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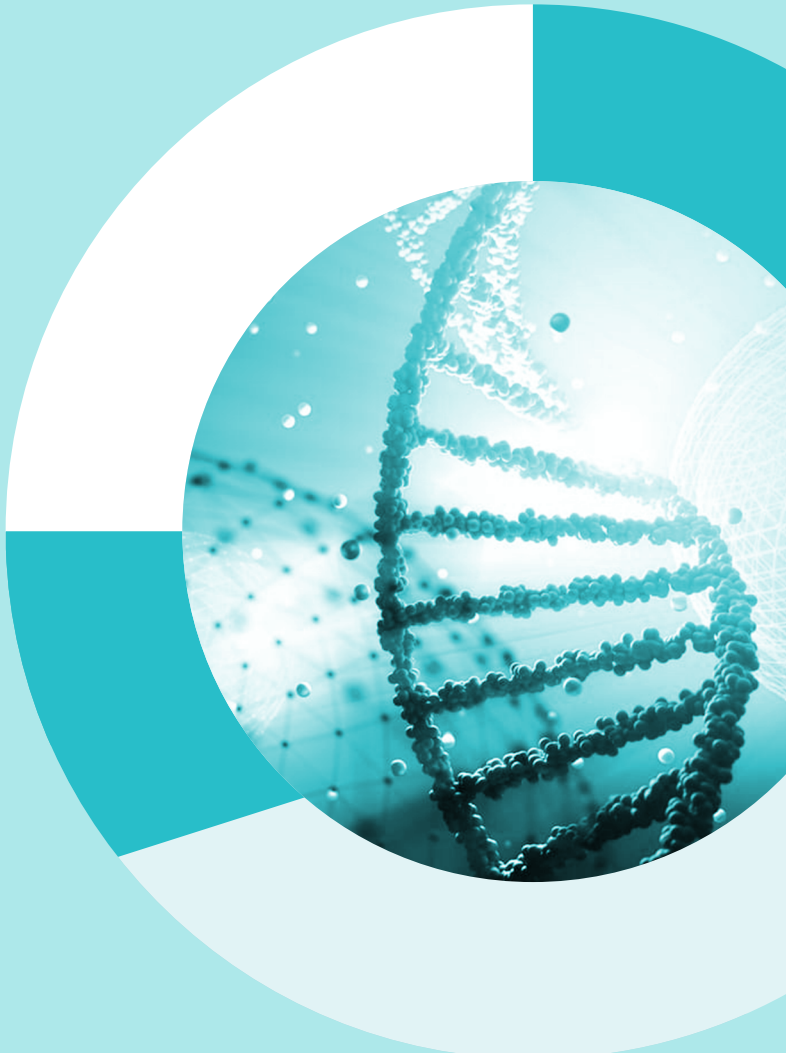

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REVIEW

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Cyprus J Med Sci 2025;10(3):157-161

Utility of Pharmacokinetic-Pharmacodynamic Modeling to Optimize Aminoglycoside Dosing in Neonates: A Mini-Review

Ahmed S. Ali[1], Khalid A. Y. Alfaifi[2], Assmaa A. Shaker[3], Faisal K. Alfaifi[4], Sajwa A. Alfaifi[5], Abir S. Mohamed[6]

[1]Department of Pharmacology, King Abdulaziz University Faculty of Medicine, Jeddah, KSA
[2]Department of Pharmacy, Medical Services Directorate Taif, Taif, KSA
[3]Ibn Sina National Faculty of Health Science, Jeddah, KSA
[4]Taif University Faculty of Pharmacy, Taif, KSA
[5]King Khalid University Faculty of Pharmacy, Abha, KSA
[6]Department of Public Health, Jazan University Faculty of Nursing and Health Sciences, Jizan, KSA

Abstract

Aminoglycosides (AG) remain essential in the management of severe neonatal infections, but their narrow therapeutic range demands precise dosing to maximize efficacy and minimize toxicity. This review evaluates the utility of pharmacokinetic/pharmacodynamic (PK/PD) models in optimizing AG dosing in neonates. An advanced literature search was conducted in PubMed using Boolean operators for accuracy and breadth. The search query combined ("AG" OR "gentamicin" OR "amikacin") AND "PK/PD modelling" AND ("neonates" OR "infants" OR "paediatrics"). The search focused on studies published between 2010 and 2024, emphasizing PK/PD modelling in neonatal and pediatric populations. Filters were applied to include only studies with free full-text availability, yielding eight relevant articles. The findings indicate that PK/PD models, combined with therapeutic drug monitoring, enhance dosing strategies by incorporating patient-specific variables such as gestational age, birth weight, postnatal age, and renal function (e.g., glomerular filtration rate). The predictive value of metrics like peak-to-minimal inhibitory concentration (MIC) and area under the curve-to-MIC ratios was evident for efficacy, while elevated trough levels were linked to nephrotoxicity. However, limitations of current approaches include insufficient consideration of disease-specific PK alterations (e.g., sepsis), reliance on invasive monitoring methods, and the absence of advanced PD indices, such as bacterial growth kinetics and immunological status. Future research should focus on developing less-invasive sampling techniques, such as dried blood spots and urine biomarkers for renal function while integrating advanced PD indices to refine neonatal dosing strategies further.

Keywords: Gentamicin, neonates, biosensors, artificial intelligence

INTRODUCTION

In neonatal intensive care units, antibiotics such as aminoglycosides (AG) are frequently prescribed, yet optimal dosing remains a common challenge. Optimizing antibiotic use is critical to ensuring effective treatment, minimizing toxicity, and mitigating the development of antibiotic resistance.[1] AG are essential for treating severe gram-negative infections. However, their use is associated with significant risks, including dose-dependent nephrotoxicity and ototoxicity,

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ORCID IDs of the authors: A.S.A. 0000-0002-3341-8177; K.A.Y.A. 0009-0003-5055-364X; A.A.S. 0000-0002-7164-5856; F.K.A. 0009-0008-2803-3775; S.A.A. 0009-0006-8480-3928; A.S.M. 0000-0002-8743-5810.

Corresponding author: Ahmed S. Ali
E-mail: asali@kau.edu.sa
ORCID ID: orcid.org/0000-0002-3341-8177

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with higher risk in neonates due to their unique physiological vulnerabilities.[2]

Neonates, particularly preterm infants, present distinct pharmacokinetic (PK) and pharmacodynamic (PD) challenges. Factors such as immature renal function, high extracellular fluid volume, and variable body composition lead to complex PK of AG. For instance, the volume of distribution for gentamicin in neonates ranges from 0.40 to 0.45 L/kg, with even higher values observed in those with sepsis. Similarly, the half-life of AG can prolonged to 11.5 hours in extremely low birth weight neonates, compared to 8-9 hours in neonates with adequate weight.[3] These PK variations contribute to either suboptimal therapeutic levels or potential toxicity when using empirical dosing regimens.[4] Moreover, the rapidly developing yet unpredictable glomerular filtration rate (GFR) in neonates complicates AG clearance, while increased extracellular fluid volume affects drug distribution. Such factors necessitate dosing regimens with extended intervals to achieve optimal peak-to-minimal inhibitory concentration (MIC) OR area under the curve (AUC)-to-MIC ratios, which correlate with improved efficacy and reduced adaptive bacterial resistance.[5]

Figure 1 provides an overview of key PK and PD parameters related to the efficacy and toxicity of AGs. The figure highlights crucial parameters such as AUC, Cmax (peak concentration), Cmin (trough concentration), MIC and post-antibiotic effect (PAE), which is the continued suppression of bacterial growth after AG concentration falls below the MIC. For effective treatment of gram-negative infections, target ratios should be C_{max}/MIC ≥8-10 OR AUC/MIC ≥70-100. Additionally, maintaining Cmin levels <2 mg/L is associated with a reduced risk of nephrotoxicity.[6]

Traditional therapeutic drug monitoring (TDM), focused on measuring serum drug levels, often falls short in addressing the complexities of neonatal pharmacology. Factors such as gestational and postnatal age, organ immaturity, birth weight, and conditions like sepsis significantly influence drug PKs and PDs, necessitating advanced modeling techniques for precise dosing. These models refine dosing regimens by accounting for variables such as AG concentration-dependent killing, PAE, and accumulation in kidney and ear tissues among other variables.[6,7] Advanced PK/PD modelling techniques, including physiologically based PK/PD models (PBPK/PD), have further enhanced our ability to simulate neonatal drug PK and improve dosing accuracy.[8]

Figure 2 describes one of the sophisticated PK/PD models which integrates bacterial growth dynamics, adaptive resistance (AR), and PK of gentamicin. It consists of two key bacterial compartments: the susceptible (S) compartment, where drug-S bacteria proliferate, and the resting compartment, containing dormant, less drug-S bacteria. Transfer between these compartments occurs when the bacterial population exceeds a threshold, regulated by the rate constant (kSR). Both compartments experience natural bacterial death at a rate constant (kdeath), while the central system mediates drug-induced bacterial killing through the elimination rate constant (ke). The model also incorporates AR, where resistance develops at a rate constant (kon), stimulated by gentamicin, and reverses at a rate constant (koff). AR diminishes the maximum drug-induced bacterial killing effect (E_{max}).

The PK model is described as a three-compartment system (one central and two peripheral compartments PI and PII). The overall system effectively captures bacterial dynamics, and gentamicin PK offers a robust integration tool to predict gentamicin efficacy against specified infections.[9-11]

Objective

This review aims to evaluate the utility of PK/PD models in optimizing AG therapy for neonates, with a specific focus on addressing the unique challenges posed by both full-term and preterm neonates.

Methods

A comprehensive literature search was conducted in PubMed using Boolean operators. The search query included the terms (“AG” OR ”gentamicin” OR “amikacin”) AND ”PK/PD modelling” AND (“neonates” OR “infants” OR “paediatrics”). The search was restricted to studies published between 2010 and 2024, emphasizing PK/PD modeling specifically in neonatal and pediatric populations. Studies focusing on *in vitro* experiments or animal models were excluded to ensure relevance to human clinical contexts.

Pharmacokinetic Modeling of Aminoglycosides in Pediatric and Neonates

PK modelling of AG has demonstrated significant success in optimizing its clinical application in pediatric populations. For example, these models have played a crucial role in adjusting amikacin and tobramycin dosing for pediatric patients with cystic fibrosis, in whom altered PK necessitate individualized dosing regimens.[12-14] Additionally, PK modelling has highlighted important differences in AG disposition between oncology and non-oncology patients and supported tailoring dosing strategies for these distinct groups.[15,16]

However, PK modelling in neonates remains relatively underexplored. Only eight studies met the inclusion criteria, yet they provide valuable insights into optimizing AG therapy in this vulnerable population. These studies highlight the potential of PK modelling to inform dosing strategies, improve therapeutic outcomes, and minimize toxicity in neonates. Nonetheless, the limited number of studies underscores the need for further research to expand the evidence base and refine AG therapy for this age group. These studies are summarized below.

Mohamed et al. [11] developed a semi-physiological model to characterize the maturation of GFR across pediatric age groups. By analyzing data from 1,760 patients, they created a body weight-dependent exponent function for GFR maturation, which improved drug clearance predictions and supported safer dosing strategies for renally excreted drugs.

In a study by De Cock et al. [17], PBPK/PD was utilized to guide gentamicin dosing in preterm neonates. The validated model accounted for gestational age, showing that neonates with postmenstrual ages of 30-34 weeks or ≥35 weeks required higher doses with longer intervals (e.g., every 36 hours) to maintain optimal trough concentrations below 1 µg/mL.

Cristea et al.[18] highlighted that neonates with perinatal asphyxia exhibited a significant reduction in amikacin clearance, necessitating extended dosing intervals to reduce toxic trough levels while maintaining efficacy.

Cies et al.[19] analyzed gentamicin PK and PD in neonates with hypoxic-ischemic encephalopathy undergoing controlled hypothermia. A two-compartment model identified 5 mg/kg every 36 hours as the optimal regimen to achieve desired therapeutic targets.

Sridharan et al.[9] employed a semi-mechanistic model to describe gentamicin's bactericidal activity and AR in preterm neonates. Despite lower peak concentrations in preterm neonates, the drug's extended half-life enhanced bacterial killing, supporting extended dosing intervals of 36-48 hours to balance efficacy and toxicity.

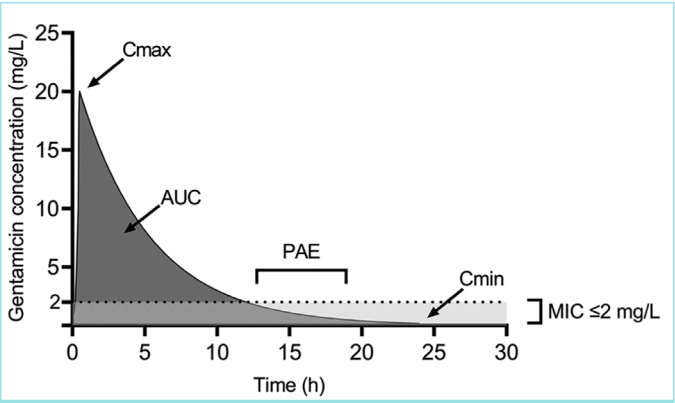

Figure 1. Overview of PK/PD parameters related to the efficacy and toxicity of AG.[6]

AUC: Area under the curve, C_{max}: Peak concentration, C_{min}:Trough concentration, MIC: Minimal inhibitory concentration, PAE: Post-antibiotic effect, defined as the continued suppression of bacterial growth after the gentamicin concentration falls below the MIC For effective treatment of gram-negative infections, target ratios include Cmax/MIC ≥8-10 and AUC/MIC ≥ 70-100. A C_{min} <2 mg/L is linked to a decreased risk of nephrotoxicity.

Neeli et al.[20] used Bayesian PK modelling and Monte Carlo simulations to evaluate gentamicin dosing in critically ill neonates. They found that increasing the dose from 4 to 5-6 mg/kg/day enhanced the likelihood of achieving both peak (C_{max}) and (AUC0-24) targets, underscoring the need for individualized dosing in this population.

Similarly, Gastine et al.[21] used PK/PD modelling to evaluate gentamicin in neonatal sepsis. The study revealed that standard dosing regimens were inadequate for *Enterobacteriaceae*, prompting a need for alternative therapies to ensure effective empirical coverage.[21]

Matcha et al.[22] validated a two-compartment PK model for amikacin in term-neonates, identifying creatinine clearance and body weight as critical covariates. This model enabled the development of dosing nomograms for neonates with varying renal function, ensuring safe and effective therapy.

Pharmacokinetic Software in Therapeutic Drug Monitoring

PK software has become indispensable in TDM, particularly within the framework of model-informed precision dosing (MIPD). A recent review identified 28 MIPD software tools, of which 18 are actively used. These tools, predominantly based on Bayesian methods, offer population models to guide dosing decisions. However, the evidence supporting their clinical utility remains limited, underscoring the need for further standardization and validation, especially in neonates. Of the 19 selected MIPD tools, 13 are web-based, with several (e.g., Autokinetics, MwPharm, PrecisePK, and RxStudio) also available as desktop versions. Tools such as DoseMeRx, MwPharm, and RxStudio offer mobile

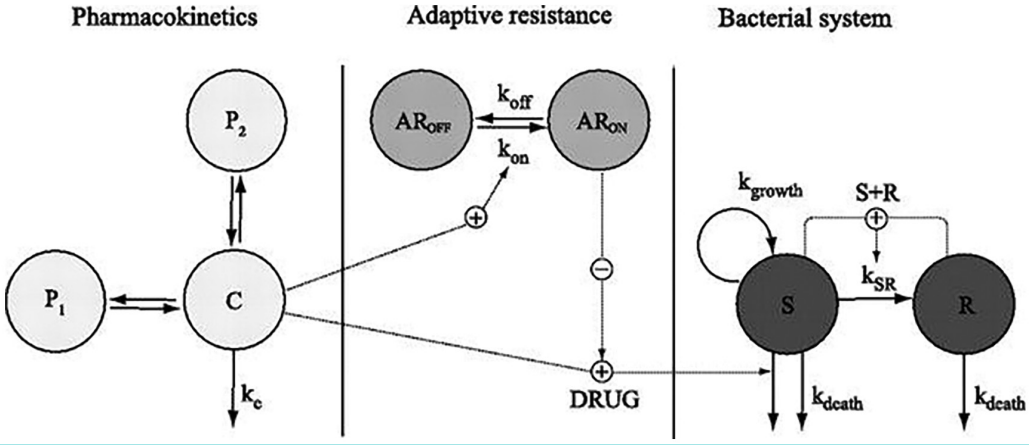

Figure 2. Schematic Illustration of the final PK/PD model the final PK/PD model comprises the following components.[11]

Bacterial compartments: susceptible compartment (S), contains proliferating and drug-susceptible bacteria, with growth occurring at a first-order rate constant. Resting compartment (R): it contains resting and drug-insusceptible bacteria. The transfer from S to R is stimulated by the total bacterial content when it exceeds a certain threshold, with a transfer rate constant (kSR).

Bacterial dynamics: both compartments experience natural bacterial death at a first-order rate constant (kdeath). The central compartment is responsible for bacterial killing, driven by a first-order elimination rate constant (ke).

Pharmacodynamic (PD) model: adaptive resistance (AR): includes a binding model with a development rate constant (kon) for AR, stimulated by gentamicin concentration, and a return rate constant (koff) for restoring susceptibility. AR reduces the maximum bacterial killing effect of gentamicin (E_{max}).

Model structure: *in vitro* experiments: utilized a two-compartment model with one peripheral compartment (P1). Predictions: applied a three-compartment model with two peripheral compartments (P1, P2).

This model captures the dynamics of bacterial growth, resistance development, and the effects of gentamicin, providing a framework for optimizing dosing strategies.

PK: Pharmacokinetic

compatibility, enhancing their usability in diverse clinical settings. These advancements highlight the growing importance of integrating MIPD tools into routine neonatal care to optimize antibiotic therapy and improve clinical outcomes.[23]

CONCLUSION

PK/PD modelling has improved AG dosing in neonates, but gaps remain, especially in complex conditions like neonatal sepsis and preterm birth. Current models often rely on extrapolated adult data, overlooking neonatal-specific physiological differences like immature renal function and variable body composition. Traditional PK-PD indices, such as C_{max}/MIC, AUC/MIC, and T% > MIC, oversimplify the relationship between drug exposure and bacterial response, highlighting the need for more tailored approaches.

Despite advances in research, neonates, particularly preterm infants, are underrepresented in research. New PBPK-PD models show promise but still rely on adult data and simplified indices that don't capture neonatal complexity. The integration of biosensors, artificial intelligence, and less invasive sampling methods, such as dried blood spots, may revolutionize dosing strategies and improve therapeutic outcomes. However, challenges in data accuracy and clinical integration remain.

MAIN POINTS

- Pharmacokinetic/pharmacodynamic models: These help adjust aminoglycoside doses in neonates, optimizing effectiveness and minimizing toxicity.
- Drug level monitoring: Regular monitoring reduces the risk of renal and ototoxicity by keeping drug levels within a safe range.
- Model limitations: Current models don't fully account for factors like infection, gestational age, and weight, which affect drug metabolism.
- Less invasive monitoring: Urine tests or spot samples could simplify monitoring for neonates, reducing discomfort.
- AI and biosensors: AI and biosensors could enable personalized, real-time treatment adjustments, improving safety and efficacy.

Footnotes

Authorship Contributions

Concept: A.S.A., K.A.Y.A., A.A.S., F.K.A., S.A.A., A.S.M., Literature Search: A.S.A., K.A.Y.A., A.A.S., F.K.A., S.A.A., A.S.M., Writing: A.S.A., K.A.Y.A., A.A.S., F.K.A., S.A.A., A.S.M.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Mian P, Flint RB, Tibboel D, van den Anker JN, Allegaert K, Koch BCP. Therapeutic drug monitoring in neonates: what makes them unique? Curr Pharm Des. 2017; 23(38): 5790-800.
2. van den Anker JN, Allegaert K. Pharmacokinetics of aminoglycosides in the newborn. Curr Pharm Des. 2012.
3. Ali AS, Farouq MF, Al-Faify KA. Pharmacokinetic approach for optimizing gentamicin use in neonates during the first week of life. Indian J Pharmacol. 2012; 44(1): 36-40.
4. Hitron AE, Sun Y, Scarpace SB. Accuracy of empiric gentamicin dosing guidelines in neonates. J Pediatr Pharmacol Ther. 2010; 15(4): 264-73.
5. Pacifici GM, Marchini G. Clinical pharmacokinetics of gentamicin in neonates. Int J Pediatr. 2017; 5(3): 4575-99.
6. Hodiamont CJ, van den Broek AK, de Vroom SL, Prins JM, Mathôt RAA, van Hest RM, et al. Clinical pharmacokinetics of gentamicin in various patient populations and consequences for optimal dosing for gram-negative infections: an updated review. Clin Pharmacokinet. 2022; 61(8): 1075-94.
7. Willems J, Hermans E, Schelstraete P, Depuydt P, De Cock P. Optimizing the use of antibiotic agents in the pediatric intensive care unit: a narrative review. Paediatr Drugs. 2021; 23(1): 39-53.
8. Zazo H, Lagarejos E, Prado-Velasco M, Sánchez-Herrero S, Serna J, Rueda-Ferreiro A, et al. Physiologically-based pharmacokinetic modelling and dosing evaluation of gentamicin in neonates using PhysPK. Front Pharmacol. 2022; 13: 977372.
9. Sridharan K, Al Jufairi M, Qader AM, Elsegai OAM. Dose optimization of gentamicin in critically Ill neonates. Curr Drug Metab. 2020; 21(4): 270-80.
10. Moffett BS, Morris J, Galati M, Munoz FM, Arikan AA. Population pharmacokinetic analysis of gentamicin in pediatric extracorporeal membrane oxygenation. Ther Drug Monit. 2018; 40(5): 581-8.
11. Mohamed AF, Nielsen EI, Cars O, Friberg LE. Pharmacokinetic-pharmacodynamic model for gentamicin and its adaptive resistance with predictions of dosing schedules in newborn infants. Antimicrob Agents Chemother. 2012; 56(1): 179-88.
12. Caceres Guido P, Perez M, Halac A, Ferrari M, Ibarra M, Licciardone N, et al. Population pharmacokinetics of amikacin in patients with pediatric cystic fibrosis. Pediatr Pulmonol. 2019; 54(11): 1801-10.
13. Downes KJ, Grim A, Shanley L, Rubenstein RC, Zuppa AF, Gastonguay MR. A pharmacokinetic analysis of tobramycin in patients less than five years of age with cystic fibrosis: assessment of target attainment with extended-interval dosing through simulation. Antimicrob Agents Chemother. 2022; 66(5): e0237721.
14. Praet A, Bourguignon L, Vetele F, Breant V, Genestet C, Dumitrescu O, et al. Population pharmacokinetic modeling and dosing simulations of tobramycin in pediatric patients with cystic fibrosis. Antimicrob Agents Chemother. 2021; 65(10): e0073721.
15. Dewandel I, Allegaert K, Renard M, Laenen A, Smits A. Covariates of amikacin disposition in a large pediatric oncology cohort. Int J Clin Pharmacol Ther. 2021; 59(1): 31-41.
16. Llanos-Paez CC, Staatz CE, Lawson R, Hennig S. Differences in the pharmacokinetics of gentamicin between oncology and nononcology pediatric patients. Antimicrob Agents Chemother. 2020; 64(2): e01730-19.
17. De Cock RF, Allegaert K, Brussee JM, Sherwin CM, Mulla H, de Hoog M, et al. Simultaneous pharmacokinetic modeling of gentamicin, tobramycin and vancomycin clearance from neonates to adults: towards a semi-physiological function for maturation in glomerular filtration. Pharm Res. 2014; 31(10): 2643-54.
18. Cristea S, Smits A, Kulo A, Knibbe CAJ, van Weissenbruch M, Krekels EHJ, et al. Amikacin pharmacokinetics to optimize dosing in neonates with perinatal asphyxia treated with hypothermia. Antimicrob Agents Chemother. 2017; 61(12): e01282-17.

19. Cies JJ, Habib T, Bains V, Young M, Menkiti OR. Population pharmacokinetics of gentamicin in neonates with hypoxemic-ischemic encephalopathy receiving controlled hypothermia. Pharmacotherapy. 2018; 38(11): 1120-9.

20. Neeli H, Hanna N, Abduljalil K, Cusumano J, Taft DR. Application of physiologically based pharmacokinetic-pharmacodynamic modeling in preterm neonates to guide gentamicin dosing decisions and predict antibacterial effect. J Clin Pharmacol. 2021; 61(10): 1356-65.

21. Gastine S, Obiero C, Kane Z, Williams P, Readman J, Murunga S, et al. Simultaneous pharmacokinetic/pharmacodynamic (PKPD) assessment of ampicillin and gentamicin in the treatment of neonatal sepsis. J Antimicrob Chemother. 2022; 77(2): 448-56.

22. Matcha S, Dillibatcha J, Raju AP, Chaudhari BB, Moorkoth S, Lewis LE, et al. Predictive performance of population pharmacokinetic models for amikacin in term neonates. Paediatr Drugs. 2023; 25(3): 365-75.

23. Del Valle-Moreno P, Suarez-Casillas P, Mejías-Trueba M, Ciudad-Gutiérrez P, Guisado-Gil AB, Gil-Navarro MV, et al. Model-informed precision dosing software tools for dosage regimen individualization: a scoping review. Pharmaceutics. 2023; 15(7): 1859.

REVIEW

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An Overview of Biotin Interference Impact on Immunoassays

Alfatih Aboalbasher Yousif[1], Nagia Suliman Ahmed[2], Ebtehal Mohamed[3], Shiema Abdemagid[4], Omar Mustafa[5], Abdelmonem Mohamed[3], Alaa Ali[6], Ahmed Omer[3], Nafisa Hassan[7], Ahmed Al Barbary[8], Abdullah Said Alghazi[8], Yassir Hamadalnil[8]

[1]Department of Medical Laboratory Science, Oman College of Health Sciences, Ruwi, Oman
[2]Department of Clinical Chemistry, University of Gezira Faculty of Medical Laboratory Science, Wad Madani, Sudan
[3]Department of Clinical Chemistry, Al-Neelain University Faculty of Medical Laboratory Science, Khartoum, Sudan
[4]Department of Microbiology, University of Alzaiem Al Azhari Faculty of Medical laboratory science, Khartoum, Sudan
[5]Department of Histopathology and Cytology, University of Medical Science and Technology Faculty of Medical Laboratories Science, Khartoum, Sudan
[6]Department of Hematology and Immunohematology, Sudan University of Science and Technology Faculty of Medical Laboratory Science, Khartoum, Sudan
[7]Department of Clinical Chemistry, University of El imam El Mahdi Faculty of Medical Laboratory Science, Aba Island, Sudan
[8]Clinic of Medical Laboratory, Ibra Hospital, Ministry of Health, Ibra, Oman

Abstract

The interference of high concentrations of biotin in patients' serum with certain immunoassays can lead to inaccurately high or low test results, which may confuse physicians and contribute to misdiagnosis and inappropriate treatment. Laboratory investigations play a crucial role in clinical decision-making by enabling physicians to manage patient outcomes effectively. Therefore, ensuring the reliability of laboratory results is of utmost importance. This paper focuses on thyroid function tests (TFTs) and the cardiac troponin test as examples of biotin interference in analytes. TFTs are essential for the routine assessment of TFT, while troponin serves as a key biomarker for heart injuries, including myocardial infarction (MI). Accurate interpretation of troponin results is critical for MI treatment strategies. Further research is needed to evaluate biotin interference in detail for other diagnostic tests. This review outlines the mechanisms by which biotin interferes with biochemical assays, highlights its impact on laboratory test accuracy, and proposes potential solutions. In conclusion, implementing precautionary measures is essential to minimize the influence of biotin interference on biochemical analytes. Different immunoassay methods, whether streptavidin-based or non-streptavidin-based, should be assessed for their susceptibility to biotin interference. Additionally, raising awareness among medical professionals and patients about this issue would aid in the early detection and management of biotin-related assay inaccuracies.

Keywords: Biotin, analytes, interference, immunoassay

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ORCID IDs of the authors: A.A.Y. 0000-0001-8159-3191, N.S.A. 0000-0002-0072-9315, E.M. 0000-0003-2690-1248, S.A. 0000-0003-0090-1514, O.M. 0000-0002-2540-1877, A.M. 0000-0002-3925-5268, A.A. 0000-0002-7482-7065, A.O. 0000-0001-9035-5558, N.H. 0000-0002-2923-0198, Y.H. 0000-0001-9705-9495, A.A.B. 0009-0002-0166-2359, A.S.A. 0009-0000-5883-9675.

Corresponding author: Nagia Suliman Ahmed
E-mail: nagiasuliman@hotmail.com
ORCID ID: orcid.org/0000-0002-0072-9315

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INTRODUCTION

Automated immunoassays are analytical procedures in which the quantification of an analyte relies on signal responses generated by the interaction between an antibody and an antigen.[1] These assays are widely used in routine laboratory diagnostics for hormonal profiling due to their high sensitivity, precision, and specificity.[2] However, regardless of the manufacturer, most immunoassays may be affected by interfering substances. The extent of interference varies depending on the specific type of interferent involved. Common sources of interference include icterus, hemolysis, lipemia, and biotin.[3] With exogenous biotin being a particularly significant concern. Biotin interference has been recognized as a critical issue by the United State of America (USA) Food and Drug Administration (FDA), highlighting the need for clinicians to consider the potential impact of interfering substances on laboratory test results.[3] In November 2017, the FDA issued a warning statement regarding the increasing number of clinically misleading results, raising concerns about biotin's potential influence on laboratory assays.[4] Biotin, also known as vitamin B7 or vitamin H, is a water-soluble vitamin that plays an essential role in several metabolic pathways, including carbohydrate, lipid, and protein metabolism.[5] Naturally, biotin has a molecular weight of 240 Da and is present in a wide range of foods, such as eggs, beef, fish, liver, pork, whole grains, soybeans, and green leafy vegetables. It is also available as a nutritional supplement and is used in certain medications for conditions such as diabetes, lipid disorders, biotinidase deficiency, peripheral neuropathy, and carboxylase deficiencies.[6,7] Additionally, biotin is commonly taken for cosmetic purposes, particularly to promote hair and nail growth. The recommended average daily intake of biotin is approximately 0.03 mg, a level that does not typically interfere with immunoassays, with blood concentrations ranging between 0.1 and 0.8 ng/mL.[7] However, due to its low molecular weight and strong affinity for streptavidin, biotin forms a stable bond with streptavidin in immunoassay analyzers. This binding mechanism allows for the detection of very small quantities of analytes in biological samples.[8] Biotin interference can lead to unexplained high or low test results, causing confusion among clinicians and laboratory professionals.[9] Consequently, biotin interference is emerging as a significant issue that, if left unaddressed, may result in serious clinical consequences, including potential misdiagnosis.[10] Interference typically occurs when serum biotin concentrations exceed 10 ng/mL, which is more than ten times the established upper limit of normal blood levels.[11] In this context, we have outlined the mechanisms by which biotin interferes with immunoassays, demonstrated its impact on biochemical tests, and proposed potential solutions to mitigate its effects.

Principle of Biotin Streptavidin Binding in Immunoassay Analyzers

Chemiluminescence immunoassay analyzers, such as the Roche Elecsys, Ortho Clinical Diagnostics VITROS, Beckman Coulter Access/DXI, and Siemens Centaur/IMMULITE/Dimension, are widely used for biochemical tests, including hormone assays, tumor markers, and therapeutic drug monitoring.[12,13] In addition to the traditional antibody-antigen system, immunoassays have incorporated other high-affinity interactions, such as the biotin-streptavidin (BAS) system, into immobilization design strategies to enhance specificity.[14] The principles of streptavidin-biotin-based immunoassays are extensively discussed in the literature. Avidins are a family of biotin-binding proteins found in both eukaryotic and prokaryotic organisms.[15] Avidin was first isolated from chicken egg whites and was named "avidin" due to its strong affinity for biotin, deriving from the combination of the words "avidity" and "biotin".[16] Streptavidin, on the other hand, was first extracted from *Streptomyces avidinii*, a bacterium known for secreting antibiotics. Both avidin and streptavidin are tetrameric proteins that share structural and functional similarities, each possessing four binding sites for biotin.[16] The additional amino acids, 12 in avidin and 8 in streptavidin, respectively, each play a crucial role in their binding strength. The BAS interaction is considered one of the most specific and stable noncovalent interactions known. Its dissociation constant (**KD**) is approximately 10^3-10^6 times lower than that of typical antigen-antibody complexes, indicating a significantly stronger interaction. For instance, the affinity constant (KD) of BAS is in the range of 10^{14}-10^{15} M^{-1}, whereas that of monoclonal antibodies typically ranges from 10^7 to 10^{11} M^{-1}. This exceptional affinity is particularly beneficial for isolating and amplifying signals, thereby enhancing the sensitivity of immunoassays for detecting extremely low analyte concentrations.[1]

The Mechanism of Biotin Interference in Immunoassay Analyzers

Excess biotin in a sample can interfere with immunoassays by preventing the target substance from binding to streptavidin-coated magnetic beads.[17] Immunoassay analyzers primarily implement two analytical approaches: the competitive assay, used for detecting small molecules, and the non-competitive (sandwich) assay, used for detecting larger molecules. The competitive immunoassay is typically applied to small hormone molecules and antibodies, including vitamin D_3, thyroxine (T4), triiodothyronine (T3), steroid hormones, thyroid-stimulating hormone (TSH) thyrotropin receptor antibody (TRAb), a-thyroid peroxidase antibody (a-TPO), and thyroglobulin antibody (a-Tg).[18] For example, in the case of T4 measurement, an excessive amount of biotin in the sample can saturate the streptavidin-binding sites, preventing streptavidin from capturing the biotinylated analyte. This leads to reduced T4-antibody complex formation on the solid phase, resulting in a falsely elevated free T4 level, a phenomenon known as pseudo-hyperthyroidism.[19] The non-competitive (sandwich) immunoassay is used for detecting larger molecules such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), TSH, human chorionic gonadotropin (HCG), parathyroid hormone (PTH), Tg, and C-peptide 2.[18] In this assay, a biotinylated antibody binds to streptavidin and the analyte of interest, which is sandwiched between the biotinylated antibody and a signal antibody. The signal antibody is labeled with an enzyme or molecule to enhance signal detection. Under normal conditions, the signal intensity increases in direct proportion to the analyte concentration. However, when an excessive amount of biotin is present in the sample, it competes for streptavidin-binding sites, preventing the proper formation of the antigen-antibody complex and leading to potential assay interference.[19] See Figure 1.

