder of cornification, generally with an autosomal recessive pattern of inheritance.[19] The first case of HI was described in 1750 by Oliver Heart, while first prenatally diagnosed in 1982. Currently, its incidence is 1:300.000 cases. No evidence of frequency regarding sex, race, or ethnicity distribution was found.[13,14] Its rare manifestation shows the importance of family history during evaluation in establishing the pattern of inheritance. A positive history of parental consanguinity suggests a recessive pattern, while an affected parent and a sibling would point toward autosomal dominant inheritance.[4] In our case, no previously affected family members were found during pedigree evaluation, and a negative parental consanguinity was confirmed.

Liang et al. exhibited a rare phenomenon of confirmed prenatal diagnosis of HI after a previous birth of a fetus with HI. Rathore et al. presented a HI case with a second-degree consanguineous marriage.[9,20] Habib et al.[7] presented a case of a "harlequin fetus" from a consanguineous couple, but contrary to the case mentioned above, their relatives had a similar baby years ago. Suzumori and Kanzaki[21] described a case with 3 consecutive harlequin fetuses confirmed via fetal skin biopsy with a negative history of consanguinity, suggesting the autosomal dominant trait of inheritance.

HI is presented with very typical clinical-pathological features. Dry, scaly plaques in a diamond shape, separated by deep fissures from which the origin of its name arises like a traditional harlequin's costume and a characteristic face pulled wide open resembling a harlequin's smile.[8,16] We presented a premature eutrophic harlequin fetus, born at 32+ weeks of gestation via emergency C-section. An event of suspended animation was described; the fetus was thought by the surgeons to be a dead macerated fetus, for a few seconds. Jilmudi also described the state of suspended animation and its incidence, though it still has not been reported in the available literature.[22] A clinical diagnosis was established minutes after birth, based on the typical features of HI, from scaly skin, marked fissures, and limbs in flexion contractures to prominent eclabium and bilateral ectropion. Hepatomegaly, collapsed bowel, and prominent gall bladder were reported during the evaluation of this patient.[18] Our case showed no abnormal findings on abdominal sonography.

On postmortem examinations of harlequin fetuses, lung and liver fibrosis and ileum infarction have been described. Brain autopsies of maldevelopment with ischemic-necrotic lesions have been observed, such as severe cholestasis and enlarged thymus. Also, microcephaly, growth retardation, and developmental delay have been reported.[18,22] Baldo et al.[12] reported a rare case with an early onset of juvenile idiopathic arthritis in a 7-year-old boy with HI, suggesting that polyarthritis also could be a unique manifestation of HI. These findings allow space for further research to provide evidence on whether these lesions and manifestations are a result of the initial injury or that this disease is more intricate, involving other tissues in addition to the skin. Prenatal diagnosis and genetic counseling should be offered to all couples with previously affected babies.[9,13] The gold standard diagnostic prenatal test for HI is fetal DNA direct sequence analysis of the *ABCA12* gene, derived from amniotic fluid cells at 16 weeks of gestation.[7,16]

Immune-histological studies of skin have revealed that the focal structure where the HI phenotype begins is the hair canal keratinization at 17 weeks of gestation, subsequently from 20 weeks in the entire hairy skin.[14,18,23] Years ago, prenatal diagnosis of HI relied on fetal skin biopsy. Taken from fetuses around 21-23 weeks of gestation, when abnormal skin was thought to be expressed in the entire body surface.[23]

Electron microscopy may identify atypical intraepidermal vesicles by 16 weeks of gestation; light microscopy can show premature keratinization by the 20th to 22nd week,[7] while amniocentesis could show aggregated lipidic vesicles in keratinocytes by the 17th week of gestation.

Its rare congenital nature makes sonographic early prenatal diagnosis challenging, especially without known risk factors and because of mid-gestation phenotype development. Hence, a prenatal sonographic diagnosis could be implemented by the second trimester.[14,18]

In our study, a diagnosis was established after birth based on the clinical features. A prenatal genetic test conducted during early pregnancy for certain aneuploidies and deletional syndromes resulted in a negative conclusion, and on ultrasound, no abnormal features during pregnancy were detected. On admission, a breech-presented fetus with oligohydramnios was described.