Influence of Biotin Interference on Some Biochemical Tests

The specific biotin dosage that causes interference varies across different immunoassay platforms, as reported in previously published studies. Thresholds for biotin interference differ significantly among manufacturers, ranging from 2.5 to 10,000 ng/mL.[19] Individual immunoassays exhibit varying degrees of sensitivity to biotin interference. For example, one study examined the impact of biotin on biochemical parameters, including free T3, free T4, PTH, TSH, vitamin D, prolactin, LH, FSH, and C-peptide. Participants received different biotin dosages (25-300 mg), and analyte levels were compared before and after biotin elimination. Additionally, the same parameters were measured

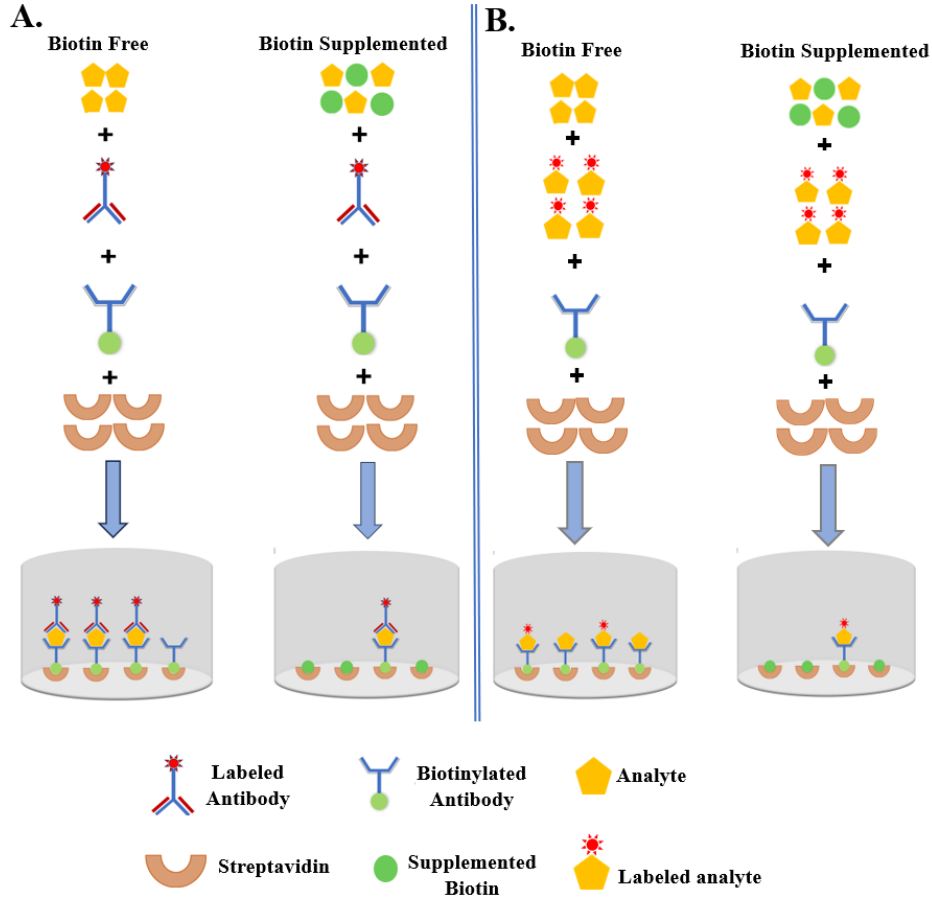

Figure 1. Shows biotin interference mechanism: (A) non-competitive (sandwich) immunoassay; (B) competitive immunoassays.

using an alternative assay without using the BAS technique to confirm the findings. The study revealed that most results were abnormal in participants taking more than 100 mg/day of biotin, with the degree of abnormality proportional to serum biotin concentration. Furthermore, analyte levels that exceeded normal reference ranges returned to normal after biotin elimination, confirming that biotin was the primary cause of the interference.[13] Biotin is primarily excreted through the kidneys; therefore, its concentration in urine may also interfere with certain analytes. For instance, one study reported that in patients undergoing urine HCG testing, biotin supplementation should be considered if repeated testing using an immunoassay device yields invalid results.[20] The following sections will discuss thyroid function tests (TFTs) and cardiac troponin (cTn) assays as examples of biotin interference in clinical analytes. TFTs are essential parameters for assessing TF, while troponin is a key biomarker for cardiac injury, including myocardial infarction (MI). Accurate interpretation of troponin levels is crucial for MI management. Further studies are needed to evaluate biotin interference in other tests, including natriuretic peptides, prostate-specific antigen, alpha-fetoprotein, vitamin B12, and so on.

Biotin Interference in Thyroid Function Test

TFT, including TSH, free T3, and free T4, along with thyroid antibodies, is a frontline parameter in the routine assessment of TF. Several studies have indicated that consuming 10 mg of biotin daily may interfere with the measurement of T4, T3, and TSH, leading to inaccurate results that could result in the misdiagnosis of hyperthyroidism or hypothyroidism.[21,22] For example, when TSH is measured using a sandwich assay technique, an excessive amount of biotin in the sample can cause signal suppression. This occurs because biotin saturation of streptavidin-binding sites reduces the attachment of immune complexes to the solid phase, leading to an artificially decreased TSH level.[23] In contrast, T3 and T4 are measured using competitive binding assays, where both endogenous and labeled analytes compete for antibody-binding sites. Higher biotin concentrations can result in falsely elevated T3 and T4 levels because biotin inhibits the binding of both the labeled analyte and the endogenous analyte to the streptavidin-coated solid phase. During the washing step, unbound antibodies are removed, eliminating any signal representing the endogenous analyte concentration. As a result, the serum concentration of the endogenous analyte becomes inversely proportional to signal intensity, leading to an artificially elevated T3 and T4 measurement.[24] Several experimental studies have evaluated biotin interference with thyroid hormone measurements across different immunoassay platforms. For instance, a study conducted by Odhaib et al.[2] examined the correlation between thyroid test results and biotin dosage using the Snibe Maglumi 800 and Roche Cobas e411 immunoassay systems. The study found that participants who ingested 20 mg or more of biotin showed altered thyroid test results. Some of the TFT findings from this study are presented in Table 1.

Table 1. Shows the results of certain thyroid parameters during and after discontinuing biotin intake (patients took ≤20 mg of biotin as prescribed)

Analyte	Unit	Result with biotin intake	Result after biotin stopped (48 hrs)	Reference range
TSH	µIU/mL	0.05	1.44	0.27-4.2
		0.04	1.11	
		<0.05	6.34	
FT4	ng/dL	2.93	1.08	0.9-1.7
		3.23	2.30	
		5.03	1.42	
FT3	pg/mL	6.09	2.31	1.21-4.18
		/	2.24	
		12.11	2.15	
Anti-TPO	IU/mL	6.33	/	0-34
		3.22	/	
		8.77	/	
TRAb	mIU/mL	4.74	/	<2
		0.68	/	
		9.3	3.32	

/: Not given. TSH: Thyroid-stimulating hormone, FT4: Free thyroxine, FT3: Free triiodothyronine, Anti-TPO: Antithyroid peroxidase, TRAb: Thyrotropin receptor antibody.

Although some parameter values in Table 1 were not provided, the findings clearly demonstrate the interference of biotin with thyroid analytes. This interference was eliminated once biotin intake was discontinued. However, in the case of TPO and TSH TRAb, a longer period of biotin withdrawal may be required before levels return to the normal range.[2] Similarly, a study, conducted by James et al.[25], measured TSH, free T4, and free T3 in a patient taking a high dose of biotin (10,000 µg) as a prescription supplement. The initial thyroid profile results were as follows: TSH=0.03 mIU/L; free T4>8.0 ng/dL; and free T3>30.0 pg/mL. However, after discontinuing biotin for five days, the results normalized to TSH=1.66 µg/dL, free T4=0.86 ng/dL, and free T3=2.9 pg/mL. The normal reference ranges for these parameters are TSH (0.4-4.0 µg/dL), free T4 (0.9-1.7 ng/dL), and free T3 (2.3-4.1 pg/mL). This provides further evidence of biotin interference in immunoassay analyzers and the potential misinterpretation of TFT results. Furthermore, a study by Li et al.[26] evaluated biotin interference using both immunoassay methods: sandwich assays for TSH, and competitive assays for free T4 (FT4), and free T3 (FT3). Various biotin concentrations (31.25-1000 ng/mL) were introduced into serum samples, and analytes were measured using the Roche Cobas 8000 e602 system. The study found that serum biotin concentrations were positively correlated with interference levels in some assays. At a biotin concentration of 250 ng/mL, the TSH level (1.65 µIU/mL) was reduced by 12.42%, while FT4, and FT3 levels were falsely elevated. Additionally, an artificial increase in TRAb titers has been reported.[27] Another study investigated biotin interference in TFTs using the Beckman UniCel DxI 800 and Roche Cobas e602 analyzers. The study found that biotin interference was present in some, but not all, thyroid parameters. Notably, interference was more pronounced in the Beckman UniCel DxI 800 system, compared to the Roche Cobas e602. For example, FT3, FT4, and total T3 levels were significantly higher in the Beckman DxI 800 system, whereas anti-TSHR, anti-TPO, and anti-Tg levels were more affected in the Roche Cobas e602 system. However, total T4 levels remained unaffected at biotin doses of up to 5 mg in the Roche Cobas e602 analyzer.[28] These findings underscore the importance of considering biotin-induced interference when monitoring TF, particularly in asymptomatic patients.

Interference of Biotin in Cardiac Troponin

cTn I (cTnI) and cTn T (cTnT) are widely recognized as the gold-standard biomarkers for diagnosing acute MI (AMI).[29] Troponin is among the clinical laboratory tests most susceptible to biotin interference[30], which can lead to falsely elevated or decreased results. For instance, sandwich immunoassays are commonly used to detect cTns, utilizing two antibodies: a biotinylated capture antibody and a detection antibody coupled to a target molecule to form cTn-antibody complexes. These complexes bind to immobilized streptavidin. In cTn assays based on this method, excess biotin can saturate streptavidin, preventing the binding of cTn-antibody complexes and leading to a falsely decreased cTn concentration. This interference can result in missed AMI diagnoses due to falsely reduced troponin levels.[31] Previous studies have reported falsely decreased troponin levels in patients taking high-dose biotin supplements. For example, one study observed that at a biotin concentration of 250 ng/mL, the measured high-sensitivity cTn T (hs-cTnT) level of 54.14 ng/L decreased by 13.77 ng/L.[25] However, evaluations of the fifth-generation troponin T assay indicated no interference at biotin concentrations up to 20 µg/L.[32] A study assessing biotin interference (>20.0 ng/mL) in the TnT Gen 5 assay (known as "Elecsys Troponin T-high sensitivity" outside the USA) concluded that the likelihood of false-negative AMI diagnoses due to biotin interference was very low.[33] Similarly, a cohort study by Mumma et al.[34] demonstrated that no biotin interference was detected at concentrations below 20.0 ng/mL in the Elecsys Troponin T-Gen 5 assay. Additionally,Nguyen et al.[35] investigated the risk of biotin interference in samples tested for hs-cTnT. Biotin concentrations in patient samples ranged from 0.02 ng/mL to 11.38 ng/mL. The study concluded that the risk of biotin interference in hs-cTnT results was minimal due to the low circulating biotin levels (<20 ng/mL). Furthermore, a study by Vroemen et al.[36] assessed biotin interference in hs-cTnT immunoassays in an acute cardiac unit using Roche Diagnostics analyzers. After removing biotin with streptavidin-coated magnetic microparticles, no significant difference was observed in the measured hs-cTnT levels when comparing values before and

after biotin removal [11.8 (5.6-24.2) ng/L vs. 11.8 (5.6-24.1) ng/L]. These findings suggest that the impact of biotin interference on troponin assays is assay-dependent. Therefore, clinicians should consider assay-specific variations when interpreting troponin results in patients taking biotin supplements.

Some Techniques to Mitigate Biotin Interference

The sensitivity of an assay to biotin interference largely depends on the assay design, particularly when using the BAS system. Immunoassays that apply preformed principles are less susceptible to biotin interference, typically exhibiting a bias of less than 10%, which is considered acceptable.[37] In preformed assays, streptavidin-coated beads are pre-conjugated with a biotinylated antibody during the manufacturing process, forming a stable complex before the patient sample is introduced into the assay. Due to the strong BAS interaction, the biotinylated bond is not easily broken, ensuring that excess serum biotin does not interfere with the binding of the capture antibody or antigen to its target analyte. Siemens platforms rely on preformed assays in their immunoassay machines.[38] In contrast, immunoassay platforms that depend on non-preformed assay designs are more susceptible to biotin interference. This is because the formation of the biotinylated antibody complex occurs after the addition of the patient sample, exposing the antibody complex to excess biotin present in the sample. Elevated serum biotin levels can competitively bind to the streptavidin-coated beads, potentially inhibiting the binding of the biotinylated capture antibody or antigen to its target analyte, leading to false results.[39] Some researchers propose substituting the BAS complex with other immune complexes. For example, the fluorescein isothiocyanate (FITC)-anti-FITC technique utilizes the interaction between an FITC label and an anti-FITC antibody for immobilization and analyte detection. Although this approach is similar to the BAS complex, FITC exhibits a lower affinity and reduced nonspecific binding compared to BAS interactions.[1] With growing awareness of biotin interference, immunoassay manufacturers are working to eliminate this issue in their platforms. For instance, Roche has introduced a high-sensitivity troponin T/TSH test kit designed to resist biotin interference at concentrations up to 1.2×10^6 pg/mL, which may improve immunoassay reliability.[40] Due to variations in signal detection methodologies, different analytical platforms exhibit varying degrees of sensitivity to biotin interference. The Abbott Architect system, which does not rely on the BAS capture principle for analyte detection, is a suitable option for patient testing regardless of biotin withdrawal. In contrast, the Roche Cobas e602 system is advised after the biotin supplementation discontinuation at doses of 5-10 mg/day for a minimum of 24 hours, while the Beckman UniCel DxI 800 platform requires a cessation period of at least 48 hours to minimize potential interference.[28] Acridinium ester immunoassays that utilize the BAS design are unaffected by biotin up to 1200 ng/mL and have been reassessed for risk of biotin interference up to a level of 3500 ng/mL.[41] Serial dilution, testing on a different platform, or stopping biotin supplementation (Roche Diagnostics, which recommends discontinuing biotin for at least 8 hours) is a possible option to avoid interference. Patients with renal impairment may require more than 48 hours for biotin clearance, as biotin is primarily excreted through the kidneys, and retesting after clearance is recommended.[42] Direct biotin measurement is also recommended for confirmation when assessing interference.[7] However, these methods have limitations, including time consumption, potential dilution errors, and the need for reference laboratory testing. To date, there is no conclusive approach to completely eliminate biotin interference; therefore, laboratories may apply one of the aforementioned techniques to confirm suspected biotin interference.

CONCLUSION

Biotin interference in certain immunoassays can lead to inaccurate results, potentially causing misdiagnosis and inappropriate therapy. Therefore, precautionary measures are essential to minimize the impact of biotin on biological analytes during analysis. To fully understand the extent of biotin interference, several factors must be considered, including biotin concentration, immunoassay principles, analyte type, and the dosage at which interference occurs. These factors highlight the need for a prompt and precise analytical method to assess biotin interference, particularly when immunoassay results do not align with clinical findings. Although there is an urgent need for a rapid analytical procedure to accurately measure biotin interference, the complete replacement of current immunoassay analyzers may take several years. In the meantime, patients are advised to discontinue biotin supplementation before undergoing certain laboratory tests, for at least 48 hours or even longer, depending on the specific test, dosage, and frequency of biotin intake to prevent misinterpretation of results.

MAIN POINTS

- Patients should discontinue the use of biotin supplements 1 to 2 days before laboratory tests for low doses and 3 to 7 days for high doses.
- Laboratories must determine the minimum biotin concentration that could result in clinically significant interference in their assays.
- The patient's medical history and supplement consumption should be thoroughly documented prior to conducting the tests.
- In emergencies or for unconscious patients, it is preferable to use methods with lower biotin interference for troponin testing.
- Clinicians must establish a correlation between clinical findings and laboratory results for patients who are reported to be taking biotin in doses over 5 mg.

Footnotes

Authorship Contributions

Concept: A.M., A.A., Design: N.S.A., A.A., A.O., Data Collection and/or Processing: S.A., O.M., Analysis and/or Interpretation: N.H., Y.H., A.A.B., A.S.A., Literature Search: A.A.Y., E.M., S.A., Writing: A.A.Y., E.M., S.A.

DISCLOSURES

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REFERENCES

1. Karachaliou CE, Livaniou E. Biotin homeostasis and human disorders: recent findings and perspectives. Int J Mol Sci. 2024; 25(12): 6578.
2. Odhaib SA, Mansour AA, Haddad NS. How biotin induces misleading results in thyroid bioassays: case series. Cureus. 2019; 11(5): e4727.
3. Rosner I, Rogers E, Maddrey A, Goldberg DM. Clinically significant lab errors due to vitamin B7 (biotin) supplementation: a case report following a recent FDA warning. Cureus. 2019; 11: e5470.
4. FDA (2019). Reminds patients, health care professionals and laboratory personnel about the potential for biotin interference with certain test results, especially specific tests to aid in heart attack diagnoses. Available from: https://www.fda.gov/news-events/fda-brief/fda-brief-fda-reminds-patients-health-care-professionals-and-laboratory-personnel-about-potential#
5. Piketty ML, Prie D, Sedel F, Bernard D, Hercend C, Chanson P, et al. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. Clin Chem Lab Med. 2017; 55(6): 817-25.
6. Li D, Ferguson A, Cervinski MA, Lynch KL, Kyle PB. AACC Guidance document on biotin interference in laboratory tests. J Appl Lab Med. 2020; 5(3): 575-87.
7. NCBI(2023).BiotinDeficiency. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547751/
8. Ostrowska M, Bartoszewicz Z, Bednarczuk T, Walczak K, Zgliczyński W, Glinicki P. The effect of biotin interference on the results of blood hormone assays. Endokrynol Pol. 2019; 70(1): 102-21.
9. Kabiri P, Weiskirchen R, van Helden J. The biotin interference within interference suppressed immunoassays. J Clin Lab Anal. 2021; 35(9): e23940.
10. Luong JHT, Vashist SK. Chemistry of biotin-streptavidin and the growing concern of an emerging biotin interference in clinical immunoassays. ACS Omega. 2020; 5(1): 10-8.
11. Katzman BM, Lueke AJ, Donato LJ, Jaffe AS, Baumann NA. Prevalence of biotin supplement usage in outpatients and plasma biotin concentrations in patients presenting to the emergency department. Clin Biochem. 2018; 60: 11-6.
12. Mrosewski I, Urbank M, Stauch T, Switkowski R. Interference from high-dose biotin intake in immunoassays for potentially time-critical analytes by roche. Arch Pathol Lab Med. 2020; 144(9): 1108-17.
13. Colon PJ, Greene DN. Biotin interference in clinical immunoassays. J Appl Lab Med. 2018; 2(6): 941-51.
14. Balzer AHA, Whitehurst CB. An analysis of the biotin-(strept)avidin system in immunoassays: interference and Mmtigation strategies. Curr Issues Mol Biol. 2023; 45(11): 8733-54.
15. Laitinen OH, Kuusela TP, Kukkurainen S, Nurminen A, Sinkkonen A, Hytönen VP. Bacterial avidins are a widely distributed protein family in Actinobacteria, Proteobacteria and Bacteroidetes. BMC Ecol Evol. 2021; 21(1): 53.
16. Meir A, Bayer EA, Livnah O. Structural adaptation of a thermostable biotin-binding protein in a psychrophilic environment. J Biol Chem. 2012; 287(22): 17951-62.
17. Liu D, Gebreab YB, Hu J, Zhou L, Zhang N, Tong H, et al. Development and evaluation of an anti-biotin interference method in biotin-streptavidin immunoassays. Diagnostics (Basel). 2022; 12(7): 1729.
18. İşbilen Başok B. Understanding and managing biotin interference in immunoassays. Int J Med Biochem. 2020; 3(1): 40-3.
19. Bowen R, Benavides R, Colón-Franco JM, Katzman BM, Muthukumar A, Sadrzadeh H, et al. Best practices in mitigating the risk of biotin interference with laboratory testing. Clin Biochem. 2019; 74: 1-11.
20. Williams GR, Cervinski MA, Nerenz RD. Assessment of biotin interference with qualitative point-of-care hCG test devices. Clin Biochem. 2018; 53: 168-70.
21. Ylli D, Soldin SJ, Stolze B, Wei B, Nigussie G, Nguyen H, et al. Biotin interference in assays for thyroid hormones, thyrotropin and thyroglobulin. Thyroid. 2021; 31(8): 1160-70.
22. Lim SK, Pilon A, Guéchot J. Biotin interferes with free thyroid hormone and thyroglobulin, but not TSH measurements using Beckman-Access immunoassays. Ann Endocrinol (Paris). 2017; 78(3): 186-7.
23. Angelopoulos N, Paparodis RD, Androulakis I, Anagnostis P, Boniakos A, Duntas L, et al. The clinical significance of low dose biotin supplements (<300μg/day) in the treatment of patients with hypothyroidism: crucial or overestimated? Thyroid Res. 2023; 16(1): 18.
24. HAS (2023). Biotin interference with thyroid function tests. Available from: https://www.hsa.gov.sg/announcements/safety-alert/biotin-interference-with-thyroid-function-tests [Accessed 21 Sep 2023]
25. James A, Stalan J, Kuzhively J. Biotin induced biochemical hyperthyroidism: a case report and review of the literature. J Med Case Rep. 2023; 17(1): 266.
26. Li J, Wagar EA, Meng QH. Comprehensive assessment of biotin interference in immunoassays. Clin Chim Acta. 2018; 487: 293-8.
27. Simó-Guerrero O, Giménez-Pérez G, Recasens-Gracia A, Villà-Blasco C, Castells-Fusté I. False overt hyperthyroidism by interference in immunoassay. Endocrinol Nutr. 2016; 63(8): 431-2.
28. Zhang Y, Wang R, Dong Y, Huang G, Ji B, Wang Q. Assessment of biotin interference in thyroid function tests. Medicine (Baltimore). 2020; 99(9): e19232.
29. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic disease. Cardiovasc Res. 2017; 113(14): 1708-18.
30. Saenger AK, Jaffe AS, Body R, Collinson PO, Kavsak PA, Lam CSP, et al. Cardiac troponin and natriuretic peptide analytical interferences from hemolysis and biotin: educational aids from the IFCC Committee on Cardiac Biomarkers (IFCC C-CB). Clin Chem Lab Med. 2019; 57(5): 633-40.
31. Frame IJ, Joshi PH, Mwangi C, Gunsolus I, De Lemos JA, Das SR, et al. Susceptibility of cardiac troponin assays to biotin interference. Am J Clin Pathol. 2019; 151(5): 486-93.
32. Mair J, Hammarsten O. Potential analytical interferences in cardiac troponin immunoassays. J Lab Precis Med. 2023; 8: 12.
33. Collinson P. Biotin interference in cardiac troponin immunoassay - where the wild things are? Clin Chem Lab Med. 2020; 58(11): 1769-71.
34. Mumma B, Diercks D, Twerenbold R, Valcour A, Ziegler A, Schützenmeister A, et al. Clinical risk assessment of biotin interference with a high-sensitivity cardiac troponin T assay. Clin Chem Lab Med. 2020; 58(11): 1931-40.
35. Nguyen KQN, Langevin R, Fankhauser K, Hashim IA. Assessment of risk for interference by circulating biotin in samples received for high sensitivity troponin-T, thyrotropin, and for prostate specific antigen testing by immunoassays. Clin Lab. 2020; 66(1).
36. Vroemen WHM, van Doorn WPTM, Kimenai DM, Wodzig WKWH, de Boer D, Bekers O, et al. Biotin interference in high-sensitivity cardiac troponin T testing: a real-world evaluation in acute cardiac care. Cardiovasc Res. 2019; 115(14): 1950-1.
37. Holmes EW, Samarasinghe S, Emanuele MA, Meah F. Biotin interference in clinical immunoassays: a cause for concern. Arch Pathol Lab Med. 2017; 141: 1459-60.
38. Avery G. Biotin interference in immunoassay: a review for the laboratory scientist. Ann Clin Biochem. 2019; 56: 424-30.
39. Sedel F, Papeix C, Bellanger A, Touitou V, Lebrun-Frenay C, Galanaud D, et al. High doses of biotin in chronic progressive multiple sclerosis: a pilot study. Mult Scler Relat Disord. 2015; 4(2): 159-69.

40. Petrucelli NF, D'Ambrosio C, Krouse J. Interference in immunoassays: a review of mechanisms and prevention. J Clin Lab Anal. 2018; 32(4): e22343.

41. Garg U. Biotin interference in certain immunoassays: risk of misdiagnosis and mismanagement. J Appl Lab Med. 2020; 5(3): 436-9.

42. Mzougui S, Favresse J, Soleimani R, Fillée C, Gruson D. Biotin interference: evaluation of a new generation of electrochemiluminescent immunoassays for high-sensitive troponin T and thyroid-stimulating hormone testing. Clin Chem Lab Med. 2020; 58(12): 2037-45.

RESEARCH ARTICLE

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Determination of CTLA-4 Levels in Placenta Tissue of Pregnant Women with Preeclampsia and Smoking Pregnant Women with Preeclampsia

Şükran Yediel Aras[1], Berrin Göktuğ Kadıoğlu[2], Arzu Gezer[3], Sevda Eliş Yıldız[1], Gizem Fidan Arslan[4], Ebru Karadağ Sarı[5]

[1]Department of Midwifery, Kafkas University Faculty of Health Sciences, Kars, Türkiye
[2]Clinic of Obstetrics and Gynecology, University of Health Sciences Türkiye, Erzurum Regional Training and Research Hospital, Erzurum, Türkiye
[3]Department of Histology and Embryology, Ataürk University Faculty of Health Services, Erzurum, Türkiye
[4]Kafkas University, Health Sciences Institute, Kars, Türkiye
[5]Department of Histology and Embryology, Kafkas University Faculty of Veterinary Medicine, Kars, Türkiye

Abstract

BACKGROUND/AIMS: In this study, the determination of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) levels in placenta tissue of pregnant women with preeclampsia and smoking pregnant women with preeclampsia was investigated using histological and immunohistochemical methods.

MATERIALS AND METHODS: Placenta tissues of 28 pregnant women were used in the study. The groups were formed into the categories of control, smoking, preeclampsia, and preeclampsia + smoking. Tissue samples taken at the end of delivery were fixed in 10% formalin, subjected to standard histological processing, and blocked in paraffin. Crossman's trichrome and haematoxylin-eosin staining was performed on sections taken from paraffin blocks. Immunohistochemical methods were applied to determine CTLA-4 immunoreactivity in placental tissues.

RESULTS: In the groups of smoking, preeclampsia, preeclampsia + smoking, changes such as: a decreased villous tree, congestion in the villi, and deposition of fibrin in the decidua were determined. In addition, different levels of CTLA-4 immunoreactivity were ascertained in the placental tissue and amniotic epithelium of all groups. The intensity of immunoreactivity in decidua cells and stem villi was identified to decrease in other groups compared to the control group.

CONCLUSION: It was thought that maternal immune system responses and histopathological changes in placenta tissue may cause decreased CTLA-4 immunoreactivity in smoking, preeclampsia and preeclampsia + smoking groups.

Keywords: CTLA-4, placenta, preeclampsia, pregnant, smoking, women

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ORCID IDs of the authors: Ş.Y.A. 0000-0002-3267-5251; B.G.K. 0000-0002-8712-318X; A.G. 0000-0002-1658-2098; S.E.Y. 0000-0002-3585-6648; G.F.A. 0000-0002-4316-5900; E.K.S. 0000-0001-7581-6109.

Corresponding author: Şükran Yediel Aras
E-mail: s.yediel@hotmail.com
ORCID ID: orcid.org/0000-0002-3267-5251

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INTRODUCTION

The placenta is an active and complex organ that contributes to the development and nutrition of the embryo, supports fetal and maternal immunotolerance, and has a wide variety of morphological variations among mammals.[1,2] Hypertensive disorders, one of the common complications in pregnancy, lead to serious negative consequences for both the mother and the fetus. Preeclampsia is one of the most important hypertensive disorders affecting 5-7% of all pregnancies, and preeclampsia causes an average of 70,000 maternal and 500,000 newborn deaths worldwide each year.[3] It is thought that immune system irregularities may play a role in the appearance of preeclampsia, regardless of the degree of placental abnormality or if it is early-onset or late-onset.[4,5] Smoking is the leading cause of preventable morbidity and mortality worldwide. More than five million premature deaths occur from smoking-related causes worldwide each year. This number is expected to reach eight million by 2030.[6] Smoking during pregnancy primarily affects the placenta, causing decreased blood flow to it and inhibiting the intrauterine growth of the fetus, thereby resulting in the birth of low birth weight babies. It also increases perinatal mortality.[7,8]

In general terms, the immune system, uses general and special defense mechanisms to resist foreign substances entering or given to the body, to protect itself and to destroy the harmful substance.[9] Negative costimulatory molecules are needed for the immune system to function in a balanced manner. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) plays a role in many immune control points, maintenance of tolerance of peripheral T-lymphocytes, prevention of autoimmunity, and the suppression of inflammation.[10,11] The *CTLA-4* gene is found in the chromosome 2q33 region. CTLA-4 encodes a protein that negatively regulates the T-cell response and is responsible for maintaining T-cell homeostasis.[12] When CTLA-4 is absent, peripheral T-cells can be overactive, causing fatal tissue damage.[13] In this study, CTLA-4 levels in placental tissue of pregnant women with preeclampsia and smoking pregnant women with preeclampsia were investigated using immunohistochemical methods. We think that our results will contribute to the enlightenment of the etiology of preeclampsia and to determining the effects of maternal smoking on placenta tissue.

MATERIALS AND METHODS

Material

Our study was designed prospectively and performed in compliance with the "Declaration of Helsinki". Tissue samples were obtained from Erzurum Nenehatun Obstetrics and Gynecology Hospital. In the study, placenta samples were used from pregnant women who were primigravida or multigravida, aged 20 and 40 years, who gave birth normally or via cesarean section, had no additional chronic diseases (e.g., diabetes, chronic renal insufficiency), and no early membrane rupture or chorioamnionitis, and who completed the 37th gestational week. Written consent from pregnant women was obtained, and they filled out demographic information forms developed by the researcher. Criteria for the diagnosis of preeclampsia were based on two blood pressure values of 140/90 mmHg or higher and 300 mg or more proteinuria in the urine collected over 24 hours, after the 20th week of pregnancy (the diagnosis of preeclampsia was made by the presence of at least two criteria listed below in the 2019 guideline of the "American Association of Obstetricians and Gynecologists".

Methods

Groups were designed as follows:

1. Control group (n=7): Pregnant women who did not have any health problems were included in this group.

2. Smoking group (n=7): Pregnant women who did not have any health problems and smoked during pregnancy were included in this group.

3. Preeclampsia group (n=7): Pregnant women who had been diagnosed with preeclampsia and did not smoke were included in this group.

4. Preeclampsia + smoking group (n=7): Pregnant women who were diagnosed with preeclampsia and smoked during pregnancy were included in this group.

A full-thickness section was taken from the middle part of the placenta from the fetal face to the maternal face and including the amnion and decidua for histopathological and immunohistochemical exeminations. Only one tissue sample was taken from each placenta. Tissue samples were obtained from the fetal and maternal parts of the placenta. The tissues were fixed in 10% formalin solution; a routine histological protocol was applied, and they were blocked in paraffin.

Histopathological Examinations

To examine the general structure of the placental tissue, 5 µm sections were taken from the blocks, and Crossman's triple staining and haematoxylin-eosin staining were performed. Six different areas were randomly evaluated from each tissue sample. Researchers made the evaluation independently of each other. The histopathological changes in placenta tissue were assessed according to their severity as none (0), weak (1), moderate (2), and strong (3).

Statistical Analysis

Data obtained from histopathological changes in placenta tissues, demographic characteristics, and blood pressure measurements were analyzed with SPSS version 22.00. Differences between the groups were determined by the Kruskal-Wallis test, and the Mann-Whitney U test was used to determine the group that made the difference. The results are presented with median, minimum, and maximum values. A $p<0.05$ was considered statistically significant. No corrections were made to the analysis using the SPSS software.

Immunohistochemical Examinations

The streptavidin-biotin peroxidase method was applied to the sections taken from placental tissue. During the immunohistochemistry procedure, all washing procedures were performed with PBS (0.1 M, pH 7.2) buffer. The sections were first soaked in 3% H_2O_2 for 15 minutes, then, citrate buffer solution was added and they were boiled in a microwave oven (600 watts for 10 minutes). Then large volume ultra V block solution was applied for 10 minutes. CTLA-4 (sc-376016) primary antibody was added to the sections and kept at room temperature in a humid environment for 1 hour (1/50 dilution). Then biotinylated goat anti B polyvalent and streptavidin peroxidase solutions were applied, respectively, for 30 minutes. Chromogen application was performed with diaminobenzidine hydrogen peroxide substrate solution. Contrast staining was performed with modified Gill III haematoxylin. In immunohistochemical evaluations, staining intensity and staining

characteristics of the cells were taken into account and semiquantitative scoring was performed as no staining (-), weak staining (+), moderate staining (++) and strong staining (+++) (evaluations were made by two independent observers). All sections were examined by light microscopy (Olympus BX51; Olympus Optical Co. Osaka, Japan) and photographed.

RESULTS

Statistical Results

The evaluation results of statistical and histopathological data of the women included in the study groups are presented in Table 1. When the demographic characteristics of the women in the study groups were examined (Table 1A), 60.7% had medium income, 67.9% of women gave birth by C-section, while the previous birth status of 46.4% was also C-section, 63.1% of women who gave birth by C-section were women in the preeclampsia and preeclampsia + smoking group and 85.7% of women did not working.

The age (χ^2=4.363, p>0.05) and weight (χ^2=7.057, p>0.05) were observed to not differ between the groups (Table 1C). Diastolic blood pressure (χ^2=19.572, p<0.001) and systolic blood pressure (χ^2=18.589, p<0.001) values showed statistically significant differences between the groups. In addition, the median values of the preeclampsia and preeclampsia + smoking groups were found to be higher for diastolic and systolic blood pressure than the median values of the control and smoking groups (Table 1D).

Histopathological Results

Serial sections were taken from placental tissue samples, and histopathological changes were found to be different between the groups (Table 1B). While the placenta samples in the control group had a normal histological structure (Figure 1), the reduction in the villous tree (VT), congestion in the villi, and fibrin deposition in the decidua were seen in the other groups. It was determined that these histopathological changes were weak in the smoking group, moderate in the preeclampsia group, and strong in the preeclampsia + smoking group (Figures 2-4).

Immunohistochemical Results

In the amniotic epithelium of the placenta tissue, moderate CTLA-4 immunoreactivity was determined in the control, smoking, and preeclampsia groups. Strong CTLA-4 immunoreactivity was determined in the preeclampsia + smoking group. Moderate immunoreactivity was detected in the chorionic plaque in all groups (Table 2, Figure 5). In decidua cells and stem villi, strong immunoreactivity in the control group, moderate immunoreactivity

Table 1. Statistical and histopathological results

A. Demographic characteristics of women included in the study groups

Variables		f	%	Variables		f	%
Income status	Low	11	39.3	Working status	Yes	4	14.3
	Middle	17	60.7		No	24	85.7
Type of birth	Normally	9	32.1	Number of children	0	5	17.9
	C-section	19	67.9		1	3	10.7
Previous form of birth	Normally	10	35.7		2	7	25.0
	C-section	13	46.4		3	6	21.4
	None	5	17.9		4	7	25.0

B. Histopathological changes in placenta tissue samples

Groups	Decrease of the VT	Congestion	Fibrin deposition	Diastolic blood pressure	Systolic blood pressure
C	0.33±0.51^{d}	0.33±0.51^{a}	0.16±0.40^{a}	70a (60-70)*	110^{a} (100-120)*
S	1.33±0.51^{c}	0.16±0.40^{a}	1.33±0.51^{b}	70a (60-90)*	110^{a} (100-140)*
P	2.16±0.40^{b}	1.83±0.40^{b}	2.16±0.40^{c}	100b (90-120)*	150^{b} (140-190)*
PS	2.83±0.40^{a}	2.66±0.51^{c}	2.83±0.40^{d}	90b (90-115)*	140^{b} (140-190)*

*Values are shown as median (minimum-maximum). $^{a-d}$: There is a statistically significant difference between the values indicated with different letters (p<0.05).

C. Age and weight values of the women included in the study groups

	C (n=7)	S (n=7)	P (n=7)	PS (n=7)
Age	27^{a} (24-30)*	32^{a} (28-37)*	30a (21-40)*	31a (23-41)*
Weight	67^{a} (43-80)*	80^{a} (67-93)*	72a (70-87)*	83a (57-95)*

*Values are shown as median (minimum-maximum). a: There is no statistical difference between the values indicated with the same letter.

D. Comparison of diastolic and systolic blood values between groups

	C (n=7)	S (n=7)	P (n=7)	PS (n=7)
Diastolic blood pressure	70^{a} (60-70)*	70^{a} (60-90)*	100^{b} (90-120)*	90^{b} (90-115)*
Systolic blood pressure	110^{a} (100-120)*	110^{a} (100-140)*	150^{b} (140-190)*	140^{b} (140-190)*

*Values are shown as median (minimum-maximum). ^{a,b}: There is a statistically significant difference between the values indicated with different letters. C: Control group, S: Smoking group, P: Preeclampsia group, PS: Preeclampsia + smoking group.

Table 2. Semiquantitative scoring of CTLA-4 immunoreactivity

Areas	Groups			
	C (n=7)	S (n=7)	P (n=7)	PS (n=7)
Amniotic epithelium	++	++	++	+++
Chorionic plaque	++	++	++	++
Decidua cells	+++	++	+	++
Stem villi	+++	++	+	++
Terminal villi	+	+	+	+

CTLA-4: Cytotoxic T-lymphocyte antigen-4, C: Control group, S: Smoking group, P: Preeclampsia group, PS: Preeclampsia + smoking group.

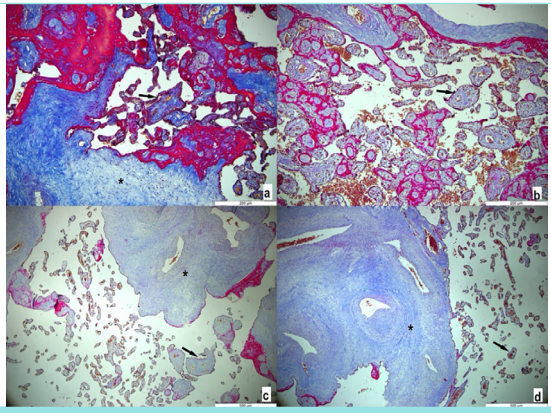

Figure 1. Placenta tissue. (a) control group, (b) smoking group, (c) preeclampsia group, (d) preeclampsia + smoking group. Crossman's trichrome staining. Asterisk: Decidua, arrow: Chorion villi.

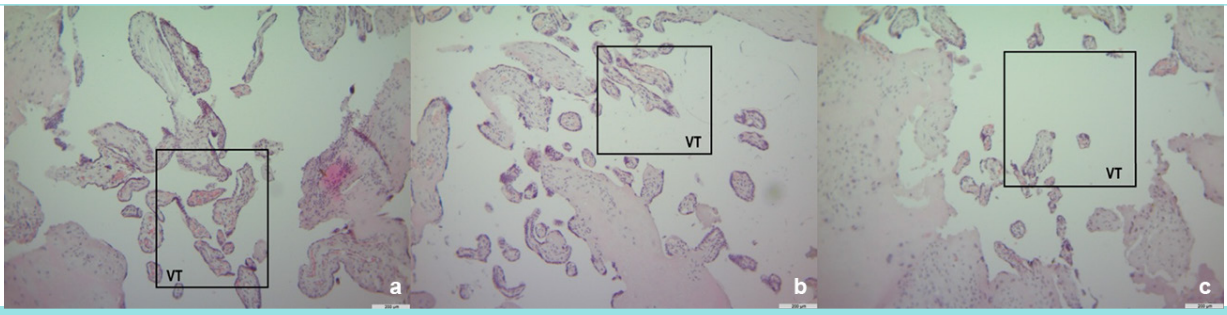

Figure 2. Placenta tissue. (a) cigarette group, weak decrease in VT; (b) preeclampsia group, moderate decrease in VT; (c) preeclampsia + smoking group, strong decrease in VT. H&E staining.

VT: Villous tree, H&E: Hematoxylin and eosin.

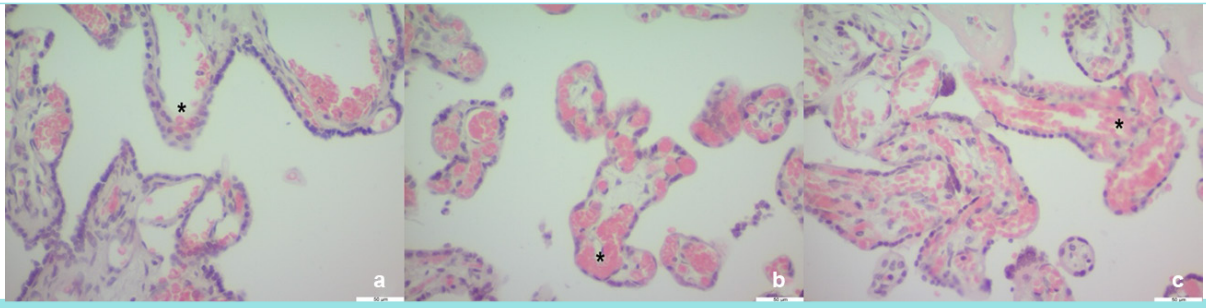

Figure 3. Placenta tissue. (a) cigarette group, weak congestion (asterisk); (b) preeclampsia group, moderate congestion (asterisks); (c) preeclampsia + smoking group. Strong congestion (asterisk), H&E staining.

H&E: Hematoxylin and eosin.

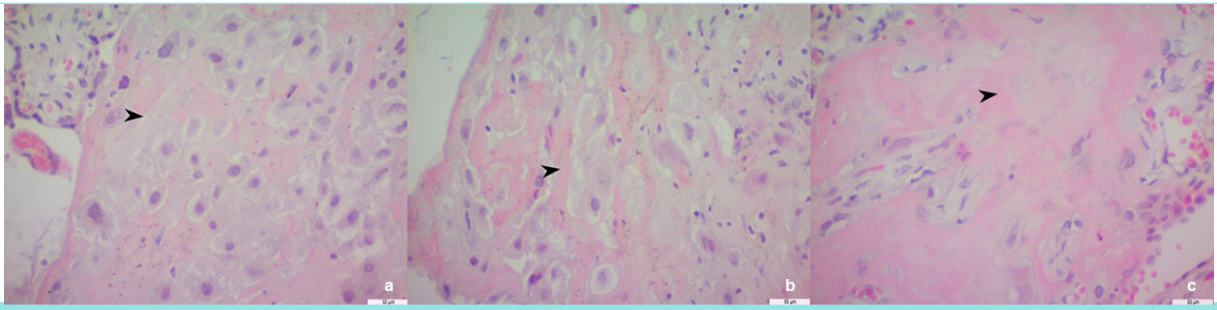

Figure 4. Placenta tissue. (a) cigarette group. Weak fibrin deposition in the decidua (arrowhead), (b) preeclampsia group. Moderate fibrin deposition in decidua (arrowhead). (c) preeclampsia + smoking group. Strong deposition of fibrin in the decidua (arrowhead). H&E staining.

H&E: Hematoxylin and eosin.

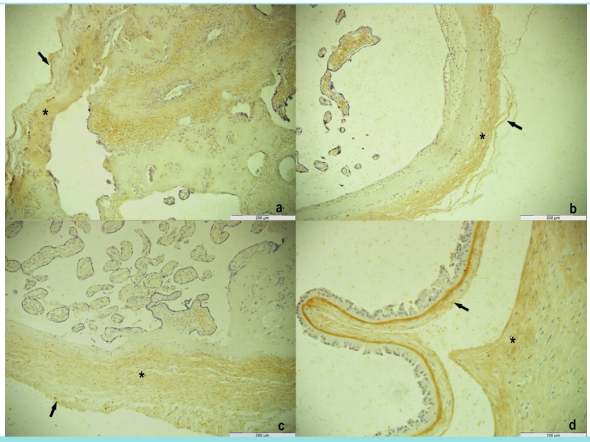

Figure 5. CTLA-4 immunoreactivity in amniotic epithelium and chorionic plate. (a) control group, (b) smoking group, (c) preeclampsia group, (d) preeclampsia + smoking group. Asterisk: Chorionic plaque, arrow: Amniotic epithelium.

CTLA-4: Cytotoxic T-lymphocyte antigen-4.

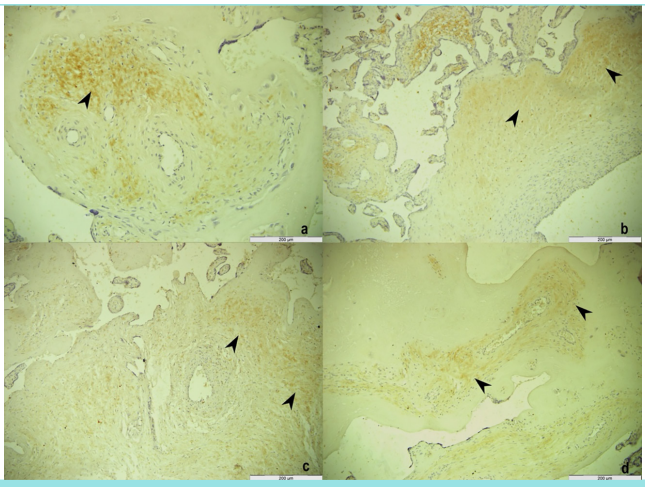

Figure 6. CTLA-4 immunoreactivity in decidua cells. (a) control group, (b) smoking group, (c) preeclampsia group, (d) preeclampsia + smoking group. Arrowhead: Decidua.

CTLA-4: Cytotoxic T-lymphocyte antigen-4.

in the smoking and preeclampsia + smoking group, and weak immunoreactivity in the preeclampsia group was observed. In terminal villi, weak immunoreactivity was detected in all groups (Table 2, Figures 6 and 7).

DISCUSSION

Although the factors that cause preeclampsia have not been completely known, the studies explain some points about the pathogenesis of preeclampsia. When the method of delivery of pregnant women with preeclampsia was examined, it was found that more than half of the patients had a cesarean delivery.[14,15] Considering whether there was a correlation between preeclampsia and age, it was determined that the average age of patients with severe preeclampsia symptoms was higher than that of the normotensive group. In addition, it was found that systolic and diastolic blood pressure levels were highest in the severe preeclampsia group, followed by the preeclampsia group, and lowest in the normotensive group.[16] In our study, the average age in the groups was homogeneous, the rate of cesarean sections was dominantly high at 67.9%, and systolic and diastolic blood pressure levels were higher in the preeclampsia and preeclampsia + smoking groups than in the

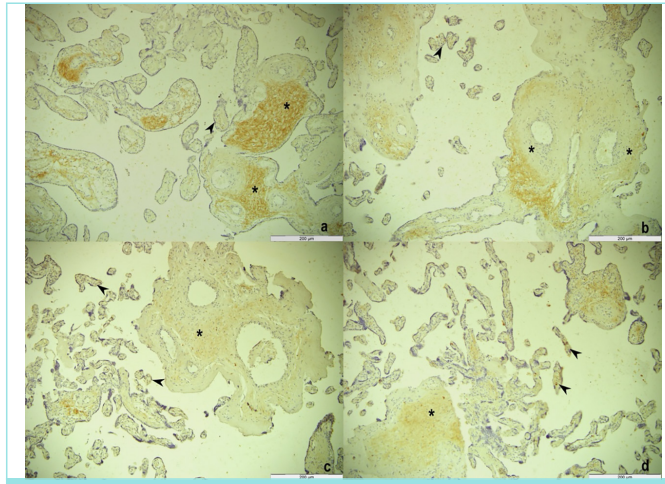

Figure 7. CTLA-4 immunoreactivity in chorionic and terminal villi. (a) control group, (b) smoking group, (c) preeclampsia group, (d) preeclampsia + smoking group. Asterisk: Stem villi, arrowhead: Terminal villi.

CTLA-4: Cytotoxic T-lymphocyte antigen-4.

other groups. These results suggested that demographic data may be meaningful for determining risk factors in patients with preeclampsia.

It has been suggested that there may be deficiencies in placental vascularization due to immunological problems in pregnant women with preeclampsia and that preeclampsia may be recessively inherited.[17-19] The storage of fibrin localized in the perivillous area in the placentas of normal pregnant women giving birth in time is not a pathological condition, but it has been reported in studies that the intensive deposition of fibrin in the placenta negatively affects fetal development.[20] It is noted that infarcts occurring in the placentas of women with preeclampsia are associated with a disorder in fetal blood flow; the thrombosis determined in the maternal vessels can cause a decrease in fetal blood flow.[21] Our study revealed the changes seen in the form of the declining VT, congestion in villi, and fibrin deposition in decidua among groups experiencing smoking, preeclampsia, and smoking + preeclampsia. These changes were found to be weak in the smoking group, moderate in the preeclampsia group, and strong in the preeclampsia + smoking group. It was thought that our results would contribute positively to the identification of the histopathological changes that smoking can produce in placental tissue, and to the illumination of the etiology of preeclampsia.

Also, 30-45% of the T-cells found in human deciduas are CD4+ T-cells and 45-75% are CD8+ T-cells, cytotoxic T lymphocytes cells.[22] CD8+ T-cells are less abundant in peripheral blood and more abundant in human decidua at term.[23,24] These cells are capable of recognizing allogeneic MHC molecules but do not attack fetal cells during pregnancy.[25] This condition is thought to be due to limited MHC class I expression in fetal trophoblast cells. CTLA-4 and CD28, which both interact with B7 belong to the immunoglobulin superfamily. It is responsible for the regulation of the immune system. It is also called CD152.[26] CTLA-4 is normally found at a low level on the surface of effector T-cells and Treg (regulatory T) cells, regulating the severity of early-stage T-cell activation.[27] When CTLA-4 is suppressed, cytotoxic T-cell activation increases and Treg cells are prevented from suppressing the immune system.[28] The successful continuation of pregnancy requires the establishment of maternal-fetal tolerance and the successful completion of placentation. When the immune balance is disturbed, spontaneous abortions, preeclampsia and intrauterine growth restriction of the fetus may occur due to inadequate placental perfusion. Extravillous trophoblasts instruct decidual immune cells to regulate fetal tolerance and promote placental development. CTLA-4 has important roles in the function of decidual immune cells. Blockade of CTLA-4 pathways results in abnormalities in the number and functionality of CD4+ T-cells, impairing the interaction of extravillous trophoblasts and decidual immune cells. It has been stated that this leads to poor placental development and increased fetal loss, and it has been emphasized that CTLA-4 plays important roles in maintaining normal pregnancy.[29,30] During pregnancy, CTLA-4 immunoreactivity was reported in numerous stromal cells in placental tissue, while immunoreactivity was not seen in trophoblast cells and endothelial cells.[31] In the placenta tissue of all groups in our study, CTLA-4 immunoreactivity was determined in the amnionic epithelium, decidual cells stem villi, chorionic plaque, and terminal villi. It was noted that CTLA-4 immunoreactivity in decidua cells and stem villi in placentas of the preeclampsia group decreased compared with the control group.

Smoking has negative effects on the immune system. Nicotine found in the composition of cigarettes is similar to acetylcholine in its chemical structure. It first stimulates transmission of stimuli in autonomic nervous system ganglia via acetylcholine, but then blocks it. Studies have determined that the nicotine metabolite "cotinine" crosses the placental barrier, as evidenced by its presence in amniotic fluid and cord blood. Although the mechanisms by which it negatively affects the fetus are not fully known, there are views that it can produce vasoconstriction in the uterine arteries, can exert direct toxic effects, or can cause placental damage.[32-34] Maternal smoking disrupts the balance between cytotrophoblast proliferation and differentiation and damages placental development.[35,36] Alkaline ribonuclease levels increase in the placentas of women who smoke, which is likely to result in impairment in protein synthesis. Moreover, there is villous hyperplasia in the placentas of these mothers.[37] The number of syncytial nodes-masses of multi-nucleated protoplasms that result from the fusion of single cells with the loss of cell membranes between them- and cytotrophoblastic cells, in pregnant smoking women, was reported to increase. Average birth weight and placental weight decreased as the number of cigarettes smoked daily increased in the third trimester.[38] It has been reported that cigarette smoking in pregnancy may lead to many adverse obstetric outcomes such as ectopic pregnancy and placental abruption, and may be a risk factor for gestational hypertension and preeclampsia.[39-42] On the other hand, some studies have suggested that the number of cigarettes smoked per day during pregnancy has a n inverse dose-response relationship to the likelihood of preeclampsia and that maternal cigarette smoking reduces the risk of pregnancy-induced hypertension and eclampsia.[43,44] The claim that smoking during pregnancy can have a protective effect against pre-eclampsia suggests that the mechanism called vascular placental pathology is a highly complex event.[45,46] It was thought that determining vigorous CTLA-4 immunoreactivity in the control group placenta tissue may result from suppression of the mother's immune responses so that the pregnancy could continue in its normal course. The decrease in CTLA-4 immunoreactivity in smoking, preeclampsia, and preeclampsia + smoking groups compared to the control group may be caused by histopathological changes in the placental tissue and deficiencies in villus development. It is also hypothesized that the increased immunoreactivity of CTLA-4 in both the smoking and the pre-eclampsia and smoking groups compared to the pre-eclampsia group could be the immune response by cells which have increased in the mother's body due to smoking.

CONCLUSION

It was thought that this research could make a positive impact on determining the impact of smoking in pregnancy on the health of the mother and fetus and illuminating the aspects of pre-eclampsia associated with the immune system. It also aims to determine the factors that may negatively affect the mother's immune system during pregnancy and the levels of immune cells and their role in pregnancy continuation.

MAIN POINTS

- Histopathologic changes occurred in placental tissue of smoking, preeclampsia and preeclampsia + smoking groups.
- Different levels of CTLA-4 immunoreactivity were detected in placental tissue and amniotic epithelium of all groups.
- Immunoreactivity intensity in decidua cells and stem villi decreased in smoking, preeclampsia, and preeclampsia + smoking groups.

ETHICS

Ethics Committee Approval: The study received approval from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (approval number: 71, date: 16.01.2020).

Informed Consent: Written consent from pregnant women was obtained, and they filled out demographic information forms developed by the researcher.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ş.Y.A., B.G.K., A.G., S.E.Y., Concept: Ş.Y.A., K.S., Design: Ş.Y.A., K.S., Data Collection and/or Processing: B.G.K., A.G., S.E.Y., G.F.A., Analysis and/or Interpretation: B.G.K., A.G., Literature Search: Ş.Y.A., Writing: S.E.Y., G.F.A., E.K.S.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Carter AM, Mess AM. Conservation of placentation during the tertiary radiation of mammals in South America. J Morphol. 2013; 274(5): 557-69.
2. Mess A. Placental Evolution within the Supraordinal Clades of Eutheria with the Perspective of Alternative Animal Models for Human Placentation. Adv Biol. 2014; 639274.
3. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circ Res. 2019; 124(7): 1094-112.
4. Gleicher N. Why much of the pathophysiology of preeclampsia-eclampsia must be of an autoimmune nature. Am J Obstet Gynecol. 2007; 196(1): 5.e1-7.
5. Prins JR, Boelens HM, Heimweg J, Van der Heide S, Dubois AE, Van Oosterhout AJ, et al. Preeclampsia is associated with lower percentages of regulatory T cells in maternal blood. Hypertens Pregnancy. 2009; 28(3): 300-11.
6. World Health Organization. WHO Global Tobacco Epidemic Report. 2009. Available at: http://whqlibdoc.who.int/publications/2009/9789241563918_eng_full.pdf, Access date: 01.08.2011.
7. Butler NR, Goldstein H, Ross EM. Cigarette smoking in pregnancy: its influence on birth weight and perinatal mortality. Br Med J. 1972; 2(5806): 127-30.
8. Andersen KV, Hermann N. Placenta flow reduction in pregnant smokers. Acta Obstet Gynecol Scand. 1984; 63(8): 707-9.
9. Chinen J, Finkelman F, Shearer WT. Advances in basic and clinical immunology. J Allergy Clin Immunol. 2006; 118: 489-95.
10. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006; 439: 682-7.
11. Boussiotis VA. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. N Engl J Med. 2016; 375(18): 1767-78.
12. Dariavach P, Mattéi MG, Golstein P, Lefranc MP. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. Eur J Immunol. 1988; 18(12): 1901-5.
13. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science. 2015; 349(6246): 436-40.
14. Zhang J, Meikle S, Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. Hypertens Pregnancy. 2003; 22(2): 203-12.
15. Kumru P, Kartal ÖP, Köse G, Aka N, Büyükoğlu B. The Evaluation of cases with preeclampsia, eclampsia and HELLP syndrome in our clinic. J Clin Obstet Gynecol. 2005; 30(2): 72-80.
16. Karaşin SS, Çift T. The Role of Ischemia-modified Albumin as a Biomarker in Preeclampsia. Rev Bras Ginecol Obstet. 2020; 42(3): 133-9.
17. Feeney JG, Scott JS. Pre-eclampsia and changed paternity. Eur J Obstet Gynecol Reprod Biol. 1980; 11(1): 35-8.
18. Klonoff-Cohen HS, Savitz DA, Cefalo RC, McCann MF. An epidemiologic study of contraception and preeclampsia. JAMA. 1989; 262(22): 3143-7.
19. Cooper DW. Immunological relationships between mother and conceptus in man. Hearn JP, editor. Immunological aspects of reproduction and fertility control. Lancaster, UK: MTP Press; 1980: 33-61.
20. Nelson DM, Crouch EC, Curran EM, Farmer DR. Trophoblast interaction with fibrin matrix. Epithelialization of perivillous fibrin deposits as a mechanism for villous repair in the human placenta. Am J Pathol. 1990; 136(4): 855-65.
21. Berkowitz K, Monteagudo A, Marks F, Jackson U, Baxi L. Mitochondrial myopathy and preeclampsia associated with pregnancy. Am J Obstet Gynecol. 1990; 162(1): 146-7.
22. Nancy P, Erlebacher A. T cell behavior at the maternal-fetal interface. Int J Dev Biol. 2014; 58(2-4): 189-98.
23. Tilburgs T, Schonkeren D, Eikmans M, Nagtzaam NM, Datema G, Swings GM, et al. Human decidual tissue contains differentiated CD8+ effector-memory T cells with unique properties. J Immunol. 2010; 185(7): 4470-7.
24. Loewendorf AI, Nguyen TA, Yesayan MN, Kahn DA. Normal human pregnancy results in maternal immune activation in the periphery and at the uteroplacental interface. PLoS One. 2014; 9(5): e96723.
25. Xu YY, Wang SC, Lin YK, Li DJ, DU MR. Tim-3 and PD-1 regulate CD8+ T cell function to maintain early pregnancy in mice. J Reprod Dev. 2017; 63(3): 289-94.
26. Chowdhury F, Dunn S, Mitchell S, Mellows T, Ashton-Key M, Gray JC. PD-L1 and $CD8^+$ $PD1^+$ lymphocytes exist as targets in the pediatric tumor microenvironment for immunomodulatory therapy. OncoImmunology. 2015; 4(10): e1029701.
27. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016; 17(12): e542-51.
28. Simsek M, Tekin SB, Bilici M. Immunological agents used in cancer treatment. Eurasian J Med. 2019; 51(1): 90-4.
29. Wang S, Chen C, Li M, Qian J, Sun F, Li Y, et al. Blockade of CTLA-4 and Tim-3 pathways induces fetal loss with altered cytokine profiles by decidual CD4+T cells. Cell Death Dis. 2019; 10(1): 15.
30. Li M, Sun F, Qian J, Chen L, Li D, Wang S, et al. Tim-3/CTLA-4 pathways regulate decidual immune cells-extravillous trophoblasts interaction by IL-4 and IL-10. FASEB J. 2021; 35(8): e21754.
31. Kaufman KA, Bowen JA, Tsai AF, Bluestone JA, Hunt JS, Ober C. The CTLA-4 gene is expressed in placental fibroblasts. Mol Hum Reprod. 1999; 5(1): 84-7.
32. Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. Semin Perinatol. 1996; 20(2): 115-26.
33. Mercelina-Roumans PE, Schouten H, Ubachs JM, van Wersch JW. Cotinine concentrations in plasma of smoking pregnant women and their infants. Eur J Clin Chem Clin Biochem. 1996; 34(7): 525-8.

34. Pastrakuljic A, Schwartz R, Simone C, Derewlany LO, Knie B, Koren G. Transplacental transfer and biotransformation studies of nicotine in the human placental cotyledon perfused in vitro. Life Sci. 1998; 63(26): 2333-42.

35. Spira A, Philippe E, Spira N, Dreyfus J, Schwartz D. Smoking during pregnancy and placental pathology. Biomedicine. 1977; 27(7): 266-70.

36. Genbacev O, McMaster MT, Zdravkovic T, Fisher SJ. Disruption of oxygen-regulated responses underlies pathological changes in the placentas of women who smoke or who are passively exposed to smoke during pregnancy. Reprod Toxicol. 2003; 17(5): 509-18.

37. Rush D, Kristal A, Blanc W, Navarro C, Chauhan P, Campbell Brown M, et al. The effects of maternal cigarette smoking on placental morphology, histomorphometry, and biochemistry. Am J Perinatol. 1986; 3(3): 263-72.

38. Demir R, Demir AY, Yinanc M. Structural changes in placental barrier of smoking mother. A quantitative and ultrastructural study. Pathol Res Pract. 1994; 190(7): 656-67.

39. Castles A, Adams EK, Melvin CL, Kelsch C, Boulton ML. Effects of smoking during pregnancy. Five meta-analyses. Am J Prev Med. 1999; 16(3): 208-15.

40. Dietz PM, England LJ, Shapiro-Mendoza CK, Tong VT, Farr SL, Callaghan WM. Infant morbidity and mortality attributable to prenatal smoking in the U.S. Am J Prev Med. 2010; 39(1): 45-52.

41. Pineles BL, Park E, Samet JM. Systematic review and meta-analysis of miscarriage and maternal exposure to tobacco smoke during pregnancy. Am J Epidemiol. 2014; 179(7): 807-23.

42. Lewandowska M, Więckowska B. The influence of various smoking categories on the risk of gestational hypertension and pre-eclampsia. J Clin Med. 2020; 9(6): 1743.

43. Kharkova OA, Grjibovski AM, Krettek A, Nieboer E, Odland JØ. First-trimester smoking cessation in pregnancy did not increase the risk of preeclampsia/eclampsia: A Murmansk County Birth Registry study. PLoS One. 2017; 12(8): e0179354.

44. Yang Q, Wen SW, Smith GN, Chen Y, Krewski D, Chen XK, et al. Maternal cigarette smoking and the risk of pregnancy-induced hypertension and eclampsia. Int J Epidemiol. 2006; 35(2): 288-93.

45. Salafia C, Shiverick K. Cigarette smoking and pregnancy II: vascular effects. Placenta. 1999; 20(4): 273-9.

46. Winer N, Hamidou M, El Kouri D, Philippe HJ. Facteurs de risque maternels et obstétricaux de pathologie vasculaire placentaire (hors facteurs biologiques et épidémiologiques) [Maternal and obstetrical risk factors of placental vascular pathology (biologic and epidemiological data excluded)]. Ann Med Interne (Paris). 2003; 154(5-6): 316-24.

RESEARCH ARTICLE

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Determination of the Effect of Coenzyme Q-10 on Spermatogenic Stem Cells by Immunohistochemical Techniques in Male Rats with Experimental Hypothyroidism

Hatice Nur Şeflek[1], Fatma Zehra Erbayram[2], Gökhan Cüce[3], Serpil Kalkan[3]

[1]Department of Histology and Embryology, KTO Karatay University Faculty of Medicine, Konya, Türkiye
[2]Department of Medical Biochemistry, KTO Karatay University Faculty of Medicine, Konya, Türkiye
[3]Department of Histology and Embryology, Necmettin Erbakan University Faculty of Medicine, Konya, Türkiye

Abstract

BACKGROUND/AIMS: Testicular dysfunction has been reported in hypothyroidism. However, the effect of hypothyroidism on spermatogonial stem cells (SSCs) is not understood. This study aimed to investigate the effect of hypothyroidism on SSCs in comparison with coenzyme Q10 (CoQ10), which is widely used in the treatment of male infertility.

MATERIALS AND METHODS: Histomorphologic examinations of rat testes, immunohistochemical analyses to detect SSCs expressions, and Enzyme-Linked Immunosorbent Assay tests for serum hormone levels were performed.

RESULTS: Hypothyroidism caused an increase in the body weight of the rats, but there was no difference between the groups when the testicular weights of the animals were evaluated. In thyroid function test evaluation, CoQ10 supplementation provided a therapeutic effect by showing positive results in thyroid stimulating hormone, triiodothyronine, and tetraiodothyronine levels. Histomorphologic evaluation showed no difference in seminiferous tubule diameters between the groups, although irregularities in the spermatogenesis process were observed. Hypothyroidism prevents SSCs from undergoing cell differentiation and thus reduces sperm production in the seminiferous tubules. CoQ10 supplementation does not affect spermatogenic stem cells, but has a beneficial effect on other cells (primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa).

CONCLUSION: Although CoQ10 supplementation does not directly affect the differentiation of SSCs, it may alleviate some of the deleterious effects of hypothyroidism in the later stages of spermatogenesis, providing a potential therapeutic benefit in maintaining sperm production.

Keywords: Coenzyme, hypothyroidism, stem cells

INTRODUCTION

Hypothyroidism is an endocrine disorder that occurs when the thyroid gland does not function properly.[1] Hypothyroidism can lead to sexual dysfunction in men and cause infertility.[2] The thyroid gland hormones triiodothyronine (T3) and tetraiodothyronine (T4) regulate testicular function through genomic and non-genomic effects.[3] Genomic effects result from the binding of T3 to its corresponding receptor thyroid hormone receptor (TR), in the nucleus of Sertoli and Leydig cells, where, after binding to thyroid hormone response elements, the hormone-receptor complex activates gene transcription and protein synthesis.[4] The non-genomic effects of thyroid hormones result from their binding to non-nuclear receptors in the cytoplasmic membrane, cytoplasm, cytoskeleton and mitochondria of the spermatozoon, increasing cyclic

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ORCID IDs of the authors: H.N.Ş. 0000-0003-2969-2322; F.Z.E. 0000-0002-9305-4782; G.C. 0000-0003-1781-5292; S.K. 0000-0003-0076-9154.

Corresponding author: Hatice Nur Şeflek
E-mail: haticenur.seflek@karatay.edu.tr
ORCID ID: orcid.org/0000-0003-2969-2322

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adenosine monophosphate synthesis, Ca^{2+} release, and ultimately sperm motility.[3-6] Although it is known that Sertoli and Leydig cells contain TRs and thus these cells are directly or indirectly affected by changes in thyroid hormone levels, the impact of hypothyroidism on the mechanisms affecting spermatogonia (SPG) in both fetal and adult life are not fully understood. In addition to this information, it is known that thyroid hormones regulate the redox state of the testis, which is mediated by various antioxidant systems.[3]

Coenzyme Q10 [(CoQ10), ubiquinone], an antioxidant, is a vitamin-like molecule found in every cell membrane. It is a normal part of our diet but is also synthesized endogenously. CoQ10 is one of the electron carriers in the mitochondrial respiratory chain and adenosine triphosphate production process.[7] In addition to this information, CoQ10 has been reported to play a role in membrane stability, cell signaling, gene expression, apoptosis control, and cell growth.[8] Studies have reported that CoQ10 has beneficial effects on male infertility by improving sperm function. A meta-analysis conducted in 2020 demonstrated that CoQ10 supplementation significantly improved sperm concentration, motility, and morphology.[9] The antioxidant properties of CoQ10 help reduce sperm DNA damage and prevent oxidative stress, which is a crucial factor in enhancing sperm quality, particularly in infertile men.[10] Human studies also support the notion that CoQ10 can protect testicular functions and enhance sperm production.[11]

Spermatogonial stem cells (SSCs) are undifferentiated germ cells responsible for spermatogenesis.[12] Throughout most of the male lifespan, SSCs provide the basis for continuous sperm production.[13] In recent years, many studies have succeeded in understanding the biology of SSCs and have drawn attention to their great potential in the field of reproductive/regenerative medicine. Recent studies on the treatment of male infertility are promising.[14,15] Recent studies have provided a wealth of information on phenotypic biomarkers of human and mouse SSCs: such as thymocyte differentiation antigen-1 (THY-1), promyelocytic leukemia zinc finger (PLZF), SRY-Box3 (SOX-3), glial cell line-derived neurotrophic factor family receptor alpha 1 (GFRα1) have been identified as biomarkers observed in human and mouse SSCs.[16,17] The identification and evaluation of these biomarkers is crucial for advancing our understanding of SSC biology and their role in male infertility. These biomarkers not only help in isolating and characterizing SSCs but also provide insights into the molecular mechanisms underlying spermatogenesis and its dysregulation in infertile men. For instance, PLZF is essential for maintaining the undifferentiated state of SSCs, and its dysregulation has been linked to impaired spermatogenesis and infertility.[18] Similarly, THY-1 and SOX-3 play critical roles in SSC self-renewal and differentiation, and their expression patterns are often altered in cases of male infertility.[19] Therefore, the evaluation of these biomarkers is crucial for the development of targeted therapies for male infertility, as it provides a better understanding of the molecular pathways involved in SSC function and spermatogenesis. Using these biomarkers, they may discover new therapies to restore fertility in men with impaired spermatogenesis, such as SSC transplantation or *in vitro* spermatogenesis.

In light of this information, we aimed to investigate the beneficial effects of CoQ10, a powerful antioxidant found in all cell membranes, vital for cellular energy, inhibiting free radicals, and protecting the cell membrane from lipid peroxidation, on testicular function, especially at the SSC level, given that its bioavailability is very high. Based on this information, the effects of CoQ10 supplementation on testicular histopathology in hypothyroidism were investigated. Additionally, the study will evaluate specific biomarkers involved in spermatogenesis, such as THY-1, PLZF, SOX-3 and GFRα1, providing greater insights into the molecular mechanisms of CoQ10's impact on male fertility, and contributing to the development of targeted therapies for male infertility.

MATERIALS AND METHODS

Study Design and Ethical Approval

In this study, 28 male *Wistar albino* rats weighing 255-304 g were divided into four groups to evaluate the effects of hypothyroidism and CoQ10 supplementation on testicular function: 1) Control, 2) hypothyroidism with PTU, 3) CoQ10 treatment, 4) hypothyroidism + CoQ10. Induction of hypothyroidism was achieved in the second and fourth groups with drinking water containing 0.05% PTU (6-n-propyl-2-thiouracil).[20] CoQ10 was administered intraperitoneally to the third and fourth groups at a dose of 10 mg/kg daily. At the end of the experiment, blood samples were taken for thyroid stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) levels. The testes were removed, weighed, and prepared for histological examination; immunohistochemical (IHC) analysis was performed on the right testis, and microscopic analysis with hematoxylin-eosin was performed on the left testis.

This study was carried out with the approval of the Necmettin Erbakan University Ethics Committee for Experimental Animal Research (approval number: 2022-036, date: 06.07.2022). The research was supported by the Scientific Research Projects Coordination Office (project no: 221418001).

Hematoxylin-Eosin Staining

For deparaffinization, sections were placed in an oven at 37 °C overnight and 60 °C for 1-hour, the next day. They were exposed to xylene (Tekkim, catalog no: 190822134001) twice for 15 minutes each. Tissues were dehydrated through 100%, 96%, and 80% ethyl alcohol (Isolab, catalog no: LR0090611AL0), for 5 minutes each, and they were washed in distilled water twice. After staining with hematoxylin (Sigma-Aldrich, catalog no: HX17558974) for 1 minute, tissues were immersed in acid-alcohol, washed in tap water, and stained with eosin (Sigma-Aldrich, catalog no: HX17224144) for 2 minutes. They were then washed in water for 1 minute and passed through increasing concentrations of ethyl alcohol (70%, 80%, 96%, 100%) for 5 minutes each, followed by dehydration in xylene (Tekkim, catalog no: 190822134001) twice for 15 minutes. Stained tissues were examined under a light microscope, and photographs were taken. Twenty seminiferous tubules per group were selected for spermatogenesis evaluation using modified Johnsen scoring, and SPG were counted in the same tubules.[21]

Morphometric Study

The diameter of 20 selected round or nearly round seminiferous tubules was measured using an Olympus BX53 light microscope with the Image Pro program and Olympus SC50 camera attachment at x20 magnification, and subsequently, the mean value was calculated.