The most common findings on ¾ dimensional ultrasound during pregnancy are ectropion, eclabium, flat nose, short limbs, swollen hands and feet, abnormal position, intrauterine growth restriction, and polyhydramnios. Less often, breech presentation, oligohydramnios, and abnormal fetal movements.[3,13,18]

In a study by Liang et al., karyotype analysis of the amniotic fluid at 20 weeks of gestation resulted in no chromosomal abnormalities, while sonography at 24 weeks of gestation showed thickened soft tissue in the anterior region of the eyeballs.[20] In a study by Rathore, a 3/4-dimensional sonographic diagnosis was established only after a confirmed HI previous pregnancy. At 26 weeks of gestation, the diagnosis was based on sonographic markers like ectropion, eclabium, short foot length, incurved toes, and polyhydramnios.[9] In Berg et al.,[18] although features of HI were encountered on ultrasound during pregnancy, they still couldn't diagnose the condition until labor, at 35 weeks of gestation, because of premature rupture of membranes. Sepsis and respiratory failure are the most common causes of death. Because of thickened skin, chest wall movements get restricted, making breathing a painful mechanism that results in poor pulmonary ventilation. The development of respiratory failure is attributed to aspiration of amniotic fluid with thick skin particles.[24]

The mortality of patients with HI is still high around the world. Most of them die in the first few hours to days of life due to secondary complications, such as infection, dehydration, and respiratory insufficiency. Many factors contribute to the survival of these patients. It has been reported that fetuses with a heterozygote mutation had a higher survival rate than those with a homozygous gene function loss. Also, milder phenotypes have been described among individuals with missense mutations of the *ABCA12* gene, in comparison to nonsense mutations.[4,6,17] In a study by Rajpopat,[24] where 45 patients participated, the survivors' ages ranged from 10 months to 25 years, and the overall survival rate was 56%. A retrospective study by Shibata et al. [25] during the 2005-2010 period concluded that early intubation can improve outcomes for harlequin fetuses due to the development of respiratory insufficiency. Also, systemic retinoids, early antibiotic administration, and intensive care units have all improved patient survival rates.[25] Rajpopat et al.[24] showed the effects of systemic retinoids. In the retinoid-treated group, 83% of patients survived, compared to the 76% mortality rate from the untreated group.

Conclusion

HI is a rare, severe chronic disorder of cornification with a poor prognosis during the neonatal period. Early life complications, such as sepsis, dehydration, and respiratory failure, can occur. Over the decades, many synonyms have been given to this skin condition, all of them resembling the phenotypical features of these babies. Usually, a diagnosis is made based on clinical appearance during delivery. Risk factors contributing to early prenatal diagnosis are positive family history, previous pregnancies with similar phenotypes, and family consanguinity. Second-trimester sonographic markers can lead to a prenatal diagnosis, especially after a previously confirmed pregnancy with HI. The typical facial features, reduced fetal movements, intrauterine growth restriction, and amniotic fluid changes are the most encountered changes during sonographic evaluation. These babies are usually born prematurely; suspended animation is a possible event during labor. Other tissues involved have been described. In addition to the skin changes, neurodevelopment delays have also been noted. Systemic retinoids and multidisciplinary immediate management have been shown to improve outcomes and increase survival rates.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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Ethical Considerations

The institutional ethical board has approved this research with registration number 3430. The patient provided written informed consent to publish case-associated data and accompanying images.