Immunohistochemical Study

Dehydrated testis tissues were washed twice with distilled water to remove alcohol. Slides were placed in a microwave-proof dish with

10% citrate buffer (Thermo Scientific, catalog no: AX201007) at 500 °C for 5 minutes a process repeated three times. After washing with PBS (BioShop, catalog no: 5B37414) for 5 minutes, hydrogen peroxide (1%, Merck, 64271) was applied for 10 minutes, followed by Super Block (ScyTek, catalog no: AAA125) for 10 minutes. Slides were incubated with primary antibodies for THY1 (CD90, Abcam, catalog no: AB203022), SOX-3 (Abcam, catalog no: AB183606), PLZF (Santa Cruz, catalog no: SC-28319), and GFRα1 (Abcam, catalog no: AB216667) (1:200 dilution) for 60 minutes. After washing with PBS, slides were incubated with secondary antibody (Santa Cruz, catalog no: SC-516102) for 20 minutes, followed by streptavidin peroxidase (Thermo Scientific, catalog no: TS-125-HR) for 20 minutes. AEC chromogen (ScyTek, catalog no: 25768) was applied for 15 minutes, and Mayer's hematoxylin stain (Sigma-Aldrich, catalog no: HX17558974) was used for 5 minutes. After washing in tap water for 3 minutes, immunoscoring was performed by three independent investigators using a scale of "-", "+", "++", and "+++". Differences between the groups, and severity, were recorded.

Biochemical Study

TSH (Elabscience, catalog no: E-EL-R0976), fT3 (Elabscience, catalog no: E-EL-0079), and fT4 (Elabscience, catalog no: E-EL-0122) levels in sera collected at the end of the experiment were measured using Enzyme-Linked Immunosorbent Assay kits on an Allsheng AMR-100 reader. TSH was calculated in ng/mL, and fT3 and fT4 in pg/mL.

Statistical Analysis

Statistical significance was determined using SPSS software (version 22; SPSS Inc., Chicago, IL). Seminiferous tubule areas, SPG cell counts, Johnsen scores, animal, and testicular weights, and biochemical analyses were compared using the Duncan test. Immunoscoring for THY-1, GFRα1, PLZF, and SOX-3 expression in spermatogonium was performed. Group comparisons were made using a one-way analysis of variance, with significance tested at $p<0.05$.

RESULTS

Weight Results

Animal weights at the beginning ($p=0.307$) and end ($p<0.001$) of the experiment were compared and the results are shown in Table 1. Weight gains were seen in groups 2 and 4 treated with PTU; there were significant differences between the initial and final weights in group 2 ($p<0.004$) and group 4 ($p=0.002$). No significant difference was found in the testicular weights (right + left), as shown in Table 1 ($p=0.0604$).

Hematoxylin-Eosin Staining Results

Germ cells were present at all stages of spermiogenesis within regular seminiferous tubule borders in groups 1 and 3. Group 2 showed irregular seminiferous tubule borders and arrested spermatogenesis. Group 4 exhibited smooth tubule borders and better germ cell arrangement than group 2. Differences between groups were observed in Johnsen scoring ($p=0.00$) (Table 1 and Figure 1).

Spermatogonial Cell Count Results

Statistical differences in mean SPG cell counts were observed between all experimental groups ($p<0.002$) (Table 1). The highest SPG cell count was in group 2, followed by group 4, group 1, and group 3.

Seminiferous Tubule Diameter Results

There was no statistical difference between the experimental groups ($p<0.186$) (Table 1).

Biochemical Results

Thyroid function test results (TSH, fT3, fT4) are shown in Table 2. Significant differences were observed between the groups in TSH ($p=0.001$), fT3 ($p=0.003$) and fT4 ($p=0.001$) levels. TSH: Normal in group 1, decreased in group 3; increased in group 2 and significantly decreased in group 4. fT3: Normal in group 1, increased in group 3, decreased in group 2, and significantly increased in group 4. fT4: Normal in group 1, increased in group 3; decreased in group 2 and significantly higher in group 4.

Immunohistochemical Results

No differences were observed in the expression of THY-1, GFRα1, and PLZF proteins between the groups ($p>0.05$). Expressions in SSCs were strong (+++) in all groups. In contrast, SOX-3 immunopositivity was strong (+++) in group 1, moderate (++) in group 2, and strong (+++) in group 3. Group 4 showed a decrease in immunopositivity similar to group 2, with moderate (++) expression, which was statistically significant ($p<0.05$). Overall, no significant difference was found in protein expression among all biomarkers (Figure 2).

DISCUSSION

Hypothyroidism negatively affects testicular function in animal models, with agents like carbimazole, methimazole, and propylthiouracil causing reduced testicular weight, impaired sperm motility and count, and disrupting spermatogenesis and steroidogenesis. Hypothyroidism also disrupts the thyroid hormone-regulated antioxidant balance, leading to increased oxidative stress and testicular damage. Antioxidants such as alpha-lipoic acid and L-carnitine have shown protective effects, reducing oxidative stress, improving sperm quality, and potentially restoring testicular function. These findings suggest antioxidant supplementation could mitigate hypothyroidism-induced testicular toxicity.[22-24]

Table 1. Histomorphological results

Parameter	Group 1	Group 2	Group 3	Group 4	p-value
Start of experiment AW (g) ± SD	$288.42^{a}\pm17.84$	$255.14^{b}\pm14.79$	$304.00^{a}\pm14.71$	$266.80^{b}\pm17.18$	p=0.307
End of experiment AW (g) ± SD	$310.57^{a}\pm21.4$	297.85±26.11	322.42±20.15	300.14±15.38	p<0.00
Testicular weight (g) ± SD	3.18±0.51	3.13±0.40	3.27±0.27	3.00±0.26	p=0.0604
SPG (cells/field) ± SD	$65^{b}\pm7.04$	$71.4^{a}\pm1.98$	$62.42^{b}\pm1.7$	$68^{a,b}\pm1.91$	p<0.002
ST (µm) ± SD	273.42±14.04	250.54±49.22	280.64±7.65	272.65±7.45	p=0.186
HE (µm) ± SD	11.3±0.5	8.4±1.4	10.5±1.2	9.4±1.4	p=0.00

AW: Animal weight, SD: Standard deviation, SPG: Spermatogonium, ST: Seminiferous tubule, H&E: Hematoxylin and eosin staining, [a]: Similar to each other, different from [b] and [c]. [b]: Different from [a], similar within itself. [c]: Different from both [a] and [b]. [ab]: Similar to both [a] and [b].

Thyroid hormones, T4 and T3, are crucial for the growth, development, and metabolism of mammalian tissues, and their imbalance can affect various organs. However, the impact of iodothyronines on human male reproduction remains controversial, as clinical signs related to male gonadal function during hypo- and hypersecretion are not clear, and controlled studies are limited. Additionally, thyroid diseases are more common in women. Recent studies have confirmed the expression of TRs in the testis at both the mRNA (reverse transcription polymerase chain reaction) and protein levels.[4,5] A study in mouse Leydig cells found that long-term T3 hormone treatment enhanced steroidogenesis in the cells in a coordinated manner.[25] Mendeluk and Rosales[6] showed that the T4 hormone added to semen increased sperm motility. In our study, experimental hypothyroidism was confirmed by analyses of TSH, fT3, and fT4. In addition, a decrease in TSH hormone levels and an increase in T3 and T4 hormone levels were observed in animals receiving CoQ10 (group 3, 4) compared to the hypothyroid group (group 2). Given that these hormones affect the process of spermatogenesis, it is logical to suggest that CoQ10 may affect the differentiation of germ cells.

Thyroid hormones are critical regulators of energy expenditure and body weight, and their deficiency usually results in decreased energy expenditure and increased adiposity.[26] Consistent with this, we observed in our study significant weight gain in animals with induced hypothyroidism. However, CoQ10 supplementation effectively attenuated this hypothyroidism-induced weight gain, suggesting its potential role in regulating thyroid hormone-related metabolic disorders. Consistent with previous studies, Yousofvand et al.[27] reported a significant decrease in testicular weight in PTU-induced hypothyroid rats. Although a decrease in testicular weight was also observed in the hypothyroid group in our study, statistical analysis did not reveal a significant difference in testicular weight between the experimental groups. This suggests that although CoQ10 supplementation attenuated the metabolic effects of hypothyroidism, it did not completely eliminate the effect on testicular weight, and, therefore, the mechanisms involved should be further investigated.

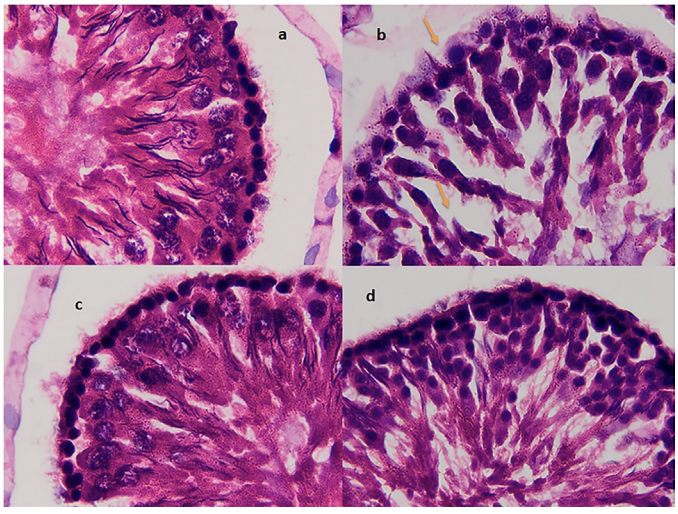

Figure 1. Hematoxylin and eosin (H&E) staining of seminiferous tubules: (a) group 1 shows regular seminiferous tubule wall and regular arrangement of spermatogenic cells. (b) group 2 shows irregular seminiferous tubule wall and disruption of spermatogenic process (orange arrow). (c) group 3 shows regular seminiferous tubule and presence of spermatogenic cell types. (d) group 4 shows a more regular seminiferous tubule structure (compared to group 2). Sections were stained using the H&E staining method, where hematoxylin stains cell nuclei blue-purple and eosin stains cytoplasm and extracellular matrix pink. Objective magnification: x100, scale bar: 20 µm.

H&E: Hematoxylin and eosin.

Lara et al.[28] reported no significant difference in tubular diameter between experimental groups in their study using PTU. In line with their findings, our study also observed a decrease in tubular diameter in the hypothyroid group. However, statistical analysis did not reveal any significant differences between the groups, suggesting that while hypothyroidism may influence tubular structure, the effect on diameter may not be substantial enough to reach statistical significance in our experimental setup.

De la Balze et al.[29] reported that testicular biopsies from adult male hypothyroid patients exhibited abnormal histological structures. Similarly, long-term hypothyroidism in adult mice resulted in a significant reduction in germ cell numbers in the epididymis, and a decreased percentage of live spermatozoa.[30] In line with these findings, our study observed in the PTU-induced hypothyroid groups a decrease in the number of seminiferous germ cells and structural defects, such as gaps between spermatogenic series cells. Interestingly, while the number of seminiferous germ cells decreased, the total number of SSCs increased. This suggests that hypothyroidism may impair the differentiation process of SSCs rather than affecting their self-renewal capacity. Notably, CoQ10 supplementation resulted in improvements in these parameters, suggesting a positive effect of CoQ10 on SSC differentiation and spermatogenesis. Consequently, our findings support the idea that hypothyroidism inhibits SSC differentiation and sperm production in the seminiferous tubules, while CoQ10 supplementation may mitigate some of these adverse effects, promoting spermatogenesis.

THY-1/CD90 (Differentiation Cluster 90) is a well-established cell surface marker of SSCs. In humans, a highly enriched population of undifferentiated SPG has been identified as β-2 microglobulin (β-2M)-SPα-6+THY1+, which further highlights the role of THY-1 in SSCs.[31] In mice, the gene expression profile of SSCs (mSSCs) also demonstrates high levels of THY-1 expression.[32] These findings indicate that THY-1 is a characteristic marker of SSCs in both humans and mice and may be involved in maintaining SSC self-renewal and differentiation. Moreover, THY-1 has been identified as a key regulator of cellular differentiation in various cell types, influencing signaling pathways and controlling cellular functions, largely through its role as an adhesion molecule on the cell membrane, rather than via intracellular pathways. Several studies have

Table 2. Biochemical results

Parameter	Group 1	Group 2	Group 3	Group 4	p-value
TSH ± SD	2.03±1.06	7.46^{a}±1.94	1.32±1.06	2.19±0.71	p=0.001
fT3 ± SD	10.05^{a}±3.57	6.85^{a}±2.21	18.32^{b}±6.66	8.44±2.91	p=0.003
fT4 ± SD	5.74^{a}±1.33	1.42^{b}±0.4	8.15^{c}±1.63	3.27±0.27	p=0.001

TSH: Thyroid-stimulating hormone, fT3: Free triiodothyronine, fT4: Freethyroxine, SD: Standard deviation, ^{a}: Similar to each other, different from ^{b} and ^{c}. ^{b}: Different from ^{a}, similar within itself. ^{c}: Different from both ^{a} and ^{b}. ^{ab}: Similar to both ^{a} and ^{b}.

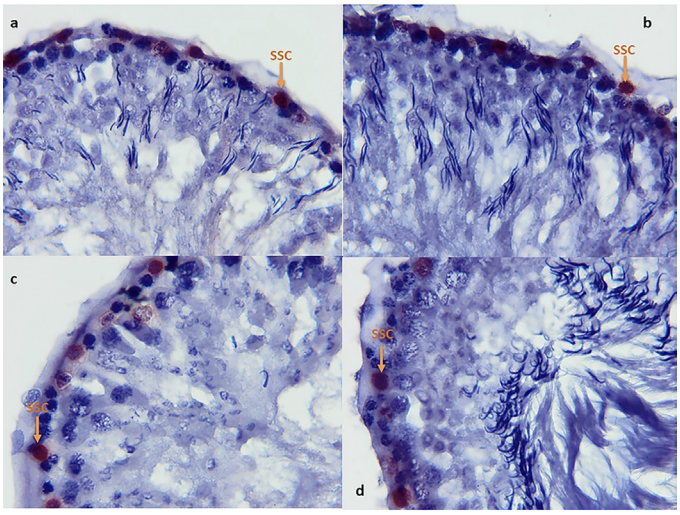

Figure 2. Immunohistochemical staining, Results: figures (a-d) show positive SOX3 staining (orange arrow). Immunohistochemical stain used: anti-SOX3. Seminiferous tubule, transverse section. Objective magnification: x100, scale bar: 20 µm.

SSC: Spermatogonial stem cell

confirmed high expression of THY-1 in undifferentiated SPG.[16,33,34] In our study, we observed strong THY-1 expression in SSCs, consistent with previous reports. To date, however, there are no studies exploring the potential effects of hypothyroidism on THY-1 expression in the male reproductive system. In contrast, Oltulu et al.[35] reported significantly decreased THY-1 expression in rat ovarian tissue in an experimental thyrotoxicosis model, suggesting a role for THY-1 in apoptosis regulation under conditions of thyroid dysfunction. In our study, THY-1 expression was strong in all experimental groups, indicating that hypothyroidism did not suppress THY-1 expression in SSCs. This suggests that the THY-1 marker may not be directly involved in apoptotic processes in response to hypothyroidism, possibly due to its role as an adhesion molecule that facilitates protein binding rather than as a direct mediator of apoptosis. Additionally, no significant differences were observed in THY-1 expression in the CoQ10-treated groups. Given that CoQ10 did not alter THY-1 expression and considering the importance of THY-1 in cellular differentiation and apoptosis, our findings suggest that CoQ10 supplementation does not significantly modulate the pathways involved in apoptosis or the cell cycle in testicular cells. Therefore, it seems unlikely that CoQ10 plays a critical role in regulating these processes under hypothyroid conditions.

SOX-3 is a key member of the SRY-related high mobility group box family of transcription factors and plays a pivotal role in testicular development and spermatogenesis.[36] It is highly expressed in SSCs, where it regulates the transition from a self-renewing state to a differentiating amplification compartment.[37] Studies have shown that SOX-3 directly targets neurogenin-3 in SPG cells, further implicating SOX-3 in the regulation of Spg differentiation. In teleost species, SOX-3 expression is upregulated during testis development, indicating its involvement in testicular function. Additionally, in black rockfish, SOX-3 plays a role in oogenesis and ovarian differentiation, exhibiting sexually dimorphic expression patterns in adult gonads.[38,39] Together, these findings suggest that SOX-3 is a critical transcription factor influencing various aspects of male reproductive development. In our study, SOX-3 protein was highly expressed in the nuclei of SSCs in groups 1 and 3, consistent with its known role in spermatogenesis. However, in groups 2 and 4 (hypothyroid and CoQ10-supplemented hypothyroid groups), a downregulation of SOX-3 protein expression was observed. This reduction in SOX-3 levels in the hypothyroid group may reflect impaired SPG differentiation, which could be a consequence of the negative effects of hypothyroidism on the regulation of the SSC differentiation process. Specifically, the decreased expression of SOX-3 in the hypothyroid group may be linked to a disruption in the differentiation of other spermatogenic lineage cells, rather than a direct effect on SSC self-renewal. Interestingly, no significant difference in SOX-3 expression was observed between the CoQ10-supplemented groups (groups 3 and 4) and the hypothyroid group (group 2). This suggests that CoQ10 supplementation did not affect SOX-3 immunoreactivity in the context of hypothyroidism. Given SOX-3's role in spermatogenesis, the lack of an effect by CoQ10 on its expression may imply CoQ10 does not significantly modulate the differentiation of SPG in hypothyroid conditions. This finding warrants further investigation into the potential mechanisms by which CoQ10 influences spermatogenesis under thyroid dysfunction.

PLZF is a SPG-specific transcription factor that plays a crucial role in regulating the self-renewal of SSCs and maintaining the SSC pool in the testis.[40] It is thought to function as a cell-autonomous factor that ensures the stability and self-renewal of SSCs.[41] PLZF also regulates SSC self-renewal by inhibiting the mammalian target of rapamycin complex 1 (mTORC1), a critical regulator of cellular processes that balances self-renewal and differentiation. Activated mTORC1 can downregulate the expression of GDNF receptors, and PLZF promotes the expression of Redd1, a negative regulator of the mTORC1 pathway, thus contributing to the maintenance of SSCs.[42]

In our study, we observed PLZF expression in SSCs, which is consistent with findings from previous studies. To our knowledge, this is the first study to report on PLZF expression in the context of hypothyroidism. We found that PLZF expression was confined to SPG, with no significant differences observed between the experimental groups. This suggests that hypothyroidism does not significantly affect PLZF expression in testicular SSCs. Similarly, CoQ10 supplementation did not influence PLZF immunoreactivity in our study. These findings suggest that PLZF may not play a direct role in DNA replication or stem cell division in the context of hypothyroidism or CoQ10 supplementation. The lack of change in PLZF expression in both the hypothyroid and CoQ10-treated groups may indicate that PLZF's regulatory role in SSCs is not impacted by these conditions.

GFRα1 is a well-known receptor for GDNF, regulating SSC self-renewal and differentiation by signaling through GFRα1, thus maintaining a balanced SSC population.[17] Meng et al.[43] who showed that GFRα1-deficient mice had a depleted SSC population, indicated that overexpression of GFRα1 led to the accumulation of undifferentiated SPG. Furthermore, the study by Meng et al.[43] highlighted the importance of the GDNF/FSH signaling axis in controlling SSC population size and maintaining the balance between self-renewal and differentiation. These findings highlight the fundamental importance of GFRα1 in regulating spermatogenesis and its potential as a therapeutic target in male infertility. Our study found that GFRα1 expression was detectable in both the hypothyroid and CoQ10-supplemented groups, which is consistent with previous reports suggesting its presence in the testis. However, no significant difference in GFRα1 expression was observed between the hypothyroid and CoQ10-treated groups. This suggests that CoQ10 supplementation may have a broader protective effect on testicular histopathology, but

does not directly affect GFRα1 expression in the testes. This result is consistent with the hypothesis that CoQ10 may attenuate metabolic and oxidative stress associated with hypothyroidism, but its effect on molecular pathways governing spermatogenesis, such as the GDNF/GFRα1 signaling pathway, may be more limited. Additionally, we observed an association between hypothyroidism and GFRα1 expression that deserves further attention. Kamyshna et al[44], previously reported a significant decrease in GFRα1 expression, in patients with primary hypothyroidism, raising the possibility that hypothyroid states may lead to decreased GFRα1 expression and thus impair SSC function and spermatogenesis. This is consistent with the findings in our study, where hypothyroidism appears to affect GFRα1 expression, potentially contributing to the observed impairments in testicular function. The relationship between thyroid dysfunction and GFRα1 expression is also supported by the study of Bilous et al.[45], who reported decreased GFRα1 levels in thyroid patients and observed GFRα1 expression in thyroid follicular cells. These findings point to a possible link between thyroid function and the regulation of spermatogenesis via GFRα1. However, in our study, CoQ10 supplementation did not significantly alter GFRα1 expression, suggesting that, although CoQ10 may exert its effects through different mechanisms, it may not directly affect the GDNF/GFRα1 signaling pathway. In conclusion, our findings reinforce the importance of GFRα1 in maintaining spermatogenesis and highlight its potential as a therapeutic target for male infertility. While hypothyroidism-induced decreases in GFRα1 expression may contribute to spermatogenic dysfunction, CoQ10 supplementation appears to offer some protective benefits, although it does not directly affect GFRα1 expression. Further research is needed to better understand the precise molecular mechanisms underlying these observations and to explore additional therapeutic strategies, including vitamin D supplementation, to restore GFRα1 expression and improve testicular function in hypothyroid individuals.

Study Limitations

Very few articles similar to the subject of this study were found in the literature review, which can be considered a limitation of this study. Although there are studies on SSC status in hypothyroid patients in the literature, they are few, and this study is one of the pioneering studies examining the relationship with antioxidants. Furthermore, the effects of vitamin D and other antioxidants were not investigated in this study, leaving room for future research.

CONCLUSION

In this study, the effects of CoQ10 supplementation on testicular tissue in adult Wistar albino rats with experimental hypothyroidism were investigated using IHC, biochemical and morphometric methods. CoQ10 given after hypothyroidism, was shown to have a beneficial effect on spermatogenesis in testicular tissue. This effect affected other SPG lineage cells rather than SSC cells. CoQ10 supplementation showed a therapeutic benefit by improving serum TSH, fT3, and fT4 levels. Since this study is a scientific study investigating SSC differentiation in hypothyroidism and also a study SSC differentiation in hypothyroidism and CoQ10, which is widely used in male infertility, on this process, we are determined to carry our research to further research topics. Low expression of the SOX-3 marker was observed after hypothyroidism. In conclusion, after this study, we plan to investigate the mechanism of SOX-3 during differentiation of SSCs with molecular and genetic studies.

MAIN POINTS

- Although there were no significant differences in testicular weight or seminiferous tubule diameter between the groups, hypothyroidism was found to inhibit differentiation of SSCs and lead to decreased sperm production in the seminiferous tubules.
- While CoQ10 supplementation does not directly affect SSCs, it has a positive effect on other stages of spermatogenesis, including primary and secondary spermatocytes, spermatids, and spermatozoa.
- CoQ10 has shown therapeutic effects on thyroid function as demonstrated by improvements in TSH, T3, and T4 levels.

ETHICS

Ethics Committee Approval: This study was carried out with the approval of the Necmettin Erbakan University Ethics Committee for Experimental Animal Research (approval number: 2022-036, date: 06.07.2022).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.N.Ş., F.Z.E., G.C., Concept: H.N.Ş., S.K., Design: H.N.Ş., G.C., Data Collection and/or Processing: H.N.Ş., F.Z.E., Analysis and/or Interpretation: H.N.Ş., F.Z.E., G.C., Literature Search: H.N.Ş., F.Z.E., G.C., S.K., Writing: H.N.Ş., F.Z.E.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Weetman AP. "Ioning" out thyroid autoimmunity. Endocr Pract. 2009; 15(1): 53-5.
2. Schwarz C, Leichtle AB, Arampatzis S, Fiedler GM, Zimmermann H, Exadaktylos AK, et al. Thyroid function and serum electrolytes: does an association exist? Swiss Med Wkly. 2012; 142: w13669.
3. La Vignera S, Vita R. Thyroid dysfunction and semen quality. Int J Immunopathol Pharmacol. 2018; 32: 2058738418775241.
4. Jannini EA, Crescenzi A, Rucci N, Screponi E, Carosa E, de Matteis A, et al. Ontogenetic pattern of thyroid hormone receptor expression in the human testis. J Clin Endocrinol Metab. 2000; 85(9): 3453-7.
5. Holsberger DR, Cooke PS. Understanding the role of thyroid hormone in Sertoli cell development: a mechanistic hypothesis. Cell Tissue Res. 2005; 322(1): 133-40.
6. Mendeluk GR, Rosales M. Thyroxin is useful to improve sperm motility. Int J Fertil Steril. 2016; 10(2): 208-14.
7. Schniertshauer D, Müller S, Mayr T, Sonntag T, Gebhard D, Bergemann J. Accelerated Regeneration of ATP Level after irradiation in human skin fibroblasts by coenzyme Q10. Photochem Photobiol. 2016; 92(3): 488-94.

8. Crane FL. Biochemical functions of coenzyme Q10. J Am Coll Nutr. 2001; 20(6): 591-8.

9. Salas-Huetos A, Rosique-Esteban N, Becerra-Tomás N, Vizmanos B, Bulló M, Salas-Salvadó J. The Effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and meta-analysis of randomized clinical trials. Adv Nutr. 2018; 9(6): 833-48.

10. Alahmar AT. Role of Oxidative Stress in Male Infertility: An Updated Review. J Hum Reprod Sci. 2019; 12(1): 4-18.

11. Safarinejad MR. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. J Urol. 2009; 182(1): 237-48.

12. de Rooij DG. The nature and dynamics of spermatogonial stem cells. Development. 2017; 144(17): 3022-30.

13. McLaren A. Primordial germ cells in the mouse. Dev Biol. 2003; 262(1): 1-15.

14. Sadri-Ardekani H, Akhondi MA, van der Veen F, Repping S, van Pelt AM. In vitro propagation of human prepubertal spermatogonial stem cells. JAMA. 2011; 305(23): 2416-8.

15. Hou J, Niu M, Liu L, Zhu Z, Wang X, Sun M, et al. Establishment and characterization of human germline stem cell line with unlimited proliferation potentials and no tumor formation. Sci Rep. 2015; 5: 16922.

16. He Z, Kokkinaki M, Jiang J, Dobrinski I, Dym M. Isolation, characterization, and culture of human spermatogonia. Biol Reprod. 2010; 82(2): 363-72.

17. Buageaw A, Sukhwani M, Ben-Yehudah A, Ehmcke J, Rawe VY, Pholpramool C, et al. GDNF family receptor alpha1 phenotype of spermatogonial stem cells in immature mouse testes. Biol Reprod. 2005; 73(5): 1011-6.

18. Costoya JA, Hobbs RM, Barna M, Cattoretti G, Manova K, Sukhwani M, et al. Essential role of Plzf in maintenance of spermatogonial stem cells. Nat Genet. 2004; 36(6): 653-9.

19. Kubota H, Avarbock MR, Brinster RL. Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells. Proc Natl Acad Sci U S A. 2004; 101(47): 16489-94.

20. Moulakakis KG, Poulakou MV, Dosios T, Dontas I, Sokolis DP, Vlachos IS, et al. Hypothyroidism and the aorta. evidence of increased oxidative DNA damage to the aorta of hypothyroid rats. In Vivo. 2008; 22(5): 603-8.

21. Johnsen SG. Testicular biopsy score counts - a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. Hormones. 1970; 1(1): 2 25.

22. Awad Hegazy A, Mohammad Morsy M, Said Moawad R, Mohammad Elsayed G. Changes in adult rats' testis structure induced by hypothyroidism and alleviating role of l-carnitine. Int J Hum Anat. 2019; 1(4): 13-28.

23. Ibrahim AA, Mohammed NA, Eid KA, Abomughaid MM, Abdelazim AM, Aboregela AM. Hypothyroidism: morphological and metabolic changes in the testis of adult albino rat and the amelioration by alpha-lipoic acid. Folia Morphol (Warsz). 2021; 80(2): 352-62.

24. Rodrigues MS, Tovo-Neto A, Rosa IF, Doretto LB, Fallah HP, Habibi HR, et al. Thyroid hormones deficiency impairs male germ cell development: a cross talk between hypothalamic-pituitary-thyroid, and-gonadal axes in zebrafish. Front Cell Dev Biol. 2022; 10: 865948.

25. Manna PR, Roy P, Clark BJ, Stocco DM, Huhtaniemi IT. Interaction of thyroid hormone and steroidogenic acute regulatory (StAR) protein in the regulation of murine Leydig cell steroidogenesis. J Steroid Biochem Mol Biol. 2001; 76(1-5): 167-77.

26. Syed MA, Thompson MP, Pachucki J, Burmeister LA. The effect of thyroid hormone on size of fat depots accounts for most of the changes in leptin mRNA and serum levels in the rat. Thyroid. 1999; 9(5): 503-12.

27. Yousofvand N, Kazemi M, Mohammadizadeh E. Effect of induced hypothyroidism by propylthiouracil (PTU) on serum levels of testosterone, luteinizing hormone, follicle stimulating hormone in adult rat. J Ilam Uni Med Sci. 2013; 21(6): 1-8.

28. Lara NLM, Silva VA Jr, Chiarini-Garcia H, Garcia SK, Debeljuk L, Hess RA, et al. Hypothyroidism induced by postnatal PTU (6-n-propyl-2-thiouracil) treatment decreases Sertoli cell number and spermatogenic efficiency in sexually mature pigs. Gen Comp Endocrinol. 2020; 299: 113593.

29. De La Balze Fa, Arrillaga F, Mancini Re, Janches M, Davidson Ow, Gurtman Ai. Male hypogonadism in hypothyroidism: a study of six cases. J Clin Endocrinol Metab. 1962; 22: 212-22.

30. Sahoo DK, Roy A, Bhanja S, Chainy GB. Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. Gen Comp Endocrinol. 2008; 156(1): 63-70.

31. Givelet M, Firlej V, Lassalle B, Gille AS, Lapoujade C, Holtzman I, et al. Transcriptional profiling of β-2M-SPα-6+THY1+ spermatogonial stem cells in human spermatogenesis. Stem Cell Reports. 2022; 17(4): 936-52.

32. Huang Y, Xiong Y, Lin Z, Feng X, Jiang X, Songyang Z, et al. Specific tandem 3'UTR patterns and gene expression profiles in mouse Thy1+ germline stem cells. PLoS One. 2015; 10(12): e0145417.

33. Kanatsu-Shinohara M, Toyokuni S, Shinohara T. CD9 is a surface marker on mouse and rat male germline stem cells. Biol Reprod. 2004; 70(1): 70-5.

34. Reding SC, Stepnoski AL, Cloninger EW, Oatley JM. THY1 is a conserved marker of undifferentiated spermatogonia in the pre-pubertal bull testis. Reproduction. 2010; 139(5): 893-903.

35. Oltulu F, Aktuğ H, Uysal A, Öktem G, Turgan N, Karabay Yavaşoğlu NÜ, et al. Thyrotoxicosis in experimental rat model of ovarian tissue regeneration capability and capacity. Ege Journal of Medicine. 2015; 54(1): 19-25.

36. Kamachi Y, Kondoh H. Sox proteins: regulators of cell fate specification and differentiation. Development. 2013; 140(20): 4129-44.

37. McAninch D, Mäkelä JA, La HM, Hughes JN, Lovell-Badge R, Hobbs RM, et al. SOX3 promotes generation of committed spermatogonia in postnatal mouse testes. Sci Rep. 2020; 10(1): 6751.

38. Rajakumar A, Senthilkumaran B. Expression analysis of SOX3 during testicular development, recrudescence, and after hCG induction in catfish, Clarias batrachus. Sex Dev. 2014; 8(6): 376-86.

39. Sreenivasan R, Gonen N, Sinclair A. SOX Genes and Their Role in Disorders of Sex Development. Sex Dev. 2022; 16(2-3): 80-91.

40. Costoya JA, Hobbs RM, Barna M, Cattoretti G, Manova K, Sukhwani M, et al. Essential role of Plzf in maintenance of spermatogonial stem cells. Nat Genet. 2004; 36(6): 653-9.

41. Phillips BT, Gassei K, Orwig KE. Spermatogonial stem cell regulation and spermatogenesis. Philos Trans R Soc Lond B Biol Sci. 2010; 365(1546): 1663-78.

42. Hobbs RM, Seandel M, Falciatori I, Rafii S, Pandolfi PP. Plzf regulates germline progenitor self-renewal by opposing mTORC1. Cell. 2010; 142(3): 468-79.

43. Meng X, Lindahl M, Hyvönen ME, Parvinen M, de Rooij DG, Hess MW, et al. Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science. 2000; 287(5457): 1489-93.

44. Kamyshna II, Pavlovych LB, Kamyshnyi AM. Vitamin D alters the transcriptional profile of blood cells in patients with primary hypothyroidism. Physiol J. 2022; 68(5): 16-24.

45. Bilous II, Korda MM, Krynytska IY, Kamyshnyi AM. Nerve impulse transmission pathway-focused genes expression analysis in patients with primary hypothyroidism and autoimmune thyroiditis. Endocr Regul. 2020; 54(2): 109-18.

RESEARCH ARTICLE

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Women's Gynecological Cancer Awareness: What is the Situation in North Cyprus?

Akile Tuncal[1], Ufuk Kaya[2]

[1]Department of Medical Biochemistry, Cyprus Health and Social Sciences University Faculty of Medicine, Morphou, North Cyprus
[2]Department of Nursing, Cyprus International University Faculty of Health Sciences, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: Awareness about gynecological cancers is important for cancer prevention. This study aimed to determine the awareness of women aged 20-65 living in North Cyprus regarding gynecological cancers and the relationship between these types of cancers.

MATERIALS AND METHODS: This research study was designed and conducted as a descriptive and cross-sectional study. As a result of the sample selection calculation made from the sample group whose population was known for the research, 400 voluntary women individuals were included. Participants who were living in North Cyprus, were married women between the ages of 20-65, who could read and write Turkish, and who did not have disabilities were included in the study. Data were collected using a form, and the general knowledge level of the participants as well as comparisons (socio-demographic characteristics and gynecological cancer statements) were determined. The data were collected by meeting participants face to face between 01 February and 30 April 2023.

RESULTS: The overall scale score average of women was determined as 150.76±25.98. Statistically significant findings were obtained when comparing the general average scale scores of the participants with their descriptive characteristics. A statistical significance was determined between scale general average scores, education level, occupation, income-expense status, knowledge about gynecological cancers, information source such as internet and scientific articles, reason for gynecological examination, and knowledge about early diagnosis of gynecological cancers (each, $p=0.001$) and birth control pill/medication use status ($p=0.002$) ($p<0.05$).

CONCLUSION: According to the study results, the awareness levels of the participants regarding gynecological cancers are above average. However, this result does not indicate the expected or desired level.