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CASE REPORT

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Aggressive HER2-Positive Gastric Cancer in a Young Patient, Refractory in Trastuzumab and Progressive with Trastuzumab-Emtansine Treatment

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Abstract

Gastric cancer remains a major global health problem. Gastric cancer is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related deaths in the world. The prognosis of the disease is poor because it is often diagnosed at later stages, especially at HER2-positive. Most patients diagnosed with gastric cancer present with advanced, incurable disease. This report details the case of a 30-year-old male patient diagnosed with metastatic gastric adenocarcinoma. Stage T3 N3 M1, PD-L1 0%, and HER2+. The patient was administered neoadjuvant palliative chemotherapy, eight cycles of FLOT, and the last two cycles of HER-FLOT. The patient constantly had elevated levels of liver enzymes, and therefore, endoscopic retrograde cholangiopancreatography with biliary stenting was performed. After chemotherapy followed by restaging, the tumor board, based on the findings, decided to remove the primary tumor from this young patient. The operation was performed as a palliative da Vinci-assisted total gastrectomy with lymphadenectomy. Then, trastuzumab monotherapy was prescribed. At that time, the patient's follow-up with PET-CT showed progression with hypermetabolic lymph nodes in the paraaortic and aortocaval regions, as well as left hydronephrosis. As a result, we started the second-line therapy with T-DM1, an antibody-drug conjugate trastuzumab-emtansine (KADCYLA), for five cycles. At the time of receiving the sixth cycle, the patient's condition changed dramatically due to liver and heart problems, pleural effusion, and bleeding. After two years of treatment, all oncological-specific therapies have been ended, and the patient has been put in palliative care to relieve suffering and to support the best possible quality of life.

This case underlines the importance of identifying potential therapeutic targets and developing therapies to improve the outcomes of systemic treatment beyond those currently achieved with conventional chemotherapy and targets.**(International Journal of Biomedicine. 2024;14(1):187-192.)**

Keywords: gastric cancer • HER2 resistance • trastuzumab

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Abbreviations

HER2, human epidermal growth factor receptor 2; **IHC**, immunohistochemistry; **NTRK**, neurotrophic tropomyosin-receptor kinase; **OS**, overall survival.

Introduction

Gastric cancer remains a major global health problem. Gastric cancer is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related deaths in the world.[1,2] The prognosis of the disease is poor because it is often diagnosed at later stages, especially at HER2-positive.[3-10] Over 95% of gastric cancers are adenocarcinomas.[11]

Overexpression of the HER2 protein or amplification of the *ERBB2* gene has been implicated in the development of gastric adenocarcinoma.[12] Some studies suggest that HER2 positivity is associated with poor prognosis.[5-10] In contrast,

others have shown that it is not an independent prognostic factor of patient outcome, except in a very small subgroup of patients with intestinal histology.[13-15] The reported rate of HER2 positivity in patients with gastric cancer ranges from 12% to 23%,[6,7,14,15] and in Europe, it is less than or equal to 20%. HER2 positivity was significantly higher in males than in females.[16]

Chemotherapy remains the standard care treatment approach for patients with advanced-stage disease; however, response rates are relatively low, and the prognosis is poor, with a median survival of only 8–10 months. Trastuzumab, in combination with chemotherapy, in the first-line setting of patients with metastatic, HER2-positive gastric cancer, represents the first targeted therapeutic method to demonstrate improvement in response rate and survival in gastric cancer. However, not all patients with HER2-positive gastric cancer respond to trastuzumab, and most patients who do initially benefit from trastuzumab develop resistance to it. Treatment with trastuzumab is based on the presence of HER2 overexpression.[16]

Case Presentation

A 30-year-old male patient with a family history unremarkable for gastric cancer was diagnosed with gastric cancer stage T3N3M1, along with multiple pathologically enlarged retroperitoneal lymph nodes, multiple small mediastinal lymph nodes, peritoneal carcinomatosis, ascites, and strong suspicion of osteolytic skeletal metastases without cortical erosion. Gastroscopy was performed with biopsies in July 2021. It showed histologically as gastric adenocarcinoma, partly solid and poorly differentiated G3, PD-L1 expression negative, NTRK IHC negative, and HER2 positive.

The patient was administered neoadjuvant palliative chemotherapy, eight cycles of FLOT, and the last two cycles of HER-FLOT. The patient constantly had elevated levels of liver enzymes, and therefore, endoscopic retrograde cholangiopancreatography with biliary stenting was performed. In December 2021, after chemotherapy followed by restaging, the tumor board, based on the findings, decided to remove the primary tumor from this young patient. Restaging shows a very good remission endoscopically and in the abdominal CT scan. The operation was performed as a palliative da Vinci-assisted total gastrectomy with lymphadenectomy. The findings in post-operative histopathology according to UIUCC-TNM classification (8th Edition, 2017): cT1b pN0(0/10) LO VO Pn0. Regression grade <10%. Then, trastuzumab monotherapy was prescribed until May 2023. At that time, the patient's follow-up with PET-CT showed progression with hypermetabolic lymph nodes in the paraaortic and aortocaval regions, as well as left hydronephrosis (Image 1).

As a result, we started the second-line therapy with T-DM1, an antibody-drug conjugate trastuzumab-emtansine (KADCYLA), for five cycles. At the time of receiving the sixth cycle, the patient's condition changed dramatically due to liver and heart problems, pleural effusion, and bleeding.

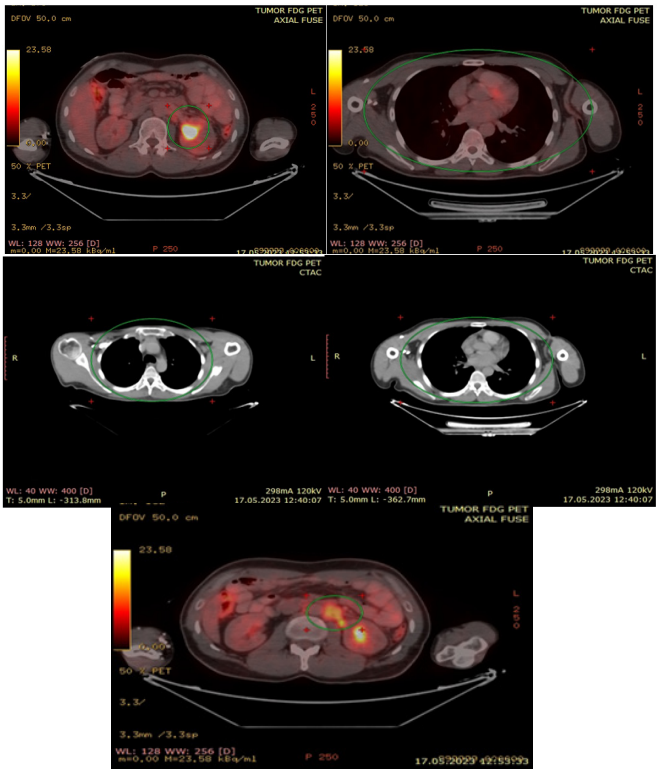
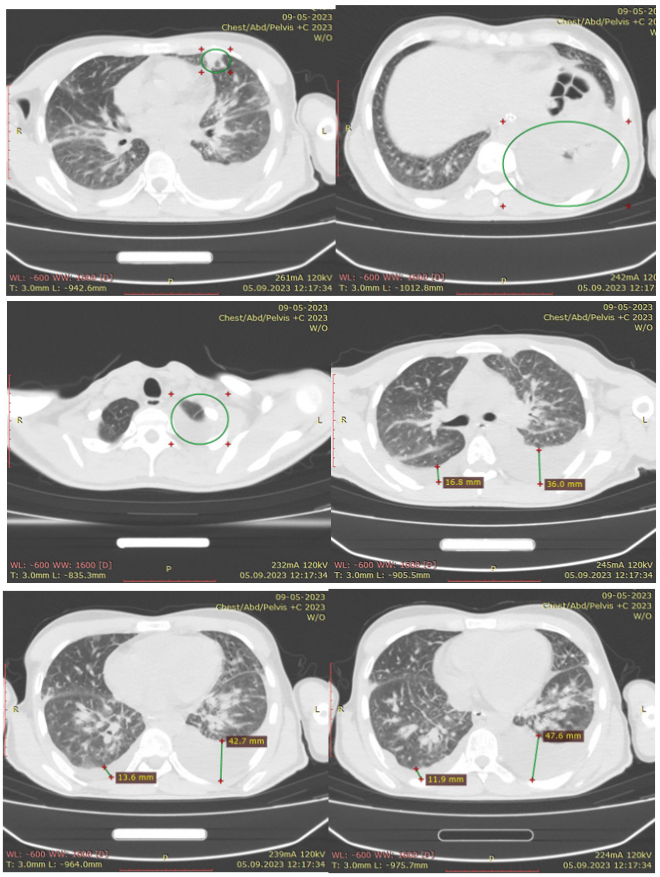