Keywords: Awareness, cancer, North Cyprus, gynecological cancer, women

INTRODUCTION

According to World Health Organization data, cancer is the world's leading cause of death and caused a total of 10 million deaths in 2020, equivalent to one in six deaths.[1] It was reported by World Cancer Research Fund International that the five most common cancer types in women as of 2020 are breast (25.8%), colorectal (9.9%), lung (8.8%), cervix uteri (6.9%) and thyroid (5.1%). In the same report, ovarian (3.6%), vulva (0.5%), and vagina (0.2%) cancers and their rates are also stated.[2]

Gynecological cancers have many risk factors. These include alcohol, body mass index, individual factors, environmental causes, genetic predisposition, inactivity, hormonal factors, occupation, perinatal development, smoking, socio-economic status, viruses, and age.[3,4] Knowing such risk factors and increasing awareness of gynecological cancers in women are among the primary health care services in cancer prevention.[5,6] Thanks to awareness, positive results can be achieved in early diagnosis, treatment, and reducing mortality among women.[7,8]

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ORCID IDs of the authors: A.T. 0000-0002-7442-1874; U.K. 0000-0002-0911-4886.

Corresponding author: Akile Tuncal
E-mail: akiletuncal@gmail.com/akile.tuncal@kstu.edu.tr
ORCID ID: orcid.org/0000-0002-7442-1874

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Based on this information, this study aimed to determine the awareness of women aged 20-65 living in North Cyprus regarding gynecological cancers. This study was conducted to determine the awareness of women aged 20-65 living in North Cyprus regarding gynecological cancers and the relationships between different types of these cancers.

MATERIALS AND METHODS

This research was designed and conducted as descriptive and cross-sectional. As a result of the sample selection calculation on a known population sample group, 400 people were included (n=400). As of the end of 2017, it was reported that the female population aged 20-65 in North Cyprus was 104,453 people.

The data were collected through a data collection form created by the researchers after reviewing the literature.[9,10] The form consists of two parts. The first section contains questions and statements indicating the descriptive characteristics of the participants. In the first section, consisting of 20 questions and statements in total, age, education level, employment status, occupation, income status, presence of health insurance, presence of children, number of children, number of pregnancies, age at first menstruation, use of birth control methods, and knowledge about gynecological cancers are collected. There is information about the patient's condition, sources of information regarding the patient, reason for going to gynecological examination, frequency of examination, smoking and alcohol use, age at menopause, knowledge about early diagnosis methods, and family history of gynecological cancer.

The second part of the form includes the Gynecological Cancer Awareness Scale (GCAS). The scale developed by Alp Dal and Ertem[11] consists of 41 items and four sub-dimensions. The Cronbach's alpha value of the scale was determined to be 0.94. 20th-41st years of GCAS. The items constitute the "Routine Control and Serious Disease Perception Awareness in Gynecological Cancers" subscale, and the Cronbach's alpha value is 0.97. Levels 3-11 of the scale. The items are the "Gynecological Cancer Risks Awareness" subscale, for which the Cronbach's alpha value is 0.84. 14th-19th on the scale. The items are the Gynecological Cancers Protection Awareness subscale, and the Cronbach's alpha value is 0.77. 1st-2nd and 12th-13th grades in the scale. The items of the "Early Diagnosis and Information Awareness in Gynecological Cancers" subscale have a Cronbach's alpha value of 0.70. The scale is evaluated on a total score basis; a minimum of 41 and a maximum of 205 points can be obtained. As the average score that individuals receive on the scale increases, their awareness also increases.[11]

In this study, the Cronbach's alpha value of the scale was determined as 0.94. Cronbach's alpha values in sub-dimensions; Routine Control and Serious Disease Perception Awareness in Gynecological Cancers was found to be 0.94, Routine Control and Serious Disease Perception Awareness was 0.94, Gynecological Cancer Risks Awareness was 0.83, Gynecological Cancers Protection Awareness was 0.69, and Early Diagnosis and Information Awareness in Gynecological Cancers was found to be was 0.74.

The data was collected by meeting participants face to face between 01 February and 30 April 2023. Application of the data collection form took an average of 10-15 minutes. Participants who were living in North Cyprus and who were married women between the ages of 20-65, could read and write Turkish, and did not have visual, auditory or mental disabilities were included in the study.

To conduct the research, ethical permission was obtained from the Cyprus Health and Social Sciences University (approval number: KSTU/2023/145, date: 01.02.2023); scale permission was obtained from the authors who conducted the GCSA Turkish validity and reliability study; and written consent was obtained from the participants who were included in the study during the data collection process.

Statistical Analysis

Statistical Package for Social Sciences 25.0 program was used for the statistical analysis of the data. The distributions of the descriptive characteristics of the participants are determined as numbers (n) and percentages (%) and are shown in tables. The average values of the scale are calculated as mean ± standard deviation and are presented in the tables. The Kolmogorov-Smirnov Z test was applied to evaluate whether the data conformed to the normal distribution. It was determined that the data did not conform to a normal distribution. Accordingly, Mann-Whitney U (U) tests were applied for binary variables, and Kruskal-Wallis H (X^2) tests were applied for more than two variables, and the results are given in the comparison tables. Pearson correlation analysis (r) was applied to determine the correlation between descriptive features and scale score average. Data were evaluated and interpreted at a 95% confidence interval and a significance level of $p<0.05$.

RESULTS

Table 1 shows the participants' mean scores on the scale and its subscales. Women's GCSA general score average was 150.76±25.98. The mean score of the Routine Control and Serious Disease Perception Awareness in Gynecological Cancers Sub-Dimension is 85.50±17.05, the Gynecological Cancer Risks Awareness Sub-Dimension is 27.71±6.92, the Gynecological Cancer Protection Awareness Sub-Dimension is 21.07±4.89, and the Early Diagnosis and Information Awareness in Gynecological Cancers Sub-Dimension is 27.71±6.92. The average score was found to be 16.47±3.29 (Table 1).

The average age of the participants in the study was found to be 40.26±11.44. 40% (n=160) of the women were university graduates, 77% (n=308) were working, and 22% (n=88) were working in the management-civil service field, 47% (n=188) had income, and it was determined that their expenses were balanced, while 52% (n=208) did not have health insurance. 67.5% (n=270) of the participants had children, 32.5% (n=130) had two children, and 31% (n=124) had two pregnancies. The average age of women's first menstrual period is

Table 1. Average score distribution of scale general and sub-dimensions (n=400)

Scale	Av.	SD	Min.	Max.
GCSA General Score Average	150.76	25.98	41	196
Routine Control and Serious Disease Perception Awareness in Gynecological Cancers	85.50	17.05	22	110
Gynecological Cancer Risk Awareness	27.71	6.92	9	43
Gynecological Cancers Protection Awareness	21.07	4.89	6	30
Early Diagnosis and Information Awareness in Gynecological Cancers	16.47	3.29	4	20

Av: Average, SD: Standard deviation, Min.: Minimum, Max.: Maximum.

12.11±2.92. Seventy-five percent (n=300) of the participants did not use birth control pills/medicines, 71% (n=284) had information about gynecological cancers, 32.5% (n=130) said their source of information was the internet, 45.5% (n=182) had a gynecological examination when they had any complaints, 34% (n=136) had a gynecological examination once a year, and 26% (n=104) smoked while 28% (n=112) consumed alcohol. This information has been detected. The average age of women at menopause is 47.55±6.45. It was determined that 42% (n=168) of the participants did not have sufficient knowledge about gynecological cancer early diagnosis methods and 30% (n=120) had a family history of gynecological cancer (Table 2).

The distribution of participants' GCSA general average scores is shown in Table 2. The variables with the five highest awareness levels are: 1) Individuals whose source of information is scientific articles (163.00±13.32) (highest), 2) Individuals whose profession is in the field of management-civil service (160.43±23.29), 3) Individuals who have a postgraduate education (158.87±15.86), 4) Individuals who are women who have three children (158.60±25.62), and 5) Individuals whose income exceeds their expenses (157.86±25.18). The variables with the lowest awareness level are 1) Literacy level (124.00 ± 25.40), which is the lowest, 2) Not having a gynecological examination (136.32±35.95), 3) Not having knowledge about gynecological cancers (139.17±26.53),

Table 2. Descriptive characteristics of the participants and statistical comparison of these characteristics and the scale general score averages (n=400)

Descriptive characteristic	n	%	Av. ± SD	Min.	Max.	Test value	p	Difference
Education level								
Literate	4	1.0	124.00±25.40	102	146	X^2=20.759	**0.001***	2-6 5-6
Primary school	38	9.5	142.57±27.50	86	189			
Middle school	16	4.0	147.00±37.29	64	179			
High school	116	29.0	154.50±29.65	46	196			
Undergraduate	160	40.0	147.68±23.51	41	191			
Graduate	66	16.5	**158.87±15.86**	126	194			
Employment status								
Yes	308	23.0	**151.01±26.45**	41	196	U=13600.000	0.559	-
No	92	77.0	149.91±24.43	64	188			
Occupation								
Education	58	14.5	154.62±15.53	122	192	X^2=22.295	**0.001***	1-3 2-3 2-4
Management/officer	88	22.0	**160.43±23.29**	90	196			
Health	66	16.5	140.12±31.51	41	186			
Other	96	24.0	147.31±27.24	69	196			
None	92	23.0	150.30±24.70	64	188			
Income status								
Low	154	38.5	144.45±24.65	64	194	X^2=20.368	**0.001***	1-2 1-3
Moderate	188	47.0	153.73±26.31	41	196			
High	58	14.5	**157.86±25.18**	117	196			
Presence of health insurance								
Yes	192	48.0	149.40±28.24	41	192	U=19784.000	0.873	-
No	208	52.0	**152.00±23.69**	69	196			
Presence of children								
Yes	270	67.0	150.38±27.16	41	194	U=17518.000	0.976	-
No	133	33.0	**155.21±25.58**	64	196			
Number of children								
One	92	23.0	149.93±29.29	46	194	X^2=5.528	0.237	-
Two	130	32.5	151.09±23.83	41	192			
Three	30	7.5	**158.60±25.62**	102	191			
Mora than three	16	4.0	142.62±25.98	103	184			
None	132	33.0	150.21±25.58	64	196			
Number of pregnancies								
One	78	19.5	151.35±30.72	46	194	X^2=1.606	0.808	-
Two	124	31.0	149.80±27.31	41	192			
Three	40	10.0	149.65±21.04	102	188			
More than three	30	7.5	151.06±23.96	103	191			
None	128	32.0	**151.59±23.58**	69	196			

Table 2.Continued

Descriptive characteristic	n	%	Av. ± SD	Min.	Max.	Test value	p	Difference
Use of birth control methods								
Yes	100	25.0	146.20±20.48	81	194	U=11840.000	**0.002***	-
No	300	75.0	**152.28±27.43**	41	196			
Knowledge about gynecological cancers								
Yes	284	71.0	**155.49±24.25**	46	196	U=9286.000	**0.001***	-
No	116	29.0	139.17±26.53	41	196			
Sources of information								
Health-care proffesionals	122	30.5	152.13±25.57	46	192	U=15230.000	0.104	-
Internet	130	32.5	157.40±21.97	90	196	U=13618.000	**0.001***	
Scientific article	44	11.0	**163.00±13.32**	142	192	U=4982.000	**0.001***	
Newspaper	18	4.5	158.66±9.22	145	172	U=2658.000	0.104	
Relative/neighbour	48	12.0	145.91±28.15	64	192	U=7592.000	0.255	
Other	20	5.0	160.50±24.44	122	191	U=3074.000	0.150	
Reasons for going to gynecological examination								
In case of unbearable distress	44	11.0	143.40±26.35	64	184	X^2=26.720	**0.001***	1-3 2-4 3-4
In case of complaint	182	45.5	152.39±21.96	46	196			
Routine	118	29.5	**157.83±22.75**	81	196			
None	56	14.0	136.32±35.95	41	189			
Frequency of examination								
Once in a six month	20	5.0	151.70±38.67	46	184	X^2=10.496	0.062	-
Once a year	136	34.0	153.51±22.07	81	192			
Once in two years	34	8.5	151.23±27.01	64	194			
Every three years	24	6.0	**154.75±22.55**	102	184			
Less frequently	30	20.0	149.25±25.56	86	196			
None	106	26.5	147.13±28.39	41	192			
Smoking status								
Yes	104	26.0	148.05±24.50	69	191	X^2=2.366	0.306	-
No	272	68.0	151.41±26.83	41	196			
Quit	24	6.0	**155.00±21.90**	115	191			
Intaking alcohol status								
Yes	112	28.0	153.25±22.47	69	194	X^2=0.715	0.699	-
No	282	70.5	149.77±27.41	41	196			
Quit	6	1.5	150.66±14.54	138	169			
Knowledge about early diagnosis methods								
Knowledgeable enough	50	12.5	154.66±26.29	81	184	X^2=23.492	**0.001***	1-3 2-3
Knowledgeable	182	45.5	**155.91±23.14**	46	196			
Not enough	168	42.0	143.92±27.35	41	196			
Family history of gynecological cancers								
Yes	120	30.0	150.15±31.97	46	196	U=16242.000	0.598	-
No	280	70.0	**151.02±23.00**	41	192			

Av.: Average, SD: Standard deviation, Min.: Minimum, Max.: Maximum, p: Significance level, X^2: Kruskal-Wallis, U: Mann-Whitney, *p<0.05.

(4) Working in the health profession group (140.12±31.51) and having more than three children (142.62±25.98) (Table 2).

Statistically significant findings, were obtained when comparing the GCSA general average scores of the participants with their descriptive characteristics. Scale general average scores and education level, occupation, income status, knowledge about gynecological cancers, information source (internet and scientific articles), reason for going to gynecological examination, knowledge about early diagnosis of gynecological cancers (each; p=0.001) and statistical significance was determined between birth control pill/medication use status (p=0.002) (p<0.05) (Table 2).

DISCUSSION

In this study, which investigated the awareness of gynecological cancers and the relationship between them among women aged 20-65 living in North Cyprus, the participants' overall scale score average was determined as 150.76±25.98. This result can indicate that women's awareness levels are above average. There are studies in the literature with similar results across different populations.

In Kıyak and Burucu[10] research examining university students' awareness of gynecological cancers and related factors, the overall score average of the scale was found to be 154.5, and in Kaya Şenol et al.[9] study on women of reproductive age and postmonopausal period, it was found to be 150.7. In Özcan and Demir Doğan[12] study, the average score was determined as 150.53, and in Teskereci et al.[13] study, it was 151.08. The reason women's awareness of gynecological cancers is above average is their high level of education. In the study, 342 women had an equivalent education level of high school or above.

In the study, statistical significance was determined among variables such as women's education level, occupation, financial status, birth control pill/medication use, knowledge about gynecological cancers, reasons for gynecological examination, and knowledge about early diagnosis of gynecological cancers. In Öztürk et al.[14] study, a statistically significant relationship was found between the general score average and financial status. In Atlas and Er Güneri[5] study, statistical significance was determined among the scale's overall score average and the variables of education level, profession, and reason for applying to the polyclinic.

In the study of Alp Dal et al.[7] statistical significance was found between the general score average, knowledge about female reproductive organ cancers, and knowledge about early diagnosis of female cancers. Socio-demographic conditions, such as education level, related profession, and economic status, can affect awareness levels positively and/or negatively. In addition, awareness of situation-specific factors such as gynecological cancers, prevention methods, and early diagnosis may affect the awareness of the subject.

The study also includes the general averages of the scale scores and the relationships resulting from the correlation analysis. Accordingly, occupation, income-expense status, birth control pill/medication use status, knowledge about gynecological cancers, internet information source, scientific article information source, and gynecological cancer early diagnosis knowledge status were determined as related factors. In the study of Teskereci et al.[13], knowledge about gynecological cancers was identified as a contributing factor. In the study of Erenoglu and Bayraktar[15], knowledge about gynecological cancers was stated as a statistical factor.

There is a limited amount of literature measuring gynecological cancer awareness. In the literature, it is possible to find awareness studies focused primarily on a single type, such as cervical, ovarian, or endometrial cancer. Gynecological cancers continue to exist as an important public health issue. It is important to determine awareness levels and increase investments in education and medical research for the future so that precautions can be taken in this area. However, it should be noted that the most important issue is education.[16]

Study Limitations

The study group, women aged 20-65 living in the North part of Cyprus, can be considered a limitation of the study. The results should not be generalized to the whole world or a larger group.

CONCLUSION

According to the study results, the awareness levels of the participants regarding gynecological cancers are above average. However, this result does not indicate the expected and/or desired level. It may be recommended to conduct more research on the lack of information on the subject in the literature, enhance public health training for awareness, focus on programs, and make educational investments, such as informative advertisements, posters, and brochures, at the public level on the subject.

MAIN POINTS

- Gynecological cancers account for a significant number of cancer-related deaths worldwide (10 million as of 2020).
- The knowledge of women who participated in the study about gynecological cancers was determined to be at a moderate level (150.76±25.98).
- Individuals' level of education, field of work, income level, birth control method, knowledge about gynecological cancers, information sources, reasons for gynecological examination, and knowledge of early diagnosis methods are significant factors affecting their knowledge levels ($p<0.05$).
- Information sources are scientific articles and the knowledge levels of people working as management/officer servants are higher.

ETHICS

Ethics Committee Approval: The study received approval from Cyprus Health and Social Sciences University Ethics Committee (approval number: KSTU/2023/145, date: 01.02.2023).

Informed Consent: Written consent was obtained from the participants who were included in the study during the data collection process.

Footnotes

Authorship Contributions

Concept: A.T., U.K., Design: A.T., U.K., Data Collection and/or Processing: A.T., U.K., Analysis and/or Interpretation: A.T., U.K., Literature Search: A.T., U.K., Writing: A.T., U.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Cancer. World Health Organization; 2022. Cancer. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer
2. World Cancer Research Fund International; 2022. Available from: https://www.wcrf.org/preventing-cancer/cancer-statistics/worldwide-cancer-data/
3. Gözüyeşil E, Arıöz DA, Ünal AKS. Kadınların serviks kanserinden korunma ve erken tanıya yönelik tutumlarının değerlendirilmesi. STED. 2019; 28(4): 228-37.
4. Teskereci G, Öncel S, Özer Arslan Ü. Evaluation of the Women Attending Vocational Courses in Terms of Risk Factors and Early Warning Signs Related to Gynaecological Cancers. Sted. 2020; 29(4): 229-238.
5. Atlas B, Er Güneri S. Kadınların Jinekolojik Kanserlerle İlgili Farkındalığı ve Farkındalığı Etkileyen Faktörler. İKÇÜSBFD. 2022; 7(1): 77-85.
6. Eroğlu K, Koç G. Jinekolojik Kanser Kontrolü ve Hemşirelik. Hacettepe Üniversitesi Hemşirelik Fakültesi Dergisi. 2015; 1(2): 77-90.
7. Alp Dal N, Akkuzu G, Çetinkaya Şen Y. UFUK ÜNİVERSİTESİ KADIN ÇALIŞANLARININ JİNEKOLOJİK KANSER FARKINDALIĞININ İNCELENMESİ. Journal of Midwifery and Health Sciences. 2020; 3(2): 91-9.
8. Köse S, Karakurt P. kadınların jinekolojik kanserler ile ilgili farkındalık düzeyinin artmasında sağlık okuryazarlığının etkisi üzerine bir araştırma. mersin üniversitesi tıp fakültesi lokman hekim tıp tarihi ve folklorik tıp dergisi. 2023; 13(1): 196-206.
9. Kaya Şenol D, Polat F, Doğan M. Gynecological Cancer Awareness: Reproductive Age and Postmenopausal Women. TJFMPC. 2021; 15(1): 56-62.
10. Kıyak S, Burucu R. Gynecological Cancer Awareness of University Students and Related Factors. Sted. 2022; 31(3): 172-182.
11. Alp Dal N, Ertem G. JİNEKOLOJİK KANSERLER FARKINDALIK ÖLÇEĞİ GELİŞTİRME ÇALIŞMASI. İnsan Ve Toplum Bilimleri Araştırmaları Dergisi. 2017; 6(5): 2351-2367.
12. Özcan H, Demir Doğan M. Gynecological Cancer Awareness Among Women. Indian Journal of Gynecologic Oncology. 2021: 19(13): 1-9.
13. Teskereci G, Arslan ÜÖ, Öncel S. The awareness levels of women for gynecologic cancer in Turkey: A cross-sectional study. Int J Gynaecol Obstet. 2022; 156(3): 539-45.
14. Öztürk R, Bakir S, Kazankaya F, Paker S, Ertem G. Awareness about Gynecologic Cancers and Related Factors among Healthy Women: a cross-sectional study. Soc Work Public Health. 2021; 36(7-8): 847-56.
15. Erenoglu R, Bayraktar E. Awareness Levels of Married Women Aged 20-60 Years about Gynaecological Cancer and the Affecting Factors. International Journal of Caring Sciences. 2020; 13(1): 457.
16. Novinson D, Puckett M, Townsend J, Reichhardt M, Tareg A, Palemar J, et al. Increasing Awareness of Gynecologic Cancer Risks and Symptoms among Asian, Native Hawaiian and Pacific Islander Women in the US-Associated Pacific Island Jurisdictions. Asian Pac J Cancer Prev. 2017; 18(8): 2127-33.

RESEARCH ARTICLE

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Evaluation of Serum Uric Acid Levels in Patients with Lichen Planus: A Case-Control Study

Doğuş Eşberk[1], İçim Kömürcügil[2], Nermin Karaosmanoğlu[3]

[1]Clinic of Dermatology, Osmaniye State Hospital, Osmaniye, Türkiye
[2]Department of Dermatology, Near East University Faculty of Medicine, Nicosia, North Cyprus
[3]Clinic of Dermatology, University of Health Sciences Türkiye, Ankara Training and Research Hospital, Ankara, Türkiye

Abstract

BACKGROUND/AIMS: The objective was to assess uric acid (UA) levels in lichen planus (LP) patients and to investigate the impact of oxidative stress in LP etiopathogenesis.

MATERIALS AND METHODS: Fiftieth participants with LP and 50 participants with tinea unguium, all aged 18 years and older, were included as the control group. Sociodemographic data including age, gender, and medical history were documented. In LP patients, disease duration, disease pattern, and severity, current, and past treatments were recorded. UA, total bilirubin, direct bilirubin, indirect bilirubin, were measured for both groups.

RESULTS: This study included 50 LP patients (35 females, 15 males) and 50 patients with tinea unguium (28 females, 22 males) for the control group. The mean age was 52.96±12.84 years for the LP group, compared to 48.44±15.14 for the control group. The difference in age and gender distribution between the LP group and the control group was not statistically significant. Among LP patients, 70% had the localized form, 26% had the oral form, and 4% had the generalized type. Topical corticosteroids were prescribed to 86% of the patients, while 14% received systemic corticosteroids. No significant difference was recorded between the LP group and the control group regarding total, direct, and indirect bilirubin levels, as well as UA levels.

CONCLUSION: No significant difference was reported in total bilirubin, direct and indirect bilirubin, and UA between LP patients and the control group.

Keywords: Bilirubin, lichen planus, oxidative stress, uric acid

INTRODUCTION

Lichen planus (LP) is a skin disease characterized by distinct clinical and histopathological features. It appears as small, shiny, polygonal; mildly erythematous, purplish papules or plaques. Besides affecting the skin and mucous membranes, LP may also involve hair and nails. Clinically, LP presents in different types, such as classic, hypertrophic, bullous, actinic, annular, and follicular types. LP typically occurs between the ages of 30 and 70, with females being affected approximately 1.5 times more often than males.

The exact cause and pathogenesis of LP remain uncertain; it is believed to arise from the attack of cytotoxic T-lymphocytes, which target basal keratinocytes, causing apoptosis. Triggers for this autoimmune reaction

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ORCID IDs of the authors: D.E. 0000-0002-2869-4070; İ.K. 0000-0002-1753-7571; N.K. 0000-0002-3462-1628.

Corresponding author: İçim Kömürcügil
E-mail: icim.komurcugil@yahoo.com
ORCID ID: orcid.org/0000-0002-1753-7571

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include microbial antigens, metal ions, and certain medications.[1,2] Some studies have also suggested a potential relationship between hepatitis C virus and LP.[3,4] Recent research has emphasised reactive oxygen species (ROS) in dermatological disorders. In LP, CD4+ T-lymphocytes are one of the sources of ROS which damages endothelial cells, that increases the intercellular adhesion molecule 1, and facilitates T-cell infiltration and exocytosis.[2,5] Oxidative stress, through elevated tumor necrosis factor-alpha, triggers pro- and anti-apoptotic pathways.[6] It also stimulates the release of molecules like perforin and granzyme, exacerbating local tissue destruction.[7]

Uric acid (UA) is a crucial antioxidant that neutralizes ROS and sequesters metal ions.[8-10] Therefore, measuring UA may be important in the clinical management of LP. The objective here is to evaluate UA levels in LP patients and investigate oxidative stress in the etiopathogenesis of LP.

MATERIALS AND METHODS

Fifty LP patients aged 18 and older, all of whom were diagnosed with LP based on clinical presentation and histopathology, were included. The participants were randomly selected from the Clinic of Dermatology and Venereology, University of Health Sciences Türkiye, Ankara Training and Research Hospital between May 2020 and August 2022. Additionally, 50 patients with tinea unguium, all aged 18 and older, were included as the control group. The diagnosis of LP was based on clinical presentation and histopathological confirmation, while tinea unguium was confirmed through clinical examination. Patients were not restricted to newly diagnosed cases and individuals with varying disease durations were included. The control group was selected without specific demographic matching, though efforts were made to ensure a comparable distribution of age and gender. Power analysis was not performed, however, a sample size of 50 patients per group was deemed appropriate based on previous studies examining oxidative stress markers in dermatological conditions and the feasibility of recruitment within the study period. The study received approval from the University of Health Sciences Türkiye, Ankara Training and Research Hospital Ethics Committee (approval number: 978/2022, date: 27.07.2022), and was conducted according to the Declaration of Helsinki and Good Clinical and Laboratory Practices. All participants gave informed consent. Individuals with renal failure, pregnancy, or breastfeeding, as well as those with conditions or medications known to elevate UA levels, were excluded. Patients with congenital, hepatic, cholestatic or hemolytic diseases or medications that could increase bilirubin levels were also excluded. Sociodemographic data including age, gender, and medical history were recorded for both groups. For the LP group, the duration of the disease, treatments received, the extent of the disease, and the pattern of involvement were thoroughly evaluated and documented. Serum UA, total bilirubin, direct and indirect bilirubin levels were measured in the LP group and the control group.

Statistical Analysis

IBM SPSS Statistics 22 software was used for statistical analyses. The normality of data distribution was evaluated with Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics (mean, standard deviation, frequency) were used to summarize the data. For comparing quantitative data, Student's t-test and ANOVA were applied to normally distributed parameters, while Mann-Whitney U test and Kruskal-Wallis test were used for non-normally distributed parameters. Qualitative data were analyzed using Fisher's exact chi-square test, Fisher-Freeman-Halton exact chi-square test, and Continuity (Yates) Correction. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 100 patients (50 LP patients and 50 control patients) participated in this study. Among LP patients, 35 were female (70%) and 15 were male (30%), while the control group included 28 females (56%) and 22 males (44%). Gender distribution was not statistically different between the LP group and the control group ($p>0.05$). The age of the LP group varied from 22 to 84 years, with a mean of 52.96±12.84. The age of the control group varied between 20 and 74 years, with a mean of 48.44±15.14. The mean age between the two groups did not show a significant difference ($p>0.05$). 42% of the LP group and 36% of the control group had additional comorbidities, with no significant difference in medical history between the two groups ($p>0.05$) (Table 1).

24% of the LP group had a disease duration of 0 to 6 months, 24% had a disease duration of greater than 6 to 12 months, and 52% had a disease duration of more than one year. In the patient group, 70% were diagnosed with localized LP, 26% with oral LP, and 8% with generalized LP. It was found that 86% received topical corticosteroid treatment, while 14% received systemic corticosteroid treatment (Table 2).

The mean UA level in the LP group was 5.01±1.32, while the mean UA level in the control group was 4.71±1.31. The difference in UA levels between the groups was not statistically significant ($p>0.05$). The median total bilirubin was 0.37 [interquartile range (IQR): 0.22] in the LP group and 0.44 (IQR: 0.28) in the control group. The median direct bilirubin was 0.15 (IQR: 0.08) in the LP group, whereas it was 0.18 (IQR: 0.12) in the control group. The median indirect bilirubin in

Table 1. Demographic characteristics of the patient and control groups

		Patient	Control	p
Gender, n (%)	Female	35 (70%)	28 (56%)	0.214[1]
	Male	15 (30%)	22 (44%)	
Personal history, n (%)	None	29 (58%)	32 (64%)	0.557[2]
	Hypertension	6 (12%)	9 (18%)	
	DM	4 (8%)	2 (4%)	
	HT + DM	1 (2%)	2 (4%)	
	Malignancy	2 (4%)	0 (0%)	
	Other	8 (16%)	5 (10%)	
Age mean ± SD		52.96±12.84	48.44±15.14	0.111[3]

[1]Continuity (Yates's) correction, [2]Fisher-Freeman-Halton exact test, [3]Student's t-test, HT: Hypertension, DM: Diabetes mellitus.

Table 2. Clinical characteristics of the patient group

		n	%
Disease duration	0-6 month	12	24
	>6-12 months	12	24
	>1 year	26	52
Subtype	Localised	35	70
	Oral	13	26
	Generalised	2	4
Treatment	Topical	43	86
	Systemic	7	14
	Total	50	100

the LP group was 0.2 (IQR: 0.17); and 0.25 (IQR: 0.21) in the control group. There was no statistically significant difference in the median total, direct, and indirect bilirubin levels between the LP and the control group (p>0.05). UA, total bilirubin, direct bilirubin and indirect bilirubin levels are shown in Table 3.

UA levels were normal in 41 patients (82%) in the LP group and 39 individuals (78%) in the control group with no significant difference between them (p>0.05). Total and indirect bilirubin levels were within normal reference ranges in all individuals in the LP group, and the control group. Direct bilirubin levels were normal in 47 patients (94%) in the LP group and 49 individuals (98%) in the control group, with no significant difference between the two groups (p>0.05) (Table 4).

UA, total bilirubin, direct bilirubin, and indirect bilirubin values of the different subtypes of LP (localized LP, oral LP, and generalized LP) were compared. No significant difference was observed in UA, total bilirubin, direct bilirubin, and indirect bilirubin levels among patients with localized LP, oral LP, and generalized LP (p>0.05) (Table 5).

DISCUSSION

LP can affect the skin, mucous membranes, hair, and nails.[1,2] Although LP can affect individuals at any age, it is mostly seen in individuals between 30 and 70 years old. The prevalence in the population is thought to range between 0.5% and 1%.[11] Some studies have shown a female predominance in the gender distribution of LP. For instance, McCartan et al.[12] reported a prevalence of 0.96% in males and 1.57% in females. In a study with 1,335 LP patients, 67.5% of the patients were female.[13] Le Cleach and Chosidow[14] reported that 60-70% of oral LP patients were female and had an average age of 50-60 years, while 50% of cutaneous LP patients were female and had an average age of 40-45 years. In a study of 232 LP patients 53.9% of the patients were female and 46.1% were male, with an average age between 40-49 years.[15] In this study, the gender distribution, age of onset, and disease duration were consistent with the literature.

Currently, there is no standardised scoring system to assess the severity of LP. In a study with 444 LP patients, the involvement of a single anatomical region was classified as localized, while involvement of two or more regions was classified as generalized, involvement of only oral, only genital, or both mucosal sites was classified as mucosal, and involvement of only the nails was classified as nail LP. 48% of patients had localized LP, 38% had generalized LP, 13% had mucosal LP, and 1% had nail LP.[16] Similarly, in this study, LP involvement was classified into 3 categories: localized LP for a single anatomical region, generalized LP for two or more regions, and oral LP for cases limited to the oral mucosa. In this study, it was found that 70% of the patients had localized LP, 26% had oral LP, and 4% had generalized LP.

UA is an antioxidant that scavenges free radicals and reduces oxidative stress. LP is an idiopathic disease with autoimmunity, infections, oxidative stress, and dental procedures as triggering factors. Recently, the role of oxidative stress has been emphasised in its disease pathogenesis.[2] However, limited studies have explored the relationship between UA and LP, with most indicating that UA levels are lower in patients with LP. A case-control study involving 58 LP patients diagnosed both clinically and histopathologically examined the relationship between LP and UA. The mean UA in the patient group was significantly lower than in the control group, and a similar decline in UA levels was observed within the patient group as disease severity and duration increased. Therefore, it was concluded that UA has a significant role in antioxidant mechanisms and in the development of LP.[17] In another case-control study involving 39 LP patients, UA levels were notably increased in the control group and during disease remission, implying that UA is an important antioxidant in LP patients.[18] In a study involving 43 oral LP patients, the link between serum and saliva UA levels and psychosocial factors was investigated. The patient group had lower saliva and serum UA levels compared to controls.[19] Additionally, a case-control study by Darczuk et al.[20] involving 40 oral LP patients evaluated the association

Table 3. Uric acid and bilirubin levels of the patient and control groups

	Patient	Control	p
	Mean ± SD/ median (IQR)	Mean ± SD/ median (IQR)	
Uric acid (mean ± SD)	5.01±1.32	4.71±1.31	0.255[1]
Total bilirubin [median, (IQR)]	0.37 (0.22)	0.44 (0.28)	0.113[2]
Direct bilirubin [median, (IQR)]	0.15 (0.08)	0.18 (0.12)	0.113[2]
Indirect bilirubin [median, (IQR)]	0.2 (0.17)	0.25 (0.21)	0.187[2]

[1]Student's t-test, [2]Mann-Whitney U test, IQR: Interquartile range, SD: Standard deviation.

Table 4. Evaluation of uric acid and bilirubin levels of the patient and control groups according to reference values

		Patient	Control	p
		n (%)	n (%)	
Uric acid	Normal	41 (82%)	39 (78%)	0.803[1]
	Out of normal range	9 (18%)	11 (22%)	
Total bilirubin	Normal	50 (100%)	50 (100%)	-
	Out of normal range	0 (0%)	0 (0%)	
Direct bilirubin	Normal	47 (94%)	49 (98%)	0.617[2]
	Out of normal range	3 (6%)	1 (2%)	
Indirect bilirubin	Normal	50 (100%)	50 (100%)	-
	Out of normal range	0 (0%)	0 (0%)	

[1]Continuity (Yates's) correction, [2]Fisher's exact test.

Table 5. Uric acid and bilirubin levels of disease subtype

	Localised lichen planus, (n=35)	Oral lichen planus, (n=15)	Generalised lichen planus, (n=2)	p
	Mean ± SD/ median (IQR)	Mean ± SD/ median (IQR)	Mean ± SD/ median (IQR)	
Uric acid (mean ± SD)	5.15±1.44	4.53±0.96	5.30±0	0.806[1]
Total bilirubin [median, (IQR)]	0.39 (0.19)	0.34 (0.39)	0.34 (-)	0.891[2]
Direct bilirubin [median, (IQR)]	0.15 (0.10)	0.13 (0.07)	0.18 (-)	0.453[2]
Indirect bilirubin [median, (IQR)]	0.24 (0.17)	0.20 (0.32)	0.16 (-)	0.530[2]

[1]ANOVA test, [2]Kruskal-Wallis test, SD: Standard deviation, IQR: Interquartile range.

between UA levels, tyrosine levels, and glutathione peroxidase activity. Consistent with previous findings, all three markers were considerably lower in the patient group. In this study, UA levels of the LP group and the control group were not statistically different from each other. Lower UA levels would be expected in LP, as the condition is thought to be associated with increased oxidative stress. However, higher UA levels in the LP group may be attributed to additional diseases like diabetes and hypertension, which are known to affect UA metabolism. Further studies with larger sample sizes and better control for comorbidities are needed to clarify the relationship between UA and LP.

There are only a few studies investigating serum bilirubin levels in dermatological conditions. In a study with 214 patients diagnosed with psoriasis vulgaris, the association between severity of the disease and serum bilirubin was explored. The study revealed that serum bilirubin levels of the patient group were significantly lower than the control group, and that they showed an inverse correlation with PASI scores.[21] In this study, unlike previous research, differences in bilirubin between LP patients and the control group and among different LP subtypes were not statistically significant. While the role of bilirubin in managing oxidative stress has been highlighted in earlier studies, this was not supported in the current study. Possible explanations for this discrepancy include the small sample size, differences in inclusion and exclusion criteria, additional diseases of both groups, the inclusion of only LP patients who had presented to the clinic in the last two years, and social and genetic variations.