Image 1. *PET-CT evaluation during Trastuzumab treatment (May 2023).*

The chest CT scan detected a pleural effusion up to 5 cm in the left lung and 1.5 cm in the right lung, lesions in the pericardiac area, and the left lung parenchyma (Image 2).



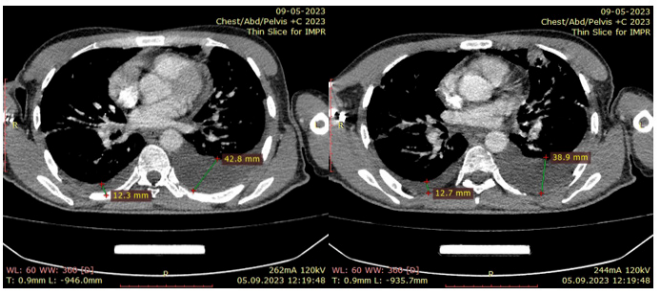


Image 2. *Chest CT scan (September 2023).*

So, after two years of treatment, all oncological-specific therapies have been ended, and the patient has been put in palliative care to relieve suffering and to support the best possible quality of life. He pursued many multidisciplinary interventions to relieve major symptoms, resulting in the prolongation of life.

Discussion

Gastric cancer represents a conglomerate of histologically and biologically heterogeneous diseases, which are characterized by various genomic alterations that result in activating molecular pathways. We know more about the biological behavior of gastric cancer and its intrinsic subtypes, particularly the *ERBB2* amplified gastric cancer subtype. HER2+ is implicated with poor prognosis and aggressiveness of gastric cancer.

Pathologic review and biomarker testing play important roles in gastric cancer diagnosis, classification, and molecular characterization. Classification based on histologic subtype and molecular features helps improve early diagnosis and has implications for therapy.[17] Presently, IHC and/or molecular testing for HER2/*ERBB2* status, MSI or MMR status, tumor mutational burden-high status, and *NTRK* gene fusion are implicated in the clinical management of advanced gastric cancer.[18] PD-L1 testing may be considered on locally advanced, recurrent, or metastatic gastric cancer in patients who are candidates for treatment with PD-1 inhibitors. An FDA-approved companion diagnostic test should be used to identify patients for treatment with PD-1 inhibitors.

Treatment of Gastric Cancer

Trastuzumab is a monoclonal antibody that binds to the extracellular domain of the HER2 receptor, blocking its downstream signaling pathway. It promotes an antibody-dependent, cell-mediated cytotoxicity by activating apoptotic signals in tumor cells.[19] Patients who underwent chemotherapy with cisplatin and fluorouracil in combination with trastuzumab had a better median OS than those who got only chemotherapy (16 months vs 11 months). This is mainly due to the survival advantage of patients with high expression of the HER2 protein.[16,20,21]

Antibody-drug conjugate is an emerging antibody bioconjugate, which is an immunoconjugate composed of a monoclonal antibody bound to a cytotoxic drug through a chemical linker, combining the antigen specificity of the antibody and the potency of the cytotoxic agent at the same time. However, patients with advanced gastric cancer treated with T-DM1 did not have a clear OS advantage over those treated with taxanes.[22,23] The issue has gained attention as most patients develop resistance to trastuzumab. Trastuzumab resistance appears to be primarily mediated by tumor heterogeneity. Treatment failure with anti-HER2 therapy is also associated with changes in receptor tyrosine kinase-RAS-PI3K signaling. To overcome this problem, various new drugs and treatments are emerging.[24,25] Intratumor heterogeneity and genomic instability processes shape tumor evolution in space and time, and growing evidence suggests a link between assessment heterogeneity and poor prognosis. This explains the mismatch between the costs and benefits of some cancer treatments.[26,27]

Surgery is the primary treatment option for patients with localized gastric cancer. Clinical staging using chest/abdominal/pelvic CT scan, with or without endoscopic ultrasound (if no metastatic disease is seen on CT), should be performed before surgery to assess the extent of the disease and degree of nodal involvement.[28]

Combined modality therapy has been shown to significantly increase survival in gastric cancer patients with locoregional disease.[29-31] Perioperative chemotherapy is recommended for localized resectable disease (category 1).[30,32-35]The survival benefit of perioperative chemotherapy in gastric cancer was first demonstrated in the landmark phase III MAGIC trial.[35] In the randomized controlled phase FLOT4 trial, Albatran et al. compared perioperative chemotherapy with fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) to the standard ECF regimen in patients with respectable, non-metastatic gastric or esophagogastric junction adenocarcinoma (≥cT2 and/or N+).[33]

Systemic therapy can provide palliation, improved survival, and enhanced quality of life in patients with locally advanced or metastatic gastric cancer.[36-38]

First-line systemic therapy regimens with two cytotoxic drugs are preferred for patients with advanced disease because of their lower toxicity. The use of three cytotoxic drugs in a regimen should be reserved for medically fit patients with excellent performance status and easy access to frequent toxicity evaluations.[40] Studies have shown that most gastric cancer recurrences occur within the first 2 years after the completion of local therapy (70%–80%), and almost all recurrences occur within 5 years (~90%).[41-43] The selection of regimens for second-line or subsequent therapy is dependent upon prior therapy and performance status.[44]

In conclusion, with the advancement of tumor immunotherapy, combined immune checkpoint inhibitors will emerge as a promising treatment, hopefully resulting in decreased tumor size and improved objective response rates. Intratumor heterogeneity may be the most important primary mechanism of anti-HER2 drug resistance. Patients with refractory HER2-positive status should be put in the new study in combination with chemotherapy and/or immunotherapy or new research approaches to participate in well-designed clinical trials investigating novel therapeutic strategies to enable further advances. Potential loss of HER2 positivity after first-line anti-HER2 treatment requires reexamining HER2 status before initiating second-line anti-HER2 therapy.

To better assess patient outcomes, we need improved diagnostic, prognostic, and disease surveillance methods despite the availability of various treatments. A combination of immunotherapy and anti-HER2 monoclonal antibodies may be required.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Matricide by Person with Borderline Personality Disorder

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Abstract

Matricide is a very rare form of murder and comprises approximately 0.68% of all murders. This scientific paper aims to present a psychiatric evaluation of one of the most macabre murders committed in Kosovo in 2023. The accused temporarily stayed in Germany and committed the act during his stay in Kosovo for vacation. Murder has been associated with marked brutality. Until the moment of committing the act, he has not behaved aggressively towards his sibling or parents because family members have avoided confrontation by fulfilling his wishes. The murder took place in a joint house, where he first stabbed his mother and then decapitated her by placing her head in the basement. After the crime, he manifested the symptoms of acting out by standing on the stairs of the house and quietly waiting for the police. Other family members reported that he never manifested aggressive behavior towards his mother, and she was the one with whom he had the best relationship. This case shows how unpredictable are borderline personality disorders and how a lack of impulse control can lead to murders.**(International Journal of Biomedicine. 2024;14(1):193-195.)**