UA and bilirubin are considered to have key roles in the pathogenesis of inflammatory diseases like LP. Measuring their serum levels in such conditions can offer valuable insights into disease prognosis, treatment options, and disease monitoring. Moreover, these tests are quick, simple, and cost-effective.

In our study, UA and bilirubin did not show significant differences between LP patients and the control group. While UA and bilirubin are recognized for their antioxidant functions in LP, research on this topic is limited. Therefore, more comparative studies with larger, more diverse patient groups and longer duration of follow-up are necessary to support this view.

Study Limitations

Possible reasons for the differing results in this study compared to the literature include the small sample size, variations in inclusion and exclusion criteria, additional diseases in the patient and control groups, the focus on patients who visited the clinic in the last two years, as well as social and geographical differences, and irregular clinic attendance during the pandemic.

MAIN POINTS

- Elevated serum UA levels in LP patients suggest that UA can be a clinical marker to assess oxidative stress.
- The results support the idea that oxidative stress may be critical in the etiopathogenesis of LP. This could lead to new possibilities for investigating antioxidant therapies in the management of LP.
- Bilirubin levels, another marker of oxidative stress, did not show significant difference between LP patients and the control group, indicating that not all oxidative stress markers may be elevated in LP.
- Monitoring UA levels in LP patients might help in understanding the disease progression and potentially guide antioxidant-based therapeutic strategies.

ETHICS

Ethics Committee Approval: The study received approval from the University of Health Sciences Türkiye, Ankara Training and Research Hospital Ethics Committee (approval number: 978/2022, date: 27.07.2022).

Informed Consent: All participants gave informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.K., Concept: D.E., İ.K., Design: D.E., İ.K., N.K., Data Collection and/or Processing: D.E., N.K., Analysis and/or Interpretation: İ.K., Literature Search: D.E., İ.K., N.K., Writing: D.E., İ.K., N.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Wagner G, Rose C, Sachse MM. Clinical variants of lichen planus. J Dtsch Dermatol Ges. 2013; 11(4): 309-19.
2. Chakraborti G, Biswas R, Chakraborti S, Sen PK. Altered serum uric Acid level in lichen planus patients. Indian J Dermatol. 2014; 59(6): 558-61.
3. Lodi G, Pellicano R, Carrozzo M. Hepatitis C virus infection and lichen planus: a systematic review with meta-analysis. Oral Dis. 2010; 16(7): 601-12.
4. Rübsam K, Schroll A, Weisenseel P, Multhaup S, Ruzicka T, Prinz JC. Lichen planus and hepatitis virus infections: causal association? J Dtsch Dermatol Ges. 2011; 9(6): 464-8.
5. Sezer E, Ozugurlu F, Ozyurt H, Sahin S, Etikan I. Lipid peroxidation and antioxidant status in lichen planus. Clin Exp Dermatol. 2007; 32(4): 430-4.
6. Shakibaei M, Schulze-Tanzil G, Takada Y, Aggarwal BB. Redox regulation of apoptosis by members of the TNF superfamily. Antioxid Redox Signal. 2005; 7(3-4): 482-96.
7. Hendel A, Hiebert PR, Boivin WA, Williams SJ, Granville DJ. Granzymes in age-related cardiovascular and pulmonary diseases. Cell Death Differ. 2010; 17(4): 596-606.
8. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. Curr Pharm Des. 2005; 11(32): 4145-51.
9. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med. 2008; 359(17): 1811-21.
10. Venkateshappa C, Harish G, Mahadevan A, Srinivas Bharath MM, Shankar SK. Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. Neurochem Res. 2012; 37(8): 1601-14.

11. Gorouhi F, Davari P, Fazel N. Cutaneous and mucosal lichen planus: a comprehensive review of clinical subtypes, risk factors, diagnosis, and prognosis. ScientificWorldJournal. 2014; 2014: 742826.

12. McCartan BE, Healy CM. The reported prevalence of oral lichen planus: a review and critique. J Oral Pathol Med. 2008; 37(8): 447-53.

13. Prakash S Mohan R, Ghanta S, Verma S, Agarwal N, Gupta N, et al. Meteorological influences on the incidence of lichen planus in a north Indian population. J Oral Sci. 2013; 55(4): 311-8.

14. Le Cleach L, Chosidow O. Clinical practice. Lichen planus. N Engl J Med. 2012; 366(8): 723-32.

15. Bhattacharya M, Kaur I, Kumar B. Lichen planus: a clinical and epidemiological study. J Dermatol. 2000; 27(9): 576-82.

16. Schwager Z, Stern M, Cohen J, Femia A. Clinical epidemiology and treatment of lichen planus: A retrospective review of 2 tertiary care centers. J Am Acad Dermatol. 2019; 81(6): 1397-9.

17. Kutzing MK, Firestein BL. Altered uric acid levels and disease states. J Pharmacol Exp Ther. 2008; 324(1): 1-7.

18. Gupta A, Mohan RP, Gupta S, Malik SS, Goel S, Kamarthi N. Roles of serum uric acid, prolactin levels, and psychosocial factors in oral lichen planus. J Oral Sci. 2017; 59(1): 139-46.

19. Shaw H, Konidena A, Malhotra A, Yumnam N, Farooq F, Bansal V. Psychological status and uric acid levels in oral lichen planus patients - A case- control study. Indian J Dent Res. 2020; 31(3): 368-75.

20. Darczuk D, Krzyściak W, Bystrowska B, Kęsek B, Kościelniak D, Chomyszyn-Gajewska M, et al. The Relationship between the Concentration of Salivary Tyrosine and Antioxidants in Patients with Oral Lichen Planus. Oxid Med Cell Longev. 2019; 2019: 5801570.

21. Zhou ZX, Chen JK, Hong YY, Zhou R, Zhou DM, Sun LY, et al. Relationship Between the Serum Total Bilirubin and Inflammation in Patients With Psoriasis Vulgaris. J Clin Lab Anal. 2016; 30(5): 768-75.

RESEARCH ARTICLE

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Bipolar Electrocautery Cryptolisis Method for Chronic Caseous Tonsillitis

Hasan Şafakoğulları, Remzi Tınazlı, Eda Tuna Yalçınozan, Pertev Gündüz

Department of Otorhinolaryngology, Near East University Faculty of Medicine, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: The aim of this study was to evaluate the efficacy and safety of bipolar electrocautery cryptolysis (BEC) in treating chronic caseous tonsillitis (CCT), a condition commonly associated with halitosis and foreign body sensation (FBS) in the throat.

MATERIALS AND METHODS: CCT patients with halitosis will be evaluated with Finkelstein's tonsil smelling test and those with FBS will be evaluated with Visual Analogue Scale scoring. Pain levels, bleeding, and the time taken for patients to start work and achieve full recovery were assessed. Forty-two patients with CCT and complaints of halitosis were included in the study. Twenty-nine of these patients also had a FBS in their throat. All patients underwent BEC and they were followed for 12 months. Data were analyzed with IBM SPSS V23.

RESULTS: After 12 months, 64.29% of patients with halitosis and 65.52% of patients with FBS experienced improvements in their symptoms.

CONCLUSION: When we compare the laser and radiofrequency (RF) cryptolysis results in the existing literature with our BEC-C results, it can be concluded that it is as safe, comfortable, and effective as both procedures in the treatment of halitosis and FBS. The primary advantage of BEC over laser and RF methods is its greater economic feasibility, making it a more accessible treatment option in a variety of clinical settings.

Keywords: Caseous, tonsillitis, bipolar electrocautery, halitosis, foreign body

INTRODUCTION

Chronic caseous tonsillitis (CCT) can cause uncomfortable halitosis and an irritating foreign body sensation (FBS) in the throat. The unique anatomical and microbiological features of palatine tonsils play a role in the development of CCT. The crypts are tubular structures covered by stratified squamous epithelium, which invaginates from the surface of the palatine tonsils to the depths of the parenchyma. Each tonsil contains about 10-20 crypts.[1] Food residues, dead cells of the tonsils, and cell debris can accumulate in these crypts over time. These accumulations result in a yellowish soft malodorous mass called the caseum. As a result, a suitable environment for anaerobic bacteria occurs in the palatine tonsils.

Regardless of the cause of CCT, signs and symptoms of halitosis and FBS are well defined in CCT. It has been reported that up to 77% of individuals suffering from CCT experience complaints of halitosis.[2] Halitosis, which can lead to social withdrawal and even depressive symptoms, is caused by odorous compounds such as volatile sulfur, hydrogen sulfide (H_2S), methyl mercaptans (CH3SH), and dimethyl sulfide, which are produced by anaerobic proteolytic bacteria. Apart from halitosis, an irritating FBS is also common in the throat. This is due to caseum accumulation in the crypts that leads to inflammation, congestion, and hypertrophy of the palatine tonsils.

Initially, CCT treatment is conservative, involving topical antiseptics, anti-inflammatory agents, or oral antibiotics.[3] If conservative treatment does not work, alternative therapeutic interventions should

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ORCID IDs of the authors: H.Ş. 0000-0001-8896-4290; R.T. 0000-0003-3066-4804; E.T.Y. 0000-0001-5392-5937; P.G. 0000-0002-8277-416X.

Corresponding author: Pertev Gündüz
E-mail: pertevgunduz@gmail.com
ORCID ID: orcid.org/0000-0002-8277-416X

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be considered, including tonsil cryptolysis (TC) or surgical excision.[2] Tonsillectomy is not considered the primary treatment option, as it necessitates general anesthesia and presents a risk of significant postoperative complications such as hemorrhage and pain. TC may be performed using various modalities, including laser, radiofrequency (RF), or electrocautery techniques. Due to its cost effectiveness, CO_2-laser cryptolysis (LC) and temperature-controlled RF (TC-RF) are not widely used. On the other hand, bipolar electrocautery (BEC) can be used more extensively because it is relatively cheaper and easier to access.

This study was designed to show the efficacy and safety of BEC-TC in the treatment of CCT. The efficacy and safety of BEC-TC on halitosis and FBS in the treatment of CCT have not been investigated in the literature.

MATERIALS AND METHODS

The study received ethical approval from the Near East University Scientific Research Ethics Committee (approval no: 2019/72, date: 19.09.2019). All patients were informed about the study protocol, and written consent was obtained from each participant. A total of 42 patients diagnosed with CCT and halitosis were enrolled in the present clinical trial. Among them, 29 patients also had a FBS in their throat.

Statistical Analysis

Data were analyzed with IBM SPSS V23. Data distribution normality was assessed using the Shapiro-Wilk test. The Mann-Whitney U test was used to compare data that were non-normally distributed, between the paired groups. The Friedman test were used to compare three or more non-normally distributed data over time. Spearman's rho correlation coefficient was used to examine the relationship between non-normally distributed quantitative data. Categorical data are presented in the form of frequency (percentage) and quantitative data are presented as mean ± standard deviation, median (minimum-maximum). The significance level was taken as $p<0.05$.

Inclusion Criteria

The inclusion criteria were as follows: being over the age of 18, positive Finklestein tonsil smell test for halitosis, relief of FBS with the removal of the caseum, complaints that do not respond to routine medical treatment (10-day course of antibiotics for anaerobic bacteria and a 2-week saline mouthwash), and normal coagulation tests.

The severity of the halitosis was evaluated as follows:

Grade score 0: No symptom.

Grade score 1: Mild: Foul odor detected during the tonsil smell test, when the material was brought close to the examiner's nose, but no bad breath perceived when sniffing exhaled air from the patient's nose or mouth.

Grade score 2: Moderate: Bad breath is felt when sniffing the air exhaled from the patient's nares or mouth, or smelling a strong fetid odor from the material at a distance of 10 cm on tonsil smelling test.

Grade score 3: Severe: When bad breath is felt during a conversation with the patient.

FBS was evaluated by a Visual Analogue Scale (VAS). Namely, score 0 showed no signs of FBS, while score 10 showed the most severe signs.

Exclusion Criteria

Patients with the following conditions were excluded from the study. Diseases related to the tooth, gingiva, nose, sinus, pharynx, larynx, lung, gastrointestinal tract, hepatorenal system, and systemic diseases such as diabetes mellitus can affect overall health. Also, patients who are at risk for surgical intervention, use of electrocautery and anesthesia, and chronic medication (for example, those with clotting disorders, taking drugs related to chorionic villus sampling) were excluded.

These procedures were performed under local anesthesia. For BEC TC, the Covidien Force FX Electrosurgical Generator was set to a power level of 20-2. BEC probes were inserted into tonsillar crypts, and energy was applied until blanching of the tonsils was achieved (Figure 1). All patients were observed in the hospital for 3 hours following the procedure. Pain levels and bleeding amounts were evaluated as outcome measures. Pain was assesed, during the intervention, and on days 1, 3, 7 and 10 following the procedure, using a VAS. The patients were also asked how many days after the procedure they started to work again and how many days after the procedure they no longer felt pain. Patients were re-examined at 1, 3, and 12 months following the procedure, and the severity of halitosis and FBS was recorded postoperatively.

RESULTS

Forty-two patients, who had a CCT with halitosis, underwent BEC-TC. Twenty-nine of these patients also had FBS in the throat. Twenty-four of the patients who had CCT with halitosis, were female, and eighteen were male. In the group where FBS was also present, there were 18 females and 11 males. Some descriptive statistics for the quantitative data of patients are shown in Table 1.

In both the halitosis and the FBS group, there was a statistically significant difference between the medians of post-operative pain levels with respect to time ($p<0.001$). The mean number of days for patients in the halitosis group to start work were 1.48 (±0.66), while the days for full recovery were 10.91 (±3.96). Likewise, the mean number of days until patients start work was 1.87 (±0.69) and for full recovery was 11.01 (±4.11) in the FBS group (Table 1).

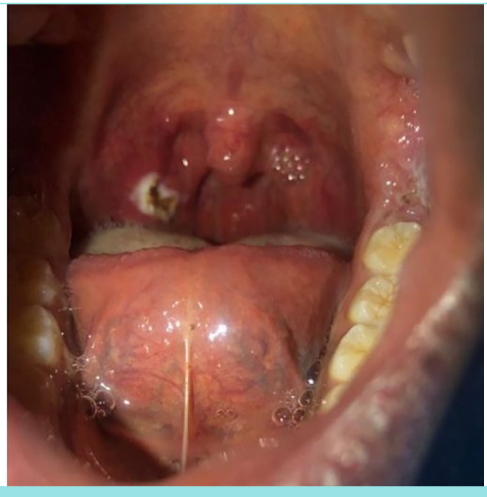

Figure 1. The view of he right tonsil after removal of caseous material and electrocauterization.

According to Mann Whitney U test, there was no significant difference between the medians of the number of tonsillitis per year and gender in both groups. And according to Spearmaans'rho test, there was no significant relationship between age and number of tonsillitis per year in both groups.

In both halitosis and FBS groups, there was a significant relationship between postoperative pain levels and the day of full recovery. Also, full recovery day and the time to start work showed a significant relationship (Table 2).

There was a statistically significant difference between halitosis improvement and postoperative times (p<0.001). Pre-operative mean halitosis scores were 2.31 (±0.67) and 1.21 (±1.10) at the 12th month. Also, it was found that 17 (40.5%) of the patients had severe halitosis during the pre-operative period and 7 (16.7%) had severe halitosis in the 12th month. At the 12th month, 27 of 42 patients with halitosis had recovered. Of these 27 patients who recovered, 13 (30.95%) had no symptoms. 14 participants (33.33%) had mild symptoms. Eight patients (19.05%) with moderate symptoms and 7 patients (16.67%) with severe symptoms were considered unsuccessful at the end of the 12 months (Table 3).

Table 1. Some descriptive statistics of quantitative data of patients with halitosis and foreign body sensation

Patients	Parameters	Mean ± SD	Median	Test statistics	p
			(min.-max.)		
Halitosis n (42)	Female (n=24) 57.1%				
	Male (n=18) 42.9%				
	Age	32.33±12.01	29 (17-67)		
	Tonsillitis per year	1.62±1.34	1.5 (0-5)		
	Full recovery day	10.91±3.96	10 (6-21)		
	Day to start work	1.48±0.66	1(1-3)		
	Post-op. mean pain level				
	1st day	0.45±0.16	0.47 (0.17-0.82)[a]		
	3rd day	0.35±0.15	0.37 (0.12-0.68)[a]	χ^2=114,714	<0.001
	7th day	0.18±0.15	0.16 (0.02-0.81)[b]		
	10th day	0.05±0.06	0.03 (0-0.27)[c]		
	Female (n=18) 62.1%				
	Male (n=11) 37.9%				
	Age	31.01±10.75	29 (17-67)		
	Tonsillitis per year	1.59±1.3	2 (0-4)		
Halitosis with foreign	Full recovery day	11.01±4.11	10 (6-21)		
Body	Day to start work	1.87±0.69	1 (1-3)		
Sensation	Post-op. mean pain level				
(FBS) n (29)	1st day	0.45±0.16	0.47 (0.17-0.82)[a]		
	3rd day	0.36±0.14	0.40 (0.12-0.68)[a]	χ^2=77,152	<0.001
	7th day	0.19±0.17	0.16 (0.03-0.81)[b]		
	10th day	0.05±0.06	0.03 (0-0.27)[c]		

χ2: Friedman test statistic, [a-c]: No difference between days with the same letter. SD: Standard deviation, min.: Minimum, max.: Maximum, FBS: Foreign body sensation.

Table 2. Relationship between post operative pain level, full recovery day to start work

Relationship between post operative pain level and full recovery day				Relationship between full recovery day and day to start work		
	Post-op. pain level	Full recovery day			Full recovery day	
		r	p		r	p
Halitosis	1. day	0.224	0.153	Start to work	0.773	**<0.001**
	3. day	0.462	**0.002**			
	7. day	0.759	**<0.001**			
	10. day	0.804	**<0.001**			
Foreign body sensation	1. day	0.194	0.313	Start to work	0.856	**<0.001**
	3. day	0.321	0.089			
	7. day	0.704	**<0.001**			
	10. day	0.775	**<0.001**			

r: Spearman's rhocor relation coefficient.

There was a statistically significant difference between pre- and post-operative medians of FBS scores (p<0.001). Pre-operative mean VAS score was 6.48, post-operative 1st month, 3.17, 3th month, 3.48, and 12th month was 3.62 (Table 4). 12th month VAS scores of FBS; 6 (20.69 %) patients had VAS score 0-1, 8 patient (27.59 %) had 2-3 scores and 7 patients (24.14 %) had 4-5 scores. However, 8 patients had an unsuccessful result of 6 or more VAS scores (Table 4).

In evaluating the impact of the treatment on symptom severity, the results show different improvements for each patient. The levels of response to treatment in patients, a very good improvement is defined as a 3-grade improvement for halitosis and an improvement of 6 or more in VAS scores for FBS. Good improvement means; only 2 grades improvement for halitosis and 4 or 5 scores improvement in VAS for FBS. Partial improvement means; only 1 grades improvement for halitosis and 2 or 3 scors improvement in VAS for FBS. No improvement means: no improvement or worse than pre-op for halitosis and FBS. Among 42 patients with halitosis, 1 patient reported significant improvement, while 18 patients showed good improvement. Eight patients had partial improvement, and fifteen patients showed no improvement, with some even experiencing worsening symptoms. Similarly, in the group of 17 patients with a sensation of a FBS, 4 patients showed very good improvement, 10 patients showed good improvement, and 5 patients showed partial improvement. However, 10 patients did not experience any improvement, and their symptoms, either stayed the same or worsened (Table 5).

DISCUSSION

The palatine tonsils serve as an ideal environment for gas-producing bacteria due to their unique anatomical and microbiological properties, making them a common source of halitosis in addition to acute localized infections.[4] The surface epithelium of the tonsils extends into the parenchyma, forming invaginations that create tonsillar crypts.[5]

Even in the core of normal or seemingly non-inflamed tonsils, there is a mixed flora of aerobic and anaerobic bacteria.[6] Among the most common organisms are gram-positive cocci[5], such as *Staphylococcus* and *Streptococcus* species, as well as anaerobes like *Prevotella, Fusobacterium,* and *Peptococcus*, which are known to produce volatile sulfur compounds such as H_2S and CH3SH.[7]

Electrocautery, a cost-effective and widely available tool, has been increasingly used for its utility in performing tonsillotomy procedures.[8] However, to our knowledge, no study has been published in the English-language literature specifically investigating the use of BEC for tonsillar cryptolysis in patients with halitosis and FBS due to CCT. Therefore, this publication will be a novel contribution to the literature. However, there are a considerable number of studies with laser or RF in the treatment of halitosis and FBS in the literature.

Finkelstein et al.[5] evaluated the effectiveness of CO_2 LC (CO_2-LC) for treating halitosis in 53 patients with CCT and found that 52.8% of patients achieved halitosis resolution after a single session. Two sessions were required for 34% of patients, and three sessions were necessary for 9%.

Table 3. Mean and number (percentage) of halitosis before and at different time points after the intervention (first, third, and twelfth month) are shown. (Halitosis severity according to Finklestein tonsil smell test: no symptom means grade score 0, mild symptoms means grade score 1, moderate symptoms means grade score 2, and severe symptoms means grade score 3.)

Halitosis	Grade scores	Median	No symptom (grade 0)	Mild (grade 1)	Moderate (grade 2)	Severe (grade 3)
	Mean ± SD	(min.-max.)	n (%)	n (%)	n (%)	n (%)
Pre-op	2.31±0.67	2 (1-3)[a]	0 (0)[a]	4 (9.5)	21 (50)	17 (40.5)
Postop 1. month	0.83±0.99	1 (0-3)[b]	18 (42.9)[b]	17 (40.5)	3 (7.1)	4 (9.5)
Postop 3. month	1.1±1.05	1 (0-3)[b]	14 (33.3)[b]	15 (35.7)	8 (19)	5 (11.9)
Postop 12. month	1.21±1.10	1 (0-3)[b]	13 (30.95)[b]	14 (33.3)	8 (19.05)	7 (16.67)
Test statistic x^2=	68,012	p<0.001				

χ^2: Friedman test statistic, [a-b]: No difference between days with the same letter. SD: Standard deviation, Min.: Minimum, Max.: Maximum.

Table 4. Mean and number (percentage) of foreign body sensation before and at different time points after the intervention (first, third and twelfth month) are shown. Foreign body sensation was evaluated with the visual analog scale (VAS). Namely, score 0 did not indicate any foreign body sensation, while score 10 indicated severe foreign body sensation

Foreign body			VAS scores				
Sensation	VAS scores	Median	0-1	2, 3	4, 5	6, 7	8, 9, 10
	Mean ± SD	min.-max.	n (%)	n (%)	n (%)	n (%)	n (%)
Preop	6.48±1.12	7 (4-8)[a]	0 (0)	0 0%	5 (17.24)	19 (65.52)	5 (17.24)
Postop 1. month	3.17±2.27	3 (0-7)[b]	7 (24.14)	9 (31.03)	5 (17.24)	8 (27.59)	0 (0)
Postop 3. month	3.48±2.37	4 (0-7)[b]	6 (20.69)	8 (27.59)	6 (20.69)	8 (27.59)	1 (3.44)
Postop 12. month	3.62±2.38	4 (0-8)[b]	6 (20.69)	8 (27.59)	7 (24.14)	7 (24.14)	1 (3.44)
Test statistic x^2=	39,861	p<0.001					

χ^2: Friedman test statistic, [a-b]: No difference between days with the same letter. Min.: Minimum, Max.: Maximum, SD: Standard deviation, VAS: Visual Analogue Scale.

Table 5. The levels of improvement in halitosis and foreign body sensation symptoms as a result of patient's response

Levels of improvement	Halitosis n (%)	FBS n (%)
Very good improvement	1 (2.38)	4 (13.80)
Good improvement	18 (42.86)	10 (34.48)
Partial improvement	8 (19.05)	5 (17.24)
No improvement	15 (35.71)	10 (34.48)

Very good improvement means a 3-grade improvement for halitosis and an improvement of 6 or more in VAS scores for FBS. Good improvement means; only 2 grades improvement for halitosis and 4 or 5 scors improvement in VAS for FBS. Partial improvement means; only 1 grades improvement for halitosis and 2 or 3 scors improvement in VAS for FBS. No improvement means; no improvement or worser than pre-op for halitosis and FBS.
VAS: Visual Analogue Scale, FBS: Foreign body sensation.

Hashemian et al.[2] used laser and RF in his study, and found a success rate of 76.9% in halitosis and 90.9% in FBS with laser therapy. Dal Rio, on the other hand, in a study investigating the effects of CO2-LC, found improvement in halitosis after the procedure in all patients and also found that volatile sulfur compounds were reduced by 30.1%. Caseum retention was also found to be significantly reduced.[9]

In another retrospective study, the efficacy of RF cryptolysis in the treatment of halitosis associated with CCT was investigated. The results showed that the mean VAS score was significantly reduced from 6.82±1.45 to 0.88±2.5 after 12 months. In addition, after one session of RF cryptolysis, 76.5% of the patients were found to be negative in the Finkelstein test.[10]

Another study comparing bipolar RF cryptolysis with monopolar RF cryptolysis in the treatment of patients with halitosis showed superior results with bipolar RF.[11]

In our study, BEC was applied in a single session. At the end of the 12th month, an improvement was observed in 27 (64.29%) of the cases with halitosis. Of these, 13 (31%) of these 27 patients had no symptoms. Considering all our patients, it was observed that the preoperative halitosis mean symptom grade decreased from 2.31 to 1.21 at the 12th month (Table 3).

We obtained similar results in the patients in the FBS group. At the end of 12 months, we had 21 (72.42%) patients who have 5 or less in VAS score. The number of patients with VAS score more than 5 was 8 (27.58%) (Table 4).

In other words, 8 patients showed a one-grade improvement in their Finkelstein smell test scores for halitosis, while 19 patients, approximately 45%, showed a good or very good (two- or three-grade) improvement. Again, in the FBS group, we found that 4 patients (13.80%) improved very well and 10 (34.48%) showed good improvement at the end of the 12th month. There was no improvement in 10 of 29 patients (34.48%) (Table 5).

These findings suggested that a second or third treatment session might be necessary in patients who show partial or no response.

Our study also assessed recovery time, with patients returning to work after a mean of 1.48 days post-BEC for halitosis alone and 1.87 days post-BEC for FBS. Pain levels, as assessed by the VAS, showed a significant decrease from day 1 (mean score of 0.45) to day 10 (mean score of 0.05). This indicates that discomfort and symptoms; including procedural pain, swallowing difficulties, and throat discomfort, resolved by a mean of 10.91 days for halitosis patients and 11.01 days for FBS patients (Table 1). These findings are consistent with those found by Finkelstein et al.[5] regarding the time of transition to a regular diet.

In their studies, Hashemian et al.[2] found that patients returned to regular diet in 1-3 days following LC.[5] In addition, Hashemian found the time to return to a regular diet following the RF procedure was longer for RF than for laser (3.1 days for RF and 1.9 days for LC).

Hashemian et al.[2] detected minimal bleeding that stopped spontaneously in one of five patients who underwent RF; and found significantly less bleeding in the laser applied group compared to RF. Similarly, in the Tanyeri study, after the RF procedure, only 1 out of 58 patients detected very little bleeding. In addition, a tonsillectomy was performed in a patient who experienced bleeding 24 hours after the procedure.

In our study, 10 of the 42 patients experienced mild, short-term spontaneous leakage during the procedure, but none required intervention. No significant bleeding was reported in the patients who were discharged. These results suggest that BEC is a safe, effective, and well-tolerated procedure for the treatment of halitosis and FBS due to CCT. It offers a viable alternative to tonsillectomy, with minimal risk of bleeding and a relatively shorter recovery time. This technique may serve as an attractive option for patients seeking treatment for these conditions.

Study Limitations

Since each individual has a different pain threshold, variations in responses to the questions and non-compliance with the prescribed diet constitute limiting factors for the study.

CONCLUSION

In conclusion, when we evaluate the results of laser and RF cryptolysis in English publications and our BEC-C results, we can conclude that all three procedures are effective in the treatment of halitosis and FBS in the throat. Also, all three procedures have minimal postoperative bleeding and low pain levels. It has been observed that after these procedures, patients can return to normal working life quickly. However, a notable advantage of the BEC-C procedure is its cost-effectiveness compared to laser and RF treatments, making it more accessible and feasible in a wider range of clinical settings. Additionally, based on the results of our study, we suggest that multiple treatment sessions may enhance the success rate of BEC-C in managing halitosis and FBS. Future studies could further explore the potential benefits of repeated sessions on the treatment of halitosis and FBS in CCT.

MAIN POINTS

- Chronic caseous tonsillitis (CCT) can cause an unpleasant halitosis and foreign body sensation in the throat.
- Bipolar electrocautery cryptolisis method is as successful as laser and radiofrequency in the treatment of CCT.
- Electrocautery is a safe, inexpensive and easily accessible instrument that can be used in every clinic.

ETHICS

Ethics Committee Approval: The study received ethical approval from the Near East University Scientific Research Ethics Committee (approval no: 2019/72, date: 19.09.2019).

Informed Consent: All patients were informed about the study protocol, and written consent was obtained from each participant.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.Ş., Concept: E.T.Y., Design: P.G., Data Collection and/or Processing: P.G., Analysis and/or Interpretation: R.T., Literature Search: R.T., Writing: R.T.

DISCLOSURES

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REFERENCES

1. Krespi YP, Kizhner V. Laser tonsil cryptolysis: in-office 500 cases review. Am J Otolaryngol. 2013; 34(5): 420-4.
2. Hashemian F, Jafari Moez H, Seif Rabiei MA, Jahanshahi J. Comparing the Efficacy of Temperature-Controlled Radiofrequency Tonsil Ablation versus CO2-Laser Cryptolysis in the Treatment of Halitosis. Iran J Otorhinolaryngol. 2018; 30(98): 159-66.
3. Ferguson M, Aydin M, Mickel J. Halitosis and the tonsils: a review of management. Otolaryngol Head Neck Surg. 2014; 151(4): 567-74.
4. Perry M, Whyte A. Immunology of the tonsils. Immunol Today. 1998; 19(9): 414-21.
5. Finkelstein Y, Talmi YP, Ophir D, Berger G. Laser cryptolysis for the treatment of halitosis. Otolaryngol Head Neck Surg. 2004; 131(4): 372-7.
6. Abbey K, Kawabata I. Computerized three-dimensional reconstruction of the crypt system of the palatine tonsil. Acta Otolaryngol Suppl. 1988; 454: 39-42.
7. Brook I, Yocum P, Foote PA Jr. Changes in the core tonsillar bacteriology of recurrent tonsillitis: 1977-1993. Clin Infect Dis. 1995; 21(1): 171-6.
8. Kazikdas KC, Safak MA. Bipolar-assisted tonsil reduction: a simple and inaexpensive tonsillotomy technique. ENT Updates. 2018; 8(1): 51-5.
9. Dal Rio AC, Passos CA, Nicola JH, Nicola EM. CO_2 laser cryptolysis by coagulation for the treatment of halitosis. Photomed Laser Surg. 2006; 24(5): 630-6.
10. Ata N, Ovet G, Alataş N. Effectiveness of radiofrequency cryptolysis for the treatment of halitosis due to caseums. Am J Otolaryngol. 2014; 35(2): 93-8.
11. Vogt K, Konuhova S, Peksis K, Markow J. Development and clinical evaluation of bipolar radiofrequency cryptolysis. Otorhinolaryngol Head Neck Surg. 2016; 2.

RESEARCH ARTICLE

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Short and Medium-Term Results of Aspirational Atherectomy in Occulative Peripheral Artery Diseases

Cem Lale, Orhan Veli Doğan

Department of Cardiovascular Surgery, Mustafa Kemal University Faculty of Tayfur Ata Sökmen Medicine, Hatay, Türkiye

Abstract

BACKGROUND/AIMS: In this prospective cross-sectional study, the short and mid-term results of aspirational atherectomy in lower extremity occlusive peripheral arterial diseases (PADs) were evaluated.

MATERIALS AND METHODS: The research was conducted between May 2017 and September 2018 at the Cardiovascular Surgery Clinic of a university hospital. A total of 34 patients who had lower extremity occlusive PAD and underwent aspirational atherectomy were included in the study. During the follow-up visits at the 24th hour, 1st month, 6th month, and 1st year, the patients' physical examinations, complaints, Rutherford and Fontaine clinical staging, and Transatlantic Intersocietal Consensus II (TASC II) classifications of the lesions before the procedure were obtained. Demographically, gender, age, smoking, peripheral procedure history, and chronic diseases were evaluated.

RESULTS: The frequency of Fontaine 3 or less, which was 47.1% before the operation, increased to 79.4% in the 6th month ($p<0.05$). The frequency of Rutherford scores of 2 or lower increased from 47.1% before the operation to 79.4% at 6 months ($p<0.05$). The rate of those with a Fontaine score of 3 or less was 85.3% in the first month after the operation, and it decreased to 79.4% in the sixth month ($p>0.05$). Similarly, while the rate of those with a Rutherford score of 2 or less was 85.3% in the first month, it decreased to 79.4% in the sixth month ($p>0.05$). The proportion of patients with Fontaine 3 and below and Rutherford 2 and below decreased from 79.4% in the 6th month to 76.5% in the 12th month. There was no significant difference between both Fontaine and Rutherford scores at 6 and 12 months after the operation ($p<0.05$)

CONCLUSION: Amputation rates may be further reduced by shortening the follow-up intervals and applying a multidisciplinary approach in diabetic patients with TASC II D lesions.

Keywords: Critical leg ischemia, aspirational atherectomy, occulative peripheral artery diseases

INTRODUCTION

Peripheral artery disease (PAD) is a health problem that begins with excessive fatty deposits on the walls of blood vessels.[1] These deposits form when blood vessels narrow due to atherosclerosis and are unable to send enough blood to the areas they supply.[2-4] Chronic diseases (CD) cause increased morbidity and mortality among pulmoner arteriyel hipertansiyon (PAH) cases.[5-7] Therefore, treatment for PAH covers a wide spectrum, including assessing, monitoring, and treating risk factors, as well as medications, surgical and endovascular interventions, and life modifications.

Endovascular aspiration atherectomy is one of the treatment methods for PAH.[8] In 1984, the American Medical Association recognized that endovascular procedures may be an alternative to coronary artery bypass grafting.[9] After becoming widely accepted in the medical world, these methods were first introduced in the United States of America in 1989, adding intervention practice to the curriculum of selected health care professionals.[10-12] Endovascular interventions include percutaneous transluminal angioplasty (PTA), stent placement, and atherectomy procedures. When using PTA and stenting, the atherosclerotic plaque is pressed against the vessel wall, and thus patency of the lumen is ensured.[13,14] However, such applications may result in complications

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ORCID IDs of the authors: C.L. 0009-0006-8380-8743; O.V.D. 0000-0001-5313-019X.

Corresponding author: Cem Lale
E-mail: cemlale@gmail.com
ORCID ID: orcid.org/0009-0006-8380-8743

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such as barotrauma and dissection that threaten lumen patency. The main mechanism of operation of atherectomy devices is to remove plaque that narrows or completely blocks the lumen using various methods.

The Rutherford and Fontaine classifications are used PAH, the most recent being the Transatlantic Intersocietal Consensus (TASC), a document that emerged from a meeting in 2000 to assess recommendations for epidemiology, treatment, and surveillance. The guidelines were revised in 2007 and renamed TASC II.[15-17] With the development of endovascular interventions, many recent studies have shown that they also produce successful results in TASC II C and D lesions. However, more clinical data are needed regarding the results of endovascular aspiration atherectomy in cases of PAH. Therefore, this study aimed to evaluate the short- and medium-term outcomes of patients who were diagnosed with PAH and followed up and underwent endovascular aspiration atherectomy.

MATERIALS AND METHODS

Patient Group

A total of 34 patients, diagnosed with PAD between January 2017 and September 2018, aged 18 to 85 years, who had near claudication, ischemic rest pain, ischemic ulcer, or tissue loss, Rutherford stage 3 and higher, Fontaine stage 2/b and higher, were included. The research data were collected within the scope of the first author's master thesis and a scientific project, for which permission was also obtained for the years 2017-2018.

Although there is no study directly compatible with our study, the closest study was conducted by Sixt et al.[17] In the power analysis performed on the sample of this study using G*power 3.1.9.2, the effect value was found to be 0.7500000. With this effect value, the minimum number of patients to be included was determined as 21, with a 95% confidence interval and a significance level of 0.05.

Patients who were seen by a physician before the procedure and were using insulin or oral antidiabetic medications were considered to have diabetes. Hypertension (HT) patients were those who were diagnosed by a physician and started antihypertensive treatment. Patients seen by a cardiologist and treated after coronary angiography were assessed as having chronic artery disease (CAD). Patients diagnosed with renal failure, receiving routine hemodialysis treatment, were assessed as having chronic kidney disease (CKD). Patients who were seen by a neurologist and had a history of stroke were assessed as having left ventricular hypertrophy based on a review of their medical records. Before the procedure, patients' height and weight were measured, and their body mass index (BMI) was calculated and recorded. Patients who smoked before the procedure were recorded as active smokers. Bleeding or hematoma in the intervention area during the first 30 days after the procedure was considered a complication. The study included patients whose lesions were suitable for interventional procedures and atherectomy, and who had short-distance claudication, ischemic pain at rest, and ischemic ulceration. Patients were excluded if they lacked consent, or had arterial dissection, loss of motor function, or known drug allergies.

Surgical Method

After obtaining informed consent from the patients and administering a local anesthetic (prilocaine 2% subcutaneously), a sheath was placed using the Seldinger technique according to the location and classification of the lesion (TASC II classification), and 5000 units of heparin were administered intravenously. A 7 French sheath was inserted using an anterograde approach in 26 patients (76.5%) and a retrograde approach in 8 patients (23.5%). After this, an appropriate amount of contrast agent (OMNIPAQUE 300 mg/100 mL) was injected, and an image was taken. The lesion was crossed using a hydrophilic guidewire (0.035-0.0014 hydrophilic Radiofocus, Terumo, Tokyo, Japan) and, if necessary, a support catheter (NAVICROSS®, also from Terumo, Tokyo, Japan). Asuction catheter for atherectomy was passed over the guidewire to access the lesion and the lumen was opened. After the procedure, a drug-coated balloon (In.Pact Admiral, Medtronic, Dublin, Ireland; Lutonix, Bard, GA, USA) was attached to the lesion. During use, the balloon was inflated to the appropriate pressure values and then allowed to rest for three minutes. Then, the balloon with the drug was deflated and control angiography was performed. After the procedure, patients were treated with heparin for 24 hours (25,000 units/24 hours) and received ASA 100 mg and clopidogrel 75 mg during follow-up.

Statistical Analysis

Nominal and ordinal data were identified by their frequency distributions. Measurement parameters were determined by the median and range. When analyzing differences, the McNemar test was used because of linearization deviations.[18,19] All analyses were conducted in SPSS 25.0 for Windows with 95% confidence intervals and a significance level of 0.05.

Ethical Considerations

This study was carried out with the approval of the Clinical Research Ethics Committee of the Faculty of Tayfur Ata Sökmen Medicine of the Hatay Mustafa Kemal University (approval number: 04, date: 01.03.2018).

RESULTS

17.6% of the patients were female and 82.4% were male. The age range was 28 to 85 years and the BMI was 21 to 35.5. Half of the patients were smokers. The distribution of comorbidities was as follows: 73.5% HT, 61.8% diabetes mellitus, 17.6% hyperlipidemia, 17.6% CAD, 8.8% CKD, and 2.9% cerebrovascular event. The length of stay was 1 to 7 days and had a median value of 1.4. The complaints included 40.6% complaints of foot and leg pain, 49.3% complaints of rest pain, 17.4% complaints of color change, and 5.8% complaints of short distance claudication (Table 1).

Fontaine score was 3 or less in 47.1% of the patients, and above 3 in 52.9% of the patients. At the end of the first month, the percentage of those with a Fountaine score of 3 or less was 85.3%, and the percentage of those with a score above 3 was 11.8%. While the Rutherford score was 2 or less in 47.1% before the operation, this rate increased to 85.2% after the operation. The differences in Fontaine and Rutherford scores before the operation and at the first month were statistically significant ($p<0.05$) (Table 2).

The frequency of Fontain 3 or less, which was 47.1% before the operation, increased to 79.4 in the 6th month ($p<0.05$). The frequency of Rutherford scores of 2 or less increased from 47.1% before the operation to 79.4% in the 6th month ($p<0.05$) (Table 3).

Table 1. Baseline and clinical parameters of patients

Parameter	Value
Gender, n (%)	
Female	6 (17.6)
Male	28 (82.4)
Age, years, median (min.-max.)	62.5 (28.0-85.0)
BMI, kg/m^2, median (min.-max.)	24.6 (21.0-35.5)
Smoking, n (%)	17 (50.0)
Comorbidity, n (%)	
HT	25 (73.5)
DM	21 (61.8)
Hyperlipidemia	6 (17.6)
CAD	6 (17.6)
CKD	3 (8.8)
CVE	1 (2.9)
Hospitalization duration, day, median (min.-max.)	1.4 (1.0-7.0)
Complaint, n (%)	
Wound on foot and leg	14 (40.6)
Rest pain	17 (49.3)
Color change	6 (17.4)
Short distance claudication	2 (5.8)
Intervention method, n (%)	
Anterograde	26 (76.5)
Retrograde	8 (23.5)
TASCII classification, n (%)	
Type B femoro-popliteal	11 (31.9)
Type C femoro-popliteal	5 (14.5)
Type C	4 (11.6)
Type C femoral, type D popliteal	1 (2.9)
Type D femoro-popliteal	4 (11.6)
Type D	6 (17.4)
Type D SFA stent occlusion	1 (2.9)
Type D long CIA occlusion	1 (2.9)
Type D long EIA whole occlusion	1 (2.9)

HT: Hypertension, DM: Diabetes Mellitus, CAD: Chronic Artery Disease, CKD: Chronic kidney disease, CVE: Cerebrovascular event, BMI: Body mass index, TASC II: Transatlantic intersocietal consensus II, CIA: Common iliac artery, EIA: External iliac artery, Min.: Minimum, Max.: Maximum.

Table 2. Fontaine and Rutherford distributions and differences between pre-operation and 1st month

	Pre-operation	1st month	p
Fontaine, n (%)			
3 and under	16 (47.1)	29 (85.3)	0.000[a]
Over 3	18 (52.9)	4 (11.8)	
Rutherford, n (%)			
2 and under	16 (47.1)	29 (85.3)	0.000[a]
Over 3	18 (52.9)	4 (11.8)	

[a]Mc Nemar test.

The rate of those with a Fontaine score of 3 or less was 85.3% in the 1st month after the operation, and it decreased to 79.4% in the 6th month (p>0.05). Similarly, while the rate of those with a Rutherford score of 2 or less was 85.3% in the 1st month, it decreased to 79.4% in the 6th month (p>0.05) (Table 4).

The proportion of patients with Fontaine 3 and below and Rutherford 2 and below decreased from 79.4% in the 6th month to 76.5% in the 12th month. There was no significant difference between the Fontaine and Rutherford scores at 6 and 12 months after the operation (p<0.05) (Table 5).

DISCUSSION

In this research, the procedural outcomes and short- and mid-term outcomes of 34 patients who underwent aspiration atherectomy for PAD were analyzed. Results showed that 6 and 12 months after operation, patients had stable Fontaine and Rutherford scores, showing success of treatment.

In studies of PA risk factors in the literature, CD and cigarette use influence disease progression.[20-22] However, in our study, the presence

Table 3. Fontaine and Rutherford distributions and differences between pre-operation and 6th month

	Pre-operation	6th month	p
Fontaine, n (%)			
3 and under	16 (47.1)	27 (79.4)	0.000[a]
Over 3	18 (52.9)	7 (20.6)	
Rutherford, n (%)			
2 and under	16 (47.1)	27 (79.4)	0.000[a]
Over 3	18 (52.9)	7 (20.6)	

[a]Mc Nemar test.

Table 4. Fontaine and Rutherford distributions and differences between 1st month and 12th month

	1st month	6th month	p
Fontaine, n (%)			
3 and under	29 (85.3)	27 (79.4)	0.250[a]
Over 3	4 (11.8)	7 (20.6)	
Rutherford, n (%)			
2 and under	29 (85.3)	27 (79.4)	0.250[a]
Over 3	4 (11.8)	7 (20.6)	

[a]Mc Nemar test.

Table 5. Fontaine and Rutherford distributions and differences between 6th month and 12th month

	6th month	12th month	p
Fontaine, n (%)			
3 and under	27 (79.4)	26 (76.5)	0.999[a]
Over 3	7 (20.6)	8 (23.5)	
Rutherford, n (%)			
2 and under	27 (79.4)	26 (76.5)	0.999[a]
Over 3	7 (20.6)	8 (23.5)	

[a]Mc Nemar test.

of diabetes, HP, coronary artery disease, duration and amount of smoking, which are among the risk factors for PAH, did not have a statistically significant effect on the recurrence of complaints after the interventional procedure and the course of clinical staging. Although there may be many reasons for this situation, it can be stated that the single-center nature of the study and the relatively small number of samples also had an impact on the study results.

TASC II C lesions are difficult to treat endovascularly.[23-25] When examining 10 patients (29.41%) in our study, it was observed that they had TASC II C lesions, the procedure was successful in all these patients and there was no change in their complaints and clinical stages during the 1st period treatment. Follow-up for one year. TASC II A and B lesions are effectively treated endovascularly with high success rates especially using new technologies.16-21 As explained in detail in the results section of our study, this is similar to the literature with a high success rate for TASC A and B lesions, and no recurrence of complaints during 1 year of follow-up. It is important to demonstrate the feasibility of endovascular interventions with low mortality, morbidity, and procedural complication rates in groups with TASC II C and D lesions and comorbidities where surgical procedures are at high risk.

Study Limitations

The most important limitation of the study is the small number of patients. The number of patients was limited in terms of both the prevalence and incidence of the case, and the follow-up period. Generally, after the diagnosis phase in public health institutions or university hospitals, the treatment process is disrupted due to consultation with other health institutions, relocation, or other reasons. This is the case in longitudinal studies and is a limiting factor in the research.

Contributions of the Study

The most important aspect of the study is that it offers an effective solution to a health problem with high mortality and morbidity, and provides source data for clinical practices with few studies in this field.

Another implication of the study is that, according to the results obtained from the study, the hospitalization period for patients after the procedure is short and the success rate of the procedure is high. The wide age range of patients in this study, from 28 to 85 years, and the fact that the study was conducted in patients with additional risk factors for PAH and high comorbidity make it important.

CONCLUSION

Aspiration atherectomy should be considered as an important option in the treatment of surgically high-risk patients. Regardless of the patient's clinical level and complaints, short- and medium-term results are quite successful, and it has been shown that amputation rates can be further reduced by shortening the follow-up intervals and applying a multidisciplinary approach in patients with TASC II D lesions. It is clear that age is an important factor in the prognosis of PAH and that the elderly will constitute the patient population that we will encounter more frequently in our clinical practice. In addition, surgical interventions generally pose a high risk in this group of patients, as additional risk factors are frequently present in these patients.

MAIN POINTS

- The research offers an effective solution to a health problem with high mortality and morbidity, and provides source data for clinical practices, in a field with few studies. Peripheral artery disease (PAD) is an important health problem, but there have not been sufficient data on the disease and its progression. This article supports clinical data on PAD.
- The hospitalization period for patients after the procedure is short and the success rate of the procedure is high. The research supports clinical usage of the procedure with quantitative data.
- The wide age range of patients in this study, from 28 to 85 years, and the fact that the study was conducted in patients with additional risk factors for PAH and high comorbidity makes it important. Thus, research provides clinical insights on PAD for a large demographic profile.

ETHICS

Ethics Committee Approval: This study was carried out with the approval of the Clinical Research Ethics Committee of the Faculty of Tayfur Ata Sökmen Medicine of the Hatay Mustafa Kemal University (approval number: 04, date: 01.03.2018).

Informed Consent: Informed consent forms have been obtained from the patients.

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Footnotes

Authorship Contributions

Surgical and Medical Practices C.L., Concept: C.L., O.V.D., Design: C.L., O.V.D., Data Collection and/or Processing: C.L., Analysis and/or Interpretation: C.L., Literature Search: C.L., Writing: C.L.

DISCLOSURES

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REFERENCES

1. Abola MTB, Golledge J, Miyata T, Rha SW, Yan BP, Dy TC, et al. Asia-Pacific Consensus Statement on the Management of Peripheral Artery Disease: A Report from the Asian Pacific Society of Atherosclerosis and Vascular Disease Asia-Pacific Peripheral Artery Disease Consensus Statement Project Committee. J Atheroscler Thromb. 2020; 27(8): 809-907.
2. Butnariu LI, Gorduza EV, Florea L, Țarcă E, Moisă ȘM, Tradafir LM, et al. The genetic architecture of the etiology of lower extremity peripheral artery disease: current knowledge and future challenges in the era of genomic medicine. Int J Mol Sci. 2022; 23(18): 10481.
3. Pabon M, Cheng S, Altin SE, Sethi SS, Nelson MD, Moreau KL, et al. Sex differences in peripheral artery disease. Circ Res. 2022; 130(4): 496-511.
4. GBD 2019 Peripheral Artery Disease Collaborators. Global burden of peripheral artery disease and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Glob Health. 2023; 11(10): e1553-65.

5. Biscetti F, Cecchini AL, Rando MM, Nardella E, Gasbarrini A, Massetti M, et al. Principal predictors of major adverse limb events in diabetic peripheral artery disease: A narrative review. Atheroscler Plus. 2021; 46: 1-14.

6. Uyagu OD, Ofoegbu C, Ikhidero J, Chukwuka E, Enwere O, Ogierakhi O, et al. Quality assessment and comparative analysis on the recommendations of current guidelines on screening and diagnosis of peripheral arterial disease: a systematic review. BMJ Open. 2022; 12(9): e061599.

7. Criqui MH, Matsushita K, Aboyans V, Hess CN, Hicks CW, Kwan TW, et al. Lower extremity peripheral artery disease: contemporary epidemiology, management gaps, and future directions: a scientific statement from the American Heart Association. Circulation. 2021; 144(9): e171-91.

8. Wardle BG, Ambler GK, Radwan RW, Hinchliffe RJ, Twine CP. Atherectomy for peripheral arterial disease. Cochrane Database Syst Rev. 2020; 9(9): CD006680.

9. Eagle KA, Guyton RA, Davidoff R, Ewy GA, Fonger J, Gardner TJ, et al. ACC/AHA guidelines for coronary artery bypass graft surgery: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1991 Guidelines for Coronary Artery Bypass Graft Surgery). American College of Cardiology/American Heart Association. J Am Coll Cardiol. 1999; 34(4): 1262-347.

10. Tunis SR, Bass EB, Steinberg EP. The use of angioplasty, bypass surgery, and amputation in the management of peripheral vascular disease. N Engl J Med. 1991; 325(8): 556-62.

11. Mark DB, Nelson CL, Califf RM, Harrell FE Jr, Lee KL, Jones RH, et al. Continuing evolution of therapy for coronary artery disease. Initial results from the era of coronary angioplasty. Circulation. 1994; 89(5): 2015-25.

12. Weintraub WS, Jones EL, Craver JM, Grosswald R, Guyton RA. In-hospital and long-term outcome after reoperative coronary artery bypass graft surgery. Circulation. 1995; 92(9 Suppl): II50-7.

13. Spreen MI, Martens JM, Hansen BE, Knippenberg B, Verhey E, van Dijk LC, et al. Percutaneous transluminal angioplasty and drug-eluting stents for infrapopliteal lesions in critical limb ischemia (PADI) Trial. Circ Cardiovasc Interv. 2016; 9(2): e002376.

14. Spreen MI, Martens JM, Knippenberg B, van Dijk LC, de Vries JPM, Vos JA, et al. Long-term follow-up of the PADI Trial: percutaneous transluminal angioplasty versus drug-eluting stents for infrapopliteal lesions in critical limb ischemia. J Am Heart Assoc. 2017; 6(4): e004877.

15. Rümenapf, Gerhard, Huppert P, Schulte KL, Lawall H. Therapy-centered classification of peripheral arterial disease. Vasa. 2014; 43(1): 3-5.

16. Antonio LM, Handa GI. Cryoplasty for the treatment of femoropopliteal arterial disease. Jornal Vascular Brasileiro. 2011; 10: 205-10.

17. Sixt S, Alawied AK, Rastan A, Schwarzwälder U, Kleim M, Noory E, et al. Acute and long-term outcome of endovascular therapy for aortoiliac occlusive lesions stratified according to the TASC classification: a single-center experience. J Endovasc Ther. 2008; 15(4): 408-16.

18. Yilmaz K, Turanlı M. A multi-disciplinary investigation on minimizing linearization deviations in different regression models. Change & Shaping The Future, IV. ASC-2022/Fall Congress. 2022.

19. Yılmaz K, Turanlı M. A multi-disciplinary investigation of linearization deviations in different regression models. Asian Journal of Probability and Statistics. 2023; 22(3): 15-9.

20. Cecchini AL, Biscetti F, Rando MM, Nardella E, Pecorini G, Eraso LH, et al. Dietary risk factors and eating behaviors in peripheral arterial disease (PAD). Int J Mol Sci. 2022; 23(18): 10814.

21. Aday AW, Matsushita K. Epidemiology of peripheral artery disease and polyvascular disease. Circ Res. 2021; 128(12): 1818-32.

22. Cardoso CRL, Melo JV, Santos TRM, Leite NC, Salles GF. Traditional and non-traditional risk factors for peripheral artery disease development/progression in patients with type 2 diabetes: the Rio de Janeiro type 2 diabetes cohort study. Cardiovasc Diabetol. 2021; 20(1): 54.

23. Iida O, Takahara M, Soga Y, Suzuki K, Hirano K, Kawasaki D, et al. Efficacy of intravascular ultrasound in femoropopliteal stenting for peripheral artery disease with TASC II class A to C lesions. J Endovasc Ther. 2014; 21(4): 485-92.

24. Baril DT, Marone LK, Kim J, Go MR, Chaer RA, Rhee RY. Outcomes of endovascular interventions for TASC II B and C femoropopliteal lesions. J Vasc Surg. 2008; 48(3): 627-33.

25. Lyden SP, Smouse HB. TASC II and the endovascular management of infrainguinal disease. J Endovasc Ther. 2009; 16(2 Suppl 2): II5-18.

RESEARCH ARTICLE

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Comparison of Postpartum Sexual Function in Patients who had Undergone Episiotomy and had Perineal Tears

Ufuk Atlıhan[1], Can Ata[2], Onur Yavuz[3], Ferruh Acet[4], Burak Ersak[5], Hüseyin Aytuğ Avşar[6], Tevfik Berk Bildacı[7], Selçuk Erkılınç[8]

[1]Clinic of Obstetrics and Gynecology, Manisa Merkezefendi State Hospital, Manisa, Türkiye
[2]Clinic of Obstetrics and Gynecology, Buca Seyfi Demirsoy Training and Research Hospital, İzmir, Türkiye
[3]Department of Obstetrics and Gynecology, Dokuz Eylül University Faculty of Medicine, İzmir, Türkiye
[4]Department of Obstetrics and Gynecology, Ege University Faculty of Medicine, İzmir, Türkiye
[5]Clinic of Obstetrics and Gynecology, Ankara Bilkent City Hospital, University of Health Sciences, Ankara, Türkiye
[6]Clinic of Obstetrics and Gynecology, İzmir Tınaztepe University Galen Hospital, İzmir, Türkiye
[7]Department of Obstetrics and Gynecology, İzmir Democracy University Faculty of Medicine, İzmir, Türkiye
[8]Department of Oncology, İzmir Democracy University Faculty of Medicine, İzmir, Türkiye

Abstract

BACKGROUND/AIMS: To make a comparison regarding postpartum sexual function according to the presence of episiotomy and the degree of perineal tears.

MATERIALS AND METHODS: Two hundred ninety-four primiparous patients in total between the ages of 18 and 45 who arrived at the obstetrics outpatient clinic between 2017 and 2024, underwent pregnancy follow-up and delivery, and fulfilled the inclusion criteria were analyzed in our research. The study included 131 women with perineal tears and 163 women who underwent episiotomy. Retrospectively, at 6 months postpartum, the Female Sexual Function Index (FSFI), Quality of Sexual Experience Scale (QSES), and visual analog scale (VAS) for dyspareunia values of all individuals were reviewed from participant files.

RESULTS: The VAS, QSES, and FSFI values were significantly greater in the episiotomy (+) group than the episiotomy (-) group ($p<0.01$). The VAS value was significantly greater in the episiotomy (+) group in comparison with the other groups ($p<0.01$). The QSES and FSFI values were significantly smaller in the tear degree 4 group than those in the other groups ($p<0.01$). The values of arousal and lubrication were significantly smaller in the tear degree 4 group than those of the other groups ($p<0.01$). The orgasm and satisfaction values were significantly smaller in the tear degree 4 group compared with the other groups ($p<0.01$).

CONCLUSION: Sexual dysfunction in the postpartum period can be affected by social, physical, psychogenic, and demographic factors. Our study showed that preventing advanced-stage tears through episiotomy might contribute positively to sexual function aspects. Prospective studies with larger samples are also needed to evaluate the effects of other maternal, demographic, and cultural factors that might influence females' sexual functions in the postpartum period.

Keywords: Episiotomy, perineal tear, postpartum, sexual function

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ORCID IDs of the authors: U.A. 0000-0002-2109-1373; C.A. 0000-0002-0841-0480; O.Y. 0000-0003-3716-2145; F.A. 0000-0003-0628-0368; B.E. 0000-0003-3301-062X; H.A.A. 0000-0003-0636-3104; T.B.B. 0000-0002-6432-6777; S.E. 0000-0002-6512-9070.

Corresponding author: Ufuk Atlıhan
E-mail: cfl.ufuk@gmail.com
ORCID ID: orcid.org/0000-0002-2109-1373

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INTRODUCTION

A complex component of total wellbeing, sexuality has social, emotional, mental, and physical components. Pregnancy and childbirth herald numerous changes in females' sense of well-being gand quality of life including physical, psychosocial, and cultural changes.[1] Studies that have been extensively evaluated indicate that many females have a drop in their sexual health following childbirth, as well as a decrease in their frequency of sexual activity, desire, and enjoyment throughout pregnancy and the postpartum period. There is evidence that childbirth alters sexual dynamics between couples and that these changes are caused by a variety of variables.[2] Perineal discomfort, exhaustion, depression, urine incontinence, and abnormalities in sexual function are common among new mothers.[3] Numerous sociodemographic factors that affect postpartum sexual health and the delivery experience also have an impact on this complex phenomenon. This might raise the chance of episiotomy or perineal tears.[4] After giving birth, nursing may have some impaction sexual function, and sexual function alterations are common during the postpartum period.[5] Both vaginal lubrication and libido may be impacted by hormonal changes that take place during lactation.[6] All aspects of sexuality, including sexual desire, vaginal bleeding or discomfort during intercourse, trouble attaining orgasm, dyspareunia, and lack of vaginal lubrication, are noticeably declining in the postpartum period.[7,8] In the first three months following birth, however, 41-83% of females had sexual dysfunction experience.[9] In the absence of an episiotomy or perineal tear, postpartum sexual function is only slightly compromised in females who give birth vaginally.[10,11] Perineal tissue damage significantly impacts women's sexual lives, leading to dyspareunia. Compared to females who suffer from perineal injuries such tears or episiotomy, those who have an intact perineum typically resume sexual activity earlier.[12] Although there is presently inadequate evidence to link episiotomy to sexual dysfunction, postpartum dyspareunia has also been associated with perineal injuries from episiotomy or tears, which may increase the risk of sexual dysfunction.[13] It is currently unclear how postpartum perineal damage, dyspareunia, and the time of postpartum sexual function recovery are related.[14] Comparing postpartum sexual function based on the degree of perineal tears and the existence of episiotomy was the goal of our study.

MATERIALS AND METHODS

The present study was designed as a retrospective case-control study. The study was designed according to the Helsinki Declaration and signed informed consent forms were obtained from all patients. The study received approval from the Non-Interventional Research Ethics Committee of Buca Seyfi Demirsoy Training and Research Hospital (approval no: 2025/404, date: 29.01.2025). Clinic between 2017 and 2024, underwent pregnancy follow-up and delivery, and fulfilled the requirements for inclusion were analyzed in our research. The research included 131 females with perineal tears and 163 who underwent episiotomy. The study's subjects were split into two groups based on whether or not they had episiotomies, and four groups according to the degree of perineal tears. Patients with perineal tears detected in stages 2, 3, and 4 were divided into subgroups. Age, body mass index (BMI), birth weight, week of birth, and time of resumption of sexual intercourse after birth were evaluated retrospectively for all patients. All data of all females who delivered birth during this time interval and satisfied the requirements for inclusion were retrospectively evaluated at the 6th month postpartum check-up. At 6 months postpartum, the Female Sexual Function Index (FSFI), Quality of Sexual Experience Scale (QSES), and visual analog scale (VAS)-dyspareunia values of each participant were reviewed from patient files retrospectively. The VAS values span from 0 (no symptoms at all) to 10 (worst possible symptoms). Participants rated dyspareunia on a scale of 0-10. The FSFI questionnaire, which use a scale ranging from 0 (no sexual activity within the previous four weeks) or 1 (very-dissatisfied) to 5 (extremely happy) to evaluate 6 distinct domains: pain/discomfort, satisfaction, orgasm, lubrication, arousal, and desire. The investigation evaluated sexual function using a full-scale score that ranged from 2.0 (severe dysfunction) to 36.0 (no dysfunction); higher FSFI scores were assumed to be linked to a reduction in symptoms. To differentiate between females with and without present sexual dysfunction, Wiegel et al.[15] established an appropriate cut-off score of 26. Higher quality is indicated by higher QSES ratings, which range from 7 to 49.[16] In the study, females with first-degree tears, those having a perineal surgery record, and the presence of fetal death, extensive congenital anomalies, genital condyloma, extensive vulvar varicose veins, lower genital tract pathology, and lacerated episiotomy were considered as criterion for exclusion.

Statistical Analysis

The SPSS software program (IBM Inc., Chicago, IL, USA) was used to conduct statistical analyses. The Kolmogorov-Smirnov test was used to assess the data distribution's normality. The Kruskal-Wallis and Mann-Whitney U post hoc tests were used to investigate parameters that were not normally distributed. Quantitative data for all patients are presented as median (range: minimum-maximum). Results were evaluated within a 95% confidence interval (CI). The p-value considered statistically significant was <0.05.

RESULTS

The BMI value was significantly higher (26.1 kg/m^2) in the episiotomy (+) group than that of the episiotomy (-) group ($p<0.01$). The birth weight was significantly lower (3.050 g) in the episiotomy (+) group than that of the episiotomy (-) group ($p<0.01$). The sexual intercourse time was significantly later (59 days) in the episiotomy (+) group in comparison with the episiotomy (-) group ($p<0.01$) (Table 1).

The VAS value was significantly greater in the episiotomy (+) group in comparison with the episiotomy (-) group ($p<0.01$). The QSES value was significantly higher in the episiotomy (+) group in comparison with the episiotomy (-) group ($p<0.01$). The FSFI value was significantly higher in the episiotomy (+) group in comparison with the episiotomy (-) group ($p<0.01$). The desire and arousal values were significantly greater in

Table 1. Comparison of demographic and obstetric characteristics of the groups

Variables	Episiotomy (+) (n=163, 55.4%)	Episiotomy (-) (n=131, 44.6%)	p-value
Age (years)	28 (18-45)	28 (18-45)	0.08
BMI (kg/m^2)	26.1 (25-26)	25 (20.7-28.2)	<0.01
Birth weight (g)	3050 (2000-3800)	3250 (2250-3950)	<0.01
Birth week	38 (32-41)	38 (32-41)	0.22
Sexual intercourse time (days)	59 (45-90)	55 (45-90)	<0.01

BMI: Body mass index.

the episiotomy (+) group in comparison with the episiotomy (-) group (p=0.02 and p<0.01, respectively). The lubrication and orgasm values were significantly greater in the episiotomy (+) group in comparison with the episiotomy (-) group (p<0.01) (Table 2).

The VAS value was significantly greater in the episiotomy (+) group than those of other groups (p<0.01). The QSES value was significantly smaller in the tear degree -4 group than those of other groups (p<0.01). The FSFI value was significantly smaller in the tear degree -4 group in comparison with the other groups (p<0.01). The values arousal and lubrication were significantly smaller in the tear degree -4 group than those of the other groups (p<0.01). The scores of orgasms and satisfaction were significantly lower in the tear degree -4 group than those of other groups (p<0.01) (Table 3).

DISCUSSION

This study examined the connection between factors associated with sexual health and the occurrence of second, third, and fourth-degree tears, as well as episiotomies, during delivery. In our study, it was found that the time to first sexual intercourse in the postpartum period was significantly longer in the episiotomy (+) group than in the episiotomy (-) group. The VAS (dyspareunia) value was significantly greater in the episiotomy (+) group than in the episiotomy (-) group. The QSES and FSFI values were significantly greater in the episiotomy (with) group than in the episiotomy (without) group. The satisfaction, orgasm, lubrication, arousal, and desire values, were significantly greater in the episiotomy (+) group than those of the episiotomy (-) group. VAS (dyspareunia) values were significantly greater in the episiotomy (+) group than those of the other groups. QSES and FSFI values were significantly smaller in the tear-degree -4 group in comparison with the other groups. Also, arousal, lubrication, orgasm, and satisfaction values, were significantly smaller in the tear degree 4 group in comparison with the other groups.

Table 2. Comparison of sexual function indexes according to the presence of episiotomy

Variables	Episiotomy (+) (n=163, 55.4%)	Episiotomy (-) (n=131, 44.6%)	p-value
VAS	4 (2-7)	3 (2-7)	<0.01
QSES	38 (36-40)	37 (32-40)	<0.01
FSFI	25 (17-30)	24 (12-29)	<0.01
Desire	5 (3-6)	4 (3-6)	0.02
Arousal	4 (2-5)	4 (2-5)	<0.01
Lubrication	4 (2-5)	4 (2-5)	<0.01
Orgasm	4 (2-5)	4 (2-5)	<0.01
Satisfaction	4 (2-5)	4 (2-5)	<0.01
Pain	4 (3-5)	4 (3-5)	0.2

VAS: Visual analog scale, QSES: Quality of sexual experience scale, FSFI: Female sexual function index.

Table 3. Comparison of sexual function indexes according to the presence of tear degree

Variables	Episiotomy (+) (n=163, 55.4%)	Tear degree -2 (n=44, 15%)	Tear degree -3 (n=45, 15.3%)	Tear degree -4 (n=42, 14.3%)	p-value
VAS	4 (2-7)	3 (2-6)	4 (2-7)	3 (2-6)	<0.01[a]
QSES	38 (36-40)	37 (33-39)	38 (36-40)	34 (32-38)	<0.01[b]
FSFI	25 (17-30)	25 (21-29)	25 (20-28)	21 (12-28)	<0.01[b]
Desire	5 (3-6)	4 (3-6)	4 (3-6)	4.5 (3-6)	0.08
Arousal	4 (2-5)	4 (4-5)	4 (4-5)	3 (2-4)	<0.01[b]
Lubrication	4 (2-5)	4 (4-5)	4 (3-5)	3 (2-5)	<0.01[b]
Orgasm	4 (2-5)	4 (4-5)	4 (4-4)	3 (2-4)	<0.01[b]
Satisfaction	4 (2-5)	4 (4-5)	4 (4-5)	3 (2-4)	<0.01[b]
Pain	4 (3-5)	4 (3-5)	5 (3-5)	4 (3-5)	0.01[c]

VAS: Visual analog scale, QSES: Quality of Sexual Experience Scale, FSFI: Female sexual function index. a: Group 1 has a statistically significant. b: Group 4 has a statistically significant. c: Statistically significant difference between group 2-3.

Rezaei et al.[17] showed that the majority (76.3%) of participants in the postpartum period experienced sexualdysfunction. Studies in the literature have reported that sexual dysfunction is seen at rates of 41-83% in postpartum women.[17] The features of the measures used to assess FSF might be one reason for the varying prevalence of postpartum sexual dysfunction. Additionally, cultural and societal disparities may affect women's postpartum sexual behavior, which might have varying effects on other communities. The research results also demonstrated a relation among FSF and demographic characteristics such age, education, family income, breastfeeding, and parity.[3,18] In our study, demographic parameters including education, family income, and breastfeeding were not evaluated.

Yee et al.[19] observed in their research that multiparous females had bettersexual function in the areas of desire, orgasm, arousal, and satisfaction. Some studies associated primiparity with a greater risk of female sexual dysfunction[20,21], but others observed a greater risk in multiparous females.[22,23] Based on our results, we cannot make any inferences about the effect of parity because only primiparous females were included in the sample group.

Botros et al.[24] argued that the most common sexual dysfunction among their research objects was found to be related to desire for sexual activity. This tendency was observed by Pourakbaran and Yazdi[25] as well. It's possible that the fatigue and emotional stress of motherhood had a role in the postpartum decline in sexual desire.[24] Barrett et al.[8] observed a decrease in sexual desire in the 3 months after childbirth and a subsequent increase by 6 months after childbirth; however, there was no full recovery to pre-pregnancy levels. In our research, contrary to the literature, no significant difference was seen in the desire scores of the participants when the presence of episiotomy or the severity of tear degree was taken into account. We attributed the difference between the literature data and our study, to the fact that the first evaluation in our patient group was made at the 6th postpartum month.

In the study by Nyaloko et al.[4], women who underwent episiotomy waited 1-2 weeks longer to initiate sexual intercourse than the other groups. Fernández and de Medina-Moragas[26] also showed a significant relation among the resumption of sexual activity after delivery and the incidence of 2nd-degree rips in the perineum in contrast toiepisiotomy. Delays in resuming sexual activity can be caused by a number of psychological factors, such as pain, low self-esteem, and worries about relationship dynamics. On the other hand, postpartumxresumption of sexual activity is also influenced by physical factors, including hormone imbalances and recuperation. Postpartum sexual activity is also influenced by sociocultural elements, including communication within the couple and cultural standards. Spaich et al.[27] reportedd existence of a significant decrease in sexual activity between 3 and 6 months after

birth and it returned to normal by 12 months. According to Banaei et al.[28] meta-analysis of 22 research, the prevalence of postpartum dyspareunia was 35%, and it declined as postpartum duration increased. In their study, Gutzeit et al.[29] showed how delivery affects sexual function. In comparison with multiparous females, primiparous females had higher rates of dyspareunia. The increased prevalence of assisted delivery and serious perineal injuries in primiparous females may help to explain this. Lagaert et al.[30] stated that primiparous females generally felt less confident regarding postpartum sexual life because of lack of experience. In our study, in order to focus on the effects of the presence of episiotomy and tear degrees, parity was not considered as a confounding factor. Therefore, only primiparous patients were evaluated.