Keywords: matricide • forensic psychiatry • mental illness • mother–son bond

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Case Presentation

The Institute of Forensic Psychiatry of Kosovo (IFPK) received a request from the Basic Court for Serious Crimes in Peja for the psychiatric evaluation of Mr. U., born in 1991. For this purpose, the IFPK Director has formed a commission for evaluation with a constitution of three members - one psychiatrist, one forensic psychiatrist, and one clinical psychologist. The duty of members was to determine the mental state of the defendant before the crime, especially during the crime, but also after the commission of the criminal offense in question, therefore, the degree of responsibility. The court charged Mr. U. with criminal offenses: Aggravated murder from article 173, part 1, subpart 1.3 and 1.4 of the PKRK.

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Personal history

Mr. U. was born on 25.11.1992. in Peja. He has a brother and a sister and is the second child. According to the information he had, his mother gave birth in a hospital, with a normal birth. He had normal psycho-physical development and was a smiling and happy child. According to Mr. U., because of the war in 1999, his family migrated to Munich, Germany, where he started first grade.

In 2000, they returned from Germany, and Mr. U. continued his studies at a school in Peja; since that time, he and his family have lived in Peja. After completing primary school, Mr. U. continued to Technical High School. During this time, Mr. U. started smoking and making small problems in school, skipping classes, and getting into fights. Since the first year of high school, at age 15, Mr. U. has started working as a butcher; he has used the income from this profession for his needs regarding smoking.

After finishing high school, Mr. U. worked various jobs in Gastronomy as a waiter, barista, assistant cook, and main cook. He “couldn’t stand the pressure from bosses in different bars and restaurants. “This is why he was changing working places

without having trouble advancing in positions. According to Mr. U., while he worked as a cook in one of the restaurants, he had a health problem when he "swallowed" his tongue, that day, according to him, his family immediately sent him to the hospital where the doctor in charge prescribed him medication "Tegredol," from which he had a severe allergic reaction. After that, he was sent to Skopje for further treatment. As a final result, Mr. U. had a pacemaker placed in his heart as he had irregular heartbeats and unstable heart rhythm.

In 2017, Mr. U. got married for the first time, but due to disagreements and contradictions in mentality, he separated from his first wife. In 2018, Mr. U. went to Germany with a work visa and started working there. During his stay in Germany, he worked various jobs, from a waiter at McDonald's to a forklift driver in a paper factory. During this time, Mr. U. lived with a German girlfriend for two years, until 2020. During this time, Mr. U. had several affairs with other women.

In 2020, Mr. U. proposed to a woman from Kosovo with whom he initially had good relations. Still, later, some problems started due to her inability to adapt to the living conditions of Germany. Once, the situation escalated so much that U. was very violent towards her, and the police were notified, who sent his ex-wife to the hospital, while Mr. U. was recommended to stay at the Hotel. His ex-wife, mediately after the incident, left Germany. Mr. U. continued to stay in Germany, working at the Cardboard Factory.

Medical history

Mr. U. denies having had previous psychiatric treatment; he also denies heredity in all aspects and denies having a history of psychiatric treatment.

Socioeconomic conditions

Living conditions are average.

Extracts from siblings' declaration

His brother declares: "U. is my younger brother. He was usually quiet, but we never upset him because he overreacted. He was a closed type, petted; before the divorce, he lived with his wife in his apartment in Germany. According to his ex-wife, he was violent, and that is the reason why he got divorced. While in Germany, he played in the Casino, and often we had to send him the money he drank away."

*His sister declares: "*U. was a quiet boy, he has been living in Germany for four or five years, there he worked different jobs. His last job was in a cardboard factory; however, we often sent him money from Kosovo. U. didn't have significant problems with our mother; it never occurred to us that U. could do something like this. U. had the best time with her."

Mental status

Male, medium height. Conscious, oriented in time and space. Appearance corresponds to age, care for hygiene and appearance is maintained, and verbal contact is maintained easily. Mr. U. speaks with a normal tone of voice. He answers questions clearly and briefly; in some questions, he tends to give acceptable and ambiguous answers, and in some questions, he reacts impulsively, especially those he considers provocative; he has tendencies to manipulate by presenting himself as a victim. There is a whine, frustration, and pronounced impulsiveness. Ideas of greatness, relationships, and persecution are evident in thinking. Denies disorders of the perceptive sphere. The mood is described as sitting with a superficial affective relationship. He currently denies homicidal and suicidal ideation.

Psychological evaluation report for Mr. U.

Mr. U. is not characterized by problems in the sphere of intellectual development. Interviewing and psychological exploration of the same have been accompanied by difficulties due to the rigid nature of thinking, impulsiveness, and distrustful and contradictory tendencies within the personality.

The Minnesota Multiphasic Personality Inventory (MMPI) was administered to measure psychopathological degrees in the sphere of personality. During the interview process, he shows resistance to answering and, in most cases, gives intermediate answers, such as "I don't know," "I can," or he answers with questions to professionals. This also points to the tendency to take control of the situation. In the questions aimed at obtaining his personal information, he gets tense and reacts with arrogance and defensiveness. This also highlights the marked difficulties in respecting boundaries in relation to others or being faced with a task or responsibility. The way he generally interprets situations shows that he is characterized by an external locus of control, which means that he always sees others as the cause of his problems. Also, the same is characterized by an affective style characterized by emotional coldness and a lack of empathy for others.

Laboratory examinations

Blood tests: normal parameters.

Native computerized tomography: In the infra and supratentorial cerebral parenchyma without densitometric changes, the ventricular system and the circulation spaces of the LCS are free of pathological content, hypertrophy of the nasal concavities, DSN with a nasal ridge on the right, the paranasal spaces are with regular development and ventilation but with thickening of the mucous membranes inflammatory of the maxillary sinuses and sphenoidal sinuses - pansinusitis, the mastoid cells have regular development and aeration, the bone structures without noticeable pathological changes.

Electroencephalogram: EEG is within normal limits, without paroxysmal discharges.

Declaration of Mr. U.

For the case, Mr. U. declares: "I didn't sleep well that night, I didn't sleep at all that night, I didn't sleep well, I didn't feel well, my mother was very sad, she saw that people didn't like me, she had a very bad time period, even during the day there at that afternoon I did it and ... what I did is very serious, I don't know when the event happened, the aunt came, and then she said call the police, the wife of the uncle's son seems to have called the police. It has happened, and life must go on... "

Conclusion

Matricide is an infrequent crime that has often raised the suspicion that the offender could suffer from a pathological mental status.[1] For Mr. U., the diagnostic criteria for "borderline

personality disorder" were used. According to the International Classification of Diseases ICD-10, this disorder is coded with code F 60.31. This disorder [1-8] is characterized by a multitude of symptoms such as emotional instability, pronounced tendencies towards impulsive actions without taking into account the consequences, anxiety, and uncertainty, in addition to self-reflection, internal goals and tendencies (including sexual ones) can be vague or disordered, irritability, impulsiveness, anger. Anger is part of the character of these people, the chronic feeling of emptiness that tries to be filled by creating intense emotional connections that last a little, the tendency to threaten and manipulate others: if their demands are not met, violent and aggressive reactions even in the weakest bullies, problems with adaptation, problems in human relationships, problems with the environment and with the family.In tense situations, this disorder can be accompanied by short psychotic symptoms. Psychotic symptoms may include disturbances in the perceptual sphere that are presented with auditory and visual hallucinations, while thinking disorders are presented with delusions of relationship and persecution.At the time of committing the criminal offense for which he is charged, based on the characteristics of the disorder and the marked intensity of psych symptomatology, his mental ability to understand and control his actions has been reduced to an essential degree.

Ethical Consent: The decision from the Institute of Forensic Psychiatry of Kosovo (IFPK) dated 10/30/2023 on confidentiality terms in this article.

Competing Interests

The authors declare that they have no competing interests.

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