There are studies in the literature with the observation that the sexual functions of females who have had an episiotomy are no different from those of females with an intact perineum.[24,31] However, there is also research suggesting that, compared to females with intactperineums or first-degree tears, those who have had an episiotomy are more likely to experience dyspareunia and reduced sexual function three months after giving birth.[32,33] In the study by Fernández and de Medina-Moragas[26], when females with second-degree tears and those who had episiotomy were compared, there was no discernible change in sexual function or dyspareunia. According to the same study, sexual initiative, arousal, orgasm, desire, absence of anticipatory anxiety, penetration, and sexual pleasure were all linked to decreased dyspareunia.[26] Romashchenko et al.[34] stated in their research that dyspareunia reduced sexual desire in females. Our study adds a new perspective to the disparate results in the literature. In our sample group, although the VAS (dyspareunia) scores were greater in the episiotomy group, no difference was found with regard to desire scores. The reason for this can be interpreted as the continued maintenance of the desire element by females, due to psychogenic and hormonal drives, regardless of their physical symptoms.

There are various results in the literature regarding the effects of episiotomy on sexual parameters. However, when independent evaluations were made using the tear degree criterion, positive results were observed in the QSES, FSFI, and general view FSFI subgroups in the episiotomy group. Signorello et al.[35] suggested that the severity of postpartum dyspareunia, reduced sexual sensation, sexual satisfaction, and the capacity for orgasm were all strongly correlated with the employment of obstetric equipment and the extent of perineal damage. However, it was generally believed that these results were caused by transient alterations in postpartum sexual function. Laganà et al.[36] showed that female sexual dysfunction resulted in significantly lower FSFI scores in females who underwent episiotomy during delivery. Regarding whether episiotomy uniquely predisposes women to sexual dysfunction, studies are divided. Connolly et al.[10] reported, in their prospective study, that in primiparous females, the manner of birth and episiotomy were not linked to anorgasmia. Hartmann et al.[13] systematic study evaluating episiotomy outcomes found no evidence to bolster the idea that episiotomy improved sexual function.

Studies in the literature have shown that the risk of female sexual dysfunction increases with instrumental vaginal birth because of the increased risk of harm that might result from this kind of delivery intervention.[22,37] Laganà et al.[36] observed that females who underwent an episiotomy during vaginal delivery had fewer sexual dysfunction issues than females without an episiotomy; this might be because the procedure lowers the incidence of third-to-fourth-degree tears, which are thought to be a contributing factor to sexual dysfunction.[37,38] Although many scientists found that sexual dysfunction and episiotomy are not directly related[21,29], there are several studies reporting that 3rd-4th degree tears are linked with pelvic floor dysfunction.[39,40] Episiotomy is a commonly performed surgical procedure to avoid severe perineal trauma. Although it is clear from the professional literature that one of the most commonly performed surgeries is episiotomy, there is ongoing debate about whether it has a protective effect against 3rd and 4th-degree tears. Gutzeit et al.[29] concluded that 3rd and 4th-degree tears were extensively linked to postpartum sexual dysfunction. In our study, especially in patients with 3rd and 4th-degree tears, both QSES and FSFI values, and generally, FSFI subgroup values, were significantly smaller than in the group that underwent episiotomy. A striking finding here is that, contrary to expectations, this result occurred due to the low VAS (dyspareunia) score in this group. The reason for this is that the decrease in the contact surface secondary to vaginal relaxation in advanced stage tears, decreases the dyspareunia score and simultaneously reduces sensation, creating a negative effect on sexual parameters.

Study Limitations

The small size of the sample of our study and the lack of a sufficient number of primary studies in the literature, leading to difficulties in determining the optimal sample size, potentially affected the representation of participants. The research's methodology evaluated participants' impressions at a single point in time, making it impossible to track changes over time. The use of self-reported measures may have been impacted by social desirability and expectation bias. Other potential confounders of sexual function that could affect the interpretability of the findings are limitations of the study.

CONCLUSION

The purpose of this investigation was to examine the connection between postpartum sexual health and perineal trauma. It is evident, that a variety of psychological, physical, and societal variables impact women's ability to engage in sexual activity following childbirth. However, our study showed that preventing advanced-stage tears through episiotomy might contribute positively to sexual function parameters. Future research should include prospective studies using a larger sample size to confirm these results and assess the impact of additional maternal, demographic, and cultural factors that can influence females' sexual functioning throughout the postpartum phase.

MAIN POINTS

- During the postpartum phase, sexual dysfunction may be impacted by physical factors and social, psychogenic, and demographic factors.
- The presence of an episiotomy should be considered in depth and with confounding factors in terms of its effects on sexual function in the postpartum period.
- We think that episiotomy, when indicated correctly to prevent third and fourth-degree tears, positively affects sexual function during the postpartum period.

ETHICS

Ethics Committee Approval: The study received approval from the Non-Interventional Research Ethics Committee of Buca Seyfi Demirsoy Training and Research Hospital (approval no: 2025/404, date: 29.01.2025).

Informed Consent: The study was designed according to the Helsinki Declaration and signed informed consent forms were obtained from all patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: U.A., Concept: C.A., H.A.A., Design: O.Y., Data Collection and/or Processing: B.E., Analysis and/or Interpretation: T.B.B., Literature Search: S.E., Writing: U.A.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Abdool Z, Thakar R, Sultan AH. Postpartum female sexual function. Eur J Obstet Gynecol Reprod Biol. 2009; 145(2): 133-7.
2. Gommesen D, Nøhr E, Qvist N, Rasch V. Obstetric perineal tears, sexual function and dyspareunia among primiparous women 12 months postpartum: a prospective cohort study. BMJ Open. 2019; 9(12): e032368.
3. Anzaku A, Mikah S. Postpartum resumption of sexual activity, sexual morbidity and use of modern contraceptives among nigerian women in jos. Ann Med Health Sci Res. 2014; 4(2): 210-6.
4. Nyaloko M, Lubbe W, Minnie K. An integrative literature review on factors affecting breastfeeding in public spaces. Open Public Health J. 2022; 15(1).
5. Rogers RG, Borders N, Leeman LM, Albers LL. Does spontaneous genital tract trauma impact postpartum sexual function? J Midwifery Womens Health. 2009; 54(2): 98-103.
6. O'Malley D, Higgins A, Begley C, Daly D, Smith V. Prevalence of and risk factors associated with sexual health issues in primiparous women at 6 and 12 months postpartum; a longitudinal prospective cohort study (the MAMMI study). BMC Pregnancy Childbirth. 2018; 18(1): 196.
7. Rosen NO, Pukall C. Comparing the prevalence, risk factors, and repercussions of postpartum genito-pelvic pain and dyspareunia. Sex Med Rev. 2016; 4(2): 126-35.
8. Barrett G, Pendry E, Peacock J, Victor C, Thakar R, Manyonda I. Women's sexual health after childbirth. BJOG. 2000; 107(2): 186-95.
9. Yeniel AO, Petri E. Pregnancy, childbirth, and sexual function: perceptions and facts. Int Urogynecol J. 2014; 25(1): 5-14.
10. Connolly A, Thorp J, Pahel L. Effects of pregnancy and childbirth on postpartum sexual function: a longitudinal prospective study. Int Urogynecol J Pelvic Floor Dysfunct. 2005; 16(4): 263-7.
11. Glazener CM. Sexual function after childbirth: women's experiences, persistent morbidity and lack of professional recognition. Br J Obstet Gynaecol. 1997; 104(3): 330-5.
12. O'Malley D, Higgins A, Smith V. Postpartum sexual health: a principle-based concept analysis. J Adv Nurs. 2015; 71(10): 2247-57.
13. Hartmann K, Viswanathan M, Palmieri R, Gartlehner G, Thorp J Jr, Lohr KN. Outcomes of routine episiotomy: a systematic review. JAMA. 2005; 293(17): 2141-8.
14. McDonald EA, Gartland D, Small R, Brown SJ. Dyspareunia and childbirth: a prospective cohort study. BJOG. 2015; 122(5): 672-9.
15. Wiegel M, Meston C, Rosen R. The female sexual function index (FSFI): cross-validation and development of clinical cutoff scores. J Sex Marital Ther. 2005; 31(1): 1-20.
16. Yassa M, Sargin MA, Dereli N, Özdemir YÖ, Akca G, Günay T, et al. The validity and reliability of the Turkish version of the Quality of Sexual Experience Scale. Anadolu Psikiyatri Dergisi-Anatolian Journal of Psychiatry. 2020; 21: 48-55.
17. Rezaei N, Azadi A, Sayehmiri K, Valizadeh R. postpartum sexual functioning and its predicting factors among iranian women. Malays J Med Sci. 2017; 24(1): 94-103.
18. Boroumandfar K, Rahmati MG, Farajzadegan Z, Hoseini H. Reviewing sexual function after delivery and its association with some of the reproductive factors. Iran J Nurs Midwifery Res. 2010; 15(4): 220-3.
19. Yee LM, Kaimal AJ, Nakagawa S, Houston K, Kuppermann M. Predictors of postpartum sexual activity and function in a diverse population of women. J Midwifery Womens Health. 2013; 58(6): 654-61.
20. Saleh DM, Hosam F, Mohamed TM. Effect of mode of delivery on female sexual function: A cross-sectional study. J Obstet Gynaecol Res. 2019; 45(6): 1143-7.
21. Banaei M, Moridi A, Dashti S. Sexual Dysfunction and its Associated Factors After Delivery: Longitudinal Study in Iranian Women. Mater Sociomed. 2018; 30(3): 198-203.
22. Dağli E, Kul Uçtu A, Özerdoğan N. Sexual dysfunction in the postpartum period: Its relationship with postpartum depression and certain other factors. Perspect Psychiatr Care. 2021; 57(2): 604-9.
23. Quoc Huy NV, Phuc An LS, Phuong LS, Tam LM. Pelvic floor and sexual dysfunction after vaginal birth with episiotomy in vietnamese women. Sex Med. 2019; 7(4): 514-21.
24. Botros SM, Abramov Y, Miller JJ, Sand PK, Gandhi S, Nickolov A, et al. Effect of parity on sexual function: an identical twin study. Obstet Gynecol. 2006; 107(4): 765-70.
25. Pourakbaran E, Yazdi SAA. A study of sexual functioning and marital satisfaction in women with and without history of labor. J Fundam Ment Health. 2015; 17(4): 202–8.
26. Fernández-Fernández MJ, de Medina-Moragas AJ. Comparative study of postpartum sexual function: Second-degree tears versus episiotomy outcomes. Arch Gynecol Obstet. 2024; 309: 2761–9.
27. Spaich S, Link G, Alvarez SO, Weiss C, Sütterlin M, Tuschy B, et al. Influence of peripartum expectations, mode of delivery, and perineal injury on women's postpartum sexuality. J Sex Med. 2020; 17(7): 1312-25.
28. Banaei M, Kariman N, Ozgoli G, Nasiri M, Ghasemi V, Khiabani A, et al. prevalence of postpartum dyspareunia: a systematic review and meta-analysis. Int J Gynaecol Obstet. 2021; 153(1): 14-24.
29. Gutzeit O, Levy G, Lowenstein L. Postpartum female sexual function: risk factors for postpartum sexual dysfunction. Sex Med. 2020; 8(1): 8-13.
30. Lagaert L, Weyers S, Van Kerrebroeck H, Elaut E. Postpartum dyspareunia and sexual functioning: a prospective cohort study. Eur J Contracept Reprod Health Care. 2017; 22(3): 200-6.
31. Holanda JBdL, Abuchaim EdSV, Coca KP, Abrão ACFdV. Sexual dysfunction and associated factors reported in the postpartum period. Acta Paulista de Enfermagem. 2014; 27(6): 573–8.

32. Hidalgo-Lopezosa P, Pérez-Marín S, Jiménez-Ruz A, López-Carrasco JC, Cubero-Luna AM, García-Fernández R, et al. Factors associated with postpartum sexual dysfunction in spanish women: a cross-sectional study. J Pers Med. 2022; 12(6): 926.

33. Alum AC, Kizza IB, Osingada CP, Katende G, Kaye DK. Factors associated with early resumption of sexual intercourse among postnatal women in Uganda. Reprod Health. 2015; 12: 107.

34. Romashchenko O, Grygorenko V, Biloholovska V, Babych O, Melnykov S, Yakovenko L. Use of platelet-rich plasma for the treatment of dyspareunia in postmenopausal women. J Sex Med. 2022; 19(11): S34.

35. Signorello LB, Harlow BL, Chekos AK, Repke JT. Postpartum sexual functioning and its relationship to perineal trauma: a retrospective cohort study of primiparous women. Am J Obstet Gynecol. 2001; 184(5): 881-8.

36. Laganà AS, Burgio MA, Ciancimino L, Sicilia A, Pizzo A, Magno C, et al. Evaluation of recovery and quality of sexual activity in women during postpartum in relation to the different mode of delivery: a retrospective analysis. Minerva Ginecol. 2015; 67(4): 315-20.

37. García-Mejido JA, Idoia-Valero I, Aguilar-Gálvez IM, Borrero González C, Fernández-Palacín A, Sainz JA. Association between sexual dysfunction and avulsion of the levator ani muscle after instrumental vaginal delivery. Acta Obstet Gynecol Scand. 2020; 99(9): 1246-52.

38. Chang SR, Lin WA, Lin HH, Shyu MK, Lin MI. Sexual dysfunction predicts depressive symptoms during the first 2 years postpartum. Women Birth. 2018; 31(6): e403-11.

39. Simic M, Cnattingius S, Petersson G, Sandström A, Stephansson O. Duration of second stage of labor and instrumental delivery as risk factors for severe perineal lacerations: population-based study. BMC Pregnancy Childbirth. 2017; 17(1): 72.

40. Safarinejad MR, Kolahi AA, Hosseini L. RETRACTED: The effect of the mode of delivery on the quality of life, sexual function, and sexual satisfaction in primiparous women and their husbands. J Sex Med. 2009; 6(6): 1645-67.

RESEARCH ARTICLE

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Psychiatric Nursing and Mental Health Course on Students' Beliefs and Attitudes Towards Mental Illness

Berna Arifoğlu[1], Sinem Dağ Canatan[1], Melihat Buldaç[2]

[1]Department of Nursing, Eastern Mediterranean University Faculty of Health Sciences, Famagusta, North Cyprus
[2]Clinic of IVF, Cyprus Center Hospital, Famagusta, North Cyprus

Abstract

BACKGROUND/AIMS: Stigmatization of mental illness impedes the understanding of psychiatric disorders among individuals. One of the objectives of the Psychiatric Nursing and Mental Health Course in the undergraduate nursing program is to identify and reduce the stigma toward mental illness. The research aimed to determine the effectiveness of the Psychiatric Nursing and Mental Health Course on nursing students' beliefs and attitudes regarding mental health conditions.

MATERIALS AND METHODS: The research involved 71 third-year nursing students from a university in North Cyprus, using a single-group pretest-posttest design. The pre-test was administered on the first day of the psychiatric nursing and mental health course, the mid-test before the clinical practice, and the post-test on the last day of the course. Data were obtained via "the spread of student information" the "Beliefs Towards Mental Illness Scale", and the "Attitudes towards Mental Problems Scale".

RESULTS: It was found that perceptions regarding mental illness changed based on factors like contact, communication, and assisting individuals with mental illness, which in turn altered attitudes.

CONCLUSION: The applied and theoretical components of the Psychiatric Nursing and Mental Health Course positively affected the perspectives of students regarding mental health issues.

Keywords: Belief, attitude, mental disorder, nursing student, psychiatric nursing and mental wellness

INTRODUCTION

Mental illnesses are characterized by imbalances, inconsistencies, and inappropriate individuals' emotions.[1,2] The effects and conduct exhibited by students diagnosed with mental illnesses are commonly considered unusual in many societies. Consequently, individuals with mental health diagnoses may face exclusion or rejection within the community.[3-5] The origins of exclusion or non-acceptance of individuals with mental illnesses date back to ancient times when mental illness symptoms were unexplained and individuals with mental health issues were feared.[6] Society often perceives individuals with mental illnesses as "dangerous" and "unpredictable". This perception forms the basis for exclusion, discrimination, negative beliefs, and attitudes toward mental disorder. The treatment process for individuals with mental health conditions is adversely affected by negative beliefs and attitudes, hindering their recovery and leading to withdrawal from psychiatric support. Additionally, it contributes to feelings of low morale, guilt, shame, and decreased self-esteem in patients.[7] Particularly, negative conviction and manner can cause students to develop mental illnesses, reject treatment, and isolate themselves from society.[8] To achieve early

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ORCID IDs of the authors: B.A. 0000-0002-6430-026X; S.D.C. 0000-0003-3746-2025; M.B. 0009-0006-1332-9691.

Corresponding author: Berna Arifoğlu
E-mail: berna.arifoglu@emu.edu.tr
ORCID ID: orcid.org/0000-0002-6430-026X

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detection, treatment, and prevention of mental illnesses, it is essential to change society's negative perceptions and attitudes towards mental illnesses.[4,9] Nursing students, as part of society, are also thought to be influenced by these prejudices.[10-12] A cross-sectional study showed that, discriminatory attitudes related to mental illnesses toward students are highly frequent among nursing students.[13] Several studies have examined the impact of mental wellness and mental wellness nursing training on students' attitudes and beliefs toward mental illnesses.[12,14-16] It is anticipated that the negative beliefs and attitudes existing among nursing students will decrease during Mental Health and Psychiatric Nursing training.[8,12,17] It is crucial to evaluate the manner and beliefs of students before and after the course, as they will help in preventing, treating, and rehabilitating mental illnesses in the future.

Aim and Research Questions

This study aims to appraise the impact of mental wellness and illness training on beliefs, attitudes towards psychiatric disorders. Related to the main aim, the research answers the following four questions:

1. Did the post-assessment score mean total of the beliefs about mental disorder measure significantly decrease for nursing students participating in psychiatric nursing training?

2. Did the post-test subsection mean total of the beliefs about mental disorder measure significantly decrease for students who obtained the Psychiatric Nursing course?

3. Did the post-test total mean score of the Community Attitudes Towards Mental Illness Scale significantly decrease for nursing students participating in Psychiatric Nursing and Mental Wellness?

4. Did the post-test subscale mean scores of the Community Attitudes Towards Mental Illness Scale significantly decrease for nursing students who took the Psychiatric Nursing course?

MATERIALS AND METHODS

In this study, which was conducted to determine the effect of the mental health and psychiatric nursing course on students' beliefs and attitudes towards psychiatric patients, a pretest-posttest model from the semi-experimental models was used in the same group. The study included 71 students enrolled in the Psychiatric Nursing and Mental Health Course in the 2021-2022 academic year, spring semester of the 3rd year of the nursing department of a university in North Cyprus. No sample selection was made, and all students enrolled in the course were included in the study. Participants were made aware of the aim and method of the study, and documented informed consent was collected from those who accepted the invitation to participate. Data were obtained via direct interaction utilizing face-to-face surveys and the administration of the scales. The pretest was distributed to the students on the first day of the course, the interim measurements were administered during the course, and the posttest was given after the course ended. All students participated in the pre-test, interim measurement, and post-test of the study simultaneously. The pre-test was administered on the first day of the Psychiatric Nursing and Mental Health Course, which consists of 14 weeks with 4 hours of theory, 3 hours of laboratory, and 7 hours of practical training each week. The theoretical part includes lectures, discussions, film analysis, case analysis, short videos, and collaboration role-playing. The practical was conducted over 11 weeks in a psychiatric clinic, an elderly care home, and a disability center. The mid-test was administered on the day before the start of the clinical practice in the third week. In practice, all students interact with patients with psychological disorders. They applied the nursing process by working, and by having regular therapeutic meetings. During the practice, students regularly discussed and created their patients' care plans with their instructors and participated in regular case meetings. Discussions were held regarding the prepared observation, interview, and care plan forms. The post-test was administered on the last day of the course.

Data were obtained utilizing The Spread of Student Information, the beliefs towards mental disorder measure [body mass index (BMI)], and the Community Attitudes Towards Mental Disorder Scale (CAMI).

The Student Information: The student information form was created by the researchers by reviewing the relevant literature. The form includes nine inquiries querying students' age, gender, parents' education level, the presence of diagnosed psychiatric disorders in themselves, their families, or their acquaintances, their encounters with individuals diagnosed with psychiatric disorders, and sources of information about psychiatry.[16,18,19]

The Beliefs Towards Mental Disorder Measure: This form was developed by Hirai and Clum in 2000 in the United States of America to assess the varying beliefs about mental illness among individuals from diverse cultural backgrounds. The Turkish validity and reliability study was conducted by Çam and Bilge.[8] beliefs towards mental disorder measure is a 6-point Likert scale featuring the following: totally disagree: 0; mostly disagree: 1; partially disagree: 2; partially agree: 3; mostly agree: 4; totally agree: 5. It includes three subscales and a total of 21 items. The lowest and highest values on the scale are 0 and 105. The BMI consists of three subscales: dangerousness, poor social and interpersonal skills, and incurability. Higher scores indicate more negative beliefs. The total Cronbach's alpha coefficient of the scale is 0.82. Concerning the subscales, for the dangerousness subscale, it was found to be 0.71; for the weak social and people abilities subscale, it was found to be 0.80; and for the incurability subscale, it was found to be 0.69.[20] In this study, the total Cronbach's alpha coefficient of the measure was found to be 0.89.

Community Attitudes Towards Mental Disorder Measure: Taylor and Dear created the form in 1979, while Bağ and Ekinci[21] carried out a Turkish reliability and validity study in 2006 in 1979. This measure consists of 3 components, including 21 elements and a 5-point Likert scale: goodwill, mental wellness ideology, and Concern (fear). The lowest and highest values measure 21 and 105, respectively. The increased values in the tool and its sections reveal an unfavorable outcome. The total Cronbach's alpha measure for the Turkish version is 0.72. Cronbach's alpha values for the subscales were: 0.78 for Goodwill, 0.76 for mental wellness ideology, and 0.75 for concern (fear).[21] The total Cronbach's alpha measure for the tool was determined to be 0.76.

The study received approval from the Board of Scientific Research and Publication Ethics Committee of Eastern Mediterranean University (approval no: ETK00-2022-0080, date: 03.03.2022).

In addition, written informed consent was obtained from the participants in the study. Permission was obtained from the researchers via e-mail for the scales used in the collection of research data.

Statistical Analysis

Statistical analyses of the data collected from the participants were performed using IBM SPSS for Windows, Version 26.0. The normality of the distributions of beliefs towards mental disorder measure and the community attitudes towards mental health problems tool total was evaluated using the Kolmogorov-Smirnov test and skewness-kurtosis values. Repeated Measures ANOVA was utilized for comparing the pre-assessment, mid-test, and post-assessment measures.

RESULTS

Table 1 shows that the 71 participants who finished the study had an average age of 22.1 (standard deviation=1.5), with 57.7% being female and 42.3% male. Among the participants, 30.9% reported that their mothers had completed primary school, 19.7% had completed secondary school, and 23.9% had completed high school. Regarding fathers' education, 26.7% had completed primary school, 21.1% had completed secondary school, 22.5% had completed high school, and 15.4% had completed higher education. Additionally, 95.5% of the students reported not having any psychiatric disorder themselves; 46.4% had encountered individuals with psychiatric diagnoses; and 23.9% had close relatives with psychiatric disorders. The students reported obtaining information about mental illnesses from the internet (35.2%), family/social circles (15.4%), and the psychiatric nursing course (30.9%).

Table 2 shows the ANOVA results for repeated measures comparing the BMI scores of the participants. The differences in scores on the dangerousness subscale were determined to be statistically significant ($p<0.05$). Post-test points on the dangerousness component were reduced compared to the preliminary test and mid-test points. Additionally, the difference between the mid-test points and the pre-test points was calculated. No significant differences were found in the inferior social and personal abilities subscales and non-curability subscales ($p>0.05$).

Table 3 shows the ANOVA results for repeated measures comparing the CAMI scores of the participants. No significant differences were found in the goodwill subscale scores across the pre-test, mid-test, and final assessment ($p>0.05$). However, vital differences were uncovered in the mental wellness ideology subscale and fear components ($p<0.05$). The mid-test scores on the mental wellness ideology subscale were higher than the preliminary test scores. The post-test scores on the fear subscale were lower than those of the pre-test and mid-test scores ($p<0.05$).

DISCUSSION

In this study, it was observed that the belief scores of nursing students towards mental illnesses decreased post-test. This result indicates that students create more positive beliefs about mental disorders. Similar findings were reported in the study Çingöl et al.[22]; Dal et al.[18], Rohaim Hamzawy et al.[23], Qtait et al.[24] where nursing students exhibited positive beliefs towards mental illnesses after educational interventions. Arslantaş et al.[25] also revealed that nursing students have positive beliefs about mental disorder following a theoretical, practical Psychiatric Nursing and Mental Health Course. In this study, the post-test measure on the dangerousness submeasure of the BMI was lower than the pre-assessment, mid-test measure. Also, similar findings were reported Richards et al.[26] study. In this study, an undergraduate mental health nursing course led to a modest decrease in nursing students' prejudice towards people with mental illness. The outcome is consistent with the results of Granados-Gámez et al.[27] in Spain, where nursing students reported lower dangerousness subscale scores after interacting with patients with mental disorders. Similar results were found in studies by Ciydem and Avci[28] and İnan et al.[29] in Türkiye, where nursing students' dangerousness subscale scores decreased after psychiatric nursing education.

In our study, significant differences were found in the CAMI scores. Evli[16] in Türkiye and Giralt Palou et al.[30] in Catalonia also reported

Table 1. Distribution of the participants according to their sociodemographic characteristics

Variable (n=71)	n	%
Gender		
Female	41	57.7
Male	30	42.2
Age (Average ± SD)	22.16±1.5	
Mother's education		
Illiterate	7	9.8
Literate	4	5.6
Primary	22	30.9
Middle	14	19.7
High	17	23.9
University	7	9.86
Father's education		
Illiterate	2	2.8
Literate	8	11.2
Primary	19	26.7
Middle	15	21.1
High	16	22.5
University	11	15.4
Mental disease		
Yes	1	1.4
No	70	98.5
Encountering an individual who has previously been Psychiatric diagnosed		
Yes	33	46.4
No	38	53.5
Psychiatric disorders in relatives		
Yes	17	23.9
No	54	76.0
Information resources for mental illnesses		
Tv	5	7.0
Internet	25	35.1
Family	11	15.4
Book	3	4.2
Psychiatric nursing course	22	30.9
Other	5	7.0

SD: Standard deviation

Table 2. Assessment measure students' beliefs towards mental ıllness scale (BMI) scores

BMI	Head period	Middle of period	End of period	F	p	η2
	$\overline{\chi}$±SD	$\overline{\chi}$±SD	$\overline{\chi}$±SD			
Dangerousness	13.2±5.4	11.0±5.5	9.7±4.3	16.86	0.001**	0.194
İnferior Social and Interpersonal Skills	19.1±8.2	18.6±7.3	17.6±7.6	1.323	0.254	0.019
Uncurability	13.1±5.5	13.5±5.5	12.8±5.6	0.513	0.600	0.007

**p<0.01, SD: Standard deviation.

Table 3. A comparison of scores students' attitudes towards mental problems scale (CAMI)

CAMI	Head period	Middle of period	End of period	F	p	η2
	$\overline{\chi}$±SD	$\overline{\chi}$±SD	$\overline{\chi}$±SD			
Goodwill	28.2±4.8	27.82±5.4	27.9±5.8	0.147	0.863	0.002
Community mental health ideology	31.8±5.4	33.93±5.4	32.3±6.5	4.232	0.043*	0.057
Fear	4.93±1.8	4.65±1.4	4.3±1.9	4.405	0.039*	0.059

*p<0.05, SD: Standard deviation.

significant differences in community attitudes toward mental illnesses among nursing students after educational interventions. Our study found that the post-test points on the mental wellness ideology subscale were higher than the pre-test scores. Şahin et al.[15] in Türkiye and Sari and Yuliastuti[31] in Indonesia also found significant increases in community mental health Ideology subscale scores among nursing students after psychiatric nursing education.

As shown by the study, nursing students experience fear before obtaining mental wellness and disorder nursing training. Additionally, the idea that participants with mental disorders are perilous is at the forefront.[15,32] This study found that the post-test scores on the fear component CAMI, were lower than the pre-test and mid-test scores. Similar findings were reported by Büyükbayram et al.[33] in Türkiye, where nursing students' fears decreased after taking the psychiatric nursing course. Abd El-Gawad and Ossman[34] in Egypt also found that fears towards patients with mental illnesses decreased among graduate nurses after completing the psychiatric nursing course. The literature and research findings suggest that psychiatric nursing education has a considerable effect on positively changing perceptions toward individuals with mental health issues.

Study Limitations

The research was carried out on a single group of nursing students from one university, and the results are not generalizable beyond this sample. The small sample size and the self-report nature of the data collection instruments are other limitations. The lack of a control group and the combined evaluation of theoretical and clinical education are significant limitations of the study.

CONCLUSION

Perceptions of mental illness vary based on conditions such as contact, communication, and assisting individuals with mental illness that, in turn, alter attitudes. The conceptual and applied components of the mental wellness and disorder nursing training were revealed to be effective in diminishing unfavorable beliefs toward mental illnesses and positively influencing attitudes toward individuals with mental health issues. Suggested to conduct an study that investigate the belief components Impacting them from the initial year of nursing training. Additionally, comparing outcomes with a control group and analyzing attitudes towards distinct mental wellness issues are suggested.

MAIN POINTS

- When the literature was examined, it is evident that nursing students experience fear before taking the mental health and psychiatric nursing course, primarily due to the perception that individuals with mental health issues are dangerous.
- The theoretical and practical components of the Mental Health and Psychiatric Nursing course positively affected attitudes towards individuals with mental health issues.
- In the study, the scores on the Dangerousness subscale of the Beliefs Toward Mental Illness Scale at the end of the term were found to be lower compared to the beginning and mid-term scores.
- It is observed that the belief scores of nursing students towards mental illness decreased in the final test. This result indicates that students have developed positive beliefs towards mental illness.
- In the study, the scores on the Community Mental Health Ideology subscale of the Community Attitudes Toward the Mentally Ill Scale were found to be higher at the end of the term compared to the beginning of the term.

ETHICS

Ethics Committee Approval: The study received approval from the Board of Scientific Research and Publication Ethics Committee of Eastern Mediterranean University (approval no: ETK00-2022-0080, date: 03.03.2022).

Informed Consent: Written informed consent was obtained from the participants in the study.

Footnotes

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Authorship Contributions

Surgical and Medical Practices: B.A., S.D.C., M.B., Concept: B.A., S.D.C., Design: B.A., S.D.C., M.B., Data Collection and/or Processing: B.A., S.D.C., Analysis and/or Interpretation: B.A., S.D.C., Literature Search: B.A., S.D.C., M.B., Writing: B.A., S.D.C.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Çam, O., and E. Engin. "Mental health and diseases nursing care art." Istanbul: Istanbul Medicine Bookstores (2014): 5-19.
2. Çam OM, Bilge A, Engin E, Akmeşe ZB, Turgut EÖ, Çakır N. Investigating the effectiveness of education of the fight against stigma on mental illness to headmen. Journal of Psychiatric Nursing, 2014; 5(3): 129-36.
3. Işık I, Nurdan S, Zeren C, Yılmaz Z. Determination of thoughts and suggestions of communication faculty students on stigma towards individuals with mental illnesses. Journal of Psychiatric Nursing. 2019; 10(1): 1-11.
4. Yüksel EG, Taşkın EO. The attitudes and knowledge of the physicians and the medical school students towards mental disorders in Turkey. Alpha Psychiatry. 2005; 6(2): 113–21.
5. Yüksel N, Yılmaz M, Temel GÖ. Opinions of faculty members regarding mental illnesses and patients. J Psy Nurs. 2015; 6(1): 26-32.
6. Article R. The fight against stigma toward mental illness. TAF Prev Med Bull. 2010; 9(1): 71-8.
7. Çam O, Çuhadar D. The stigmatization process and internalized stigma in individuals with mental illness. J Psy Nurs. 2011; 2(3): 136-40.
8. Çam O, Bilge A. The process of stigmatization and attitude, belief about mental illness and patient in Turkey: A systematic review. J Psy Nurs. 2013; 4: 91-101.
9. Malas EM. Stigma review against mental disorders. Manas Journal of Social Research. 2019; 8 (1): 1203-21.
10. Çam O, Bilge A. Determination of beliefs and attitudes toward mental illness and patients of the public who live in the western part of Turkey. New Symposium. 2011; 49(3): 131-40.
11. Yamawaki N, Pulsipher G, Moses JD, Rasmuse RK, Ringger AK. Predictors of negative attitudes toward mental health services: a general population study in Japan. The European Journal of Psychiatry. 2011; 25: 101-10.
12. Günay S, Bekitkol T, Ekitli BG, Yıldırım S. Determination of the mental disorder beliefs of students in a nursing faculty. Journal of Psychiatric Nursing. 2016; 7(3): 129-34.
13. Tan S, Liu X, Zhang, Y, Chen D. Investigation and analysis of discrimination attitudes towards people with mental illness among nursing undergraduates in southwest China. The Guide of Science and Education. 2018; 17: 186-8.
14. Öz F, İnci F. The effect of mental health and psychiatric nursing courses on the attitudes of nursing students towards individuals with mental problems. Crisis Journal. 2009; 17(2): 39-47.
15. Şahin G, Amancalı M, Sayın SA, Yakar A, Buzlu S. Undergraduate nursing students' attitudes towards patients with mental disorders and related factors. ACU Sağlık Bilimleri Dergisi. 2019; 10(2): 218-24.
16. Evli M. The effect of psychiatric nursing education on the relationship between belief and attitude towards mental diseases. Ordu University Journal of Nursing Studies. 2021; 4(1): 64-74.
17. Akdede BBA, Alptekin K, Topkaya ŞÖ, et al. The rank of stigma of schizophrenia among young people New Symposium. 2004; 42(3), 113-17.
18. Dal Ü, Güleryüz İO, Ülker E, Demiray T. Beliefs of nursing students for mental illnesses. Journal of Nursing Science. 2018; (2): 14-8.
19. Duman ZÇ, Günüşen NP, İnan FŞ, Ince SÇ, Sari A. Effects of two different psychiatric nursing courses on nursing students' attitudes towards mental illness, perceptions of psychiatric nursing, and career choices. J Prof Nurs. 2017; 33(6): 452-9.
20. Ayşegül BİLGE, Olcay ÇAM. Validity and reliability of Beliefs towards Mental Illness Scale. Alpha Psychiatry. 2008, 9(2): 91–6.
21. Bağ B, Ekinci M. Examining the scale of the community attitudes towards the mentally ill in the Turkish sample. Electronic Journal of Social Sciences. 2006; 3.82: 63-83.
22. Çingöl N, Karakaş M, Zengin S, Çelebi E. The effect of psychiatric nursing students' internships on their beliefs about and attitudes toward mental health problems; a single-group experimental study. Nurse Educ Today. 2020; 84: 104243.
23. Rohaim Hamzawy M, Abdel-Hamid Loutfi Z, Elias Abdel-Aziz A. Effect of the psychiatric-mental health nursing curriculum on students' attitude towards mental illness. Egyptian Journal of Health Care. 2022; 13(3): 923-39.
24. Qtait, Mohammad, Dima Shqerat, Hadeel Mashahrea, Mohammad Sayyad, Razan Shqerat, Saleh Azzeh, et al. Attitudes of nursing students towards individuals with mental illness before and after attending the mental health nursing course: comparative study. Seminars in Medical Writing and Education. 2025; 4: 63.
25. Arslantaş H, Abacıgil F, Adana F, Karadağ Ş, Koyak HÇ. Beliefs of nursing students about mental illnesses and social distance: the effects of theoretical and practical psychiatric nursing education. Neuropsychiatric Investigation. 2019; 57(2): 2-9.
26. Richards SJ, O'Connell KA, Dickinson JK. Acknowledging stigma: levels of prejudice among undergraduate nursing students toward people living with a mental illness-a quasi-experimental single-group study. Issues Ment Health Nurs. 2023; 44(8): 778-86.
27. Granados-Gámez G, López Rodríguez MD, Corral Granados A, Márquez-Hernández VV. Attitudes and beliefs of nursing students toward mental disorder: the significance of direct experience with patients. Perspect Psychiatr Care. 2017; 53(2): 135-43.
28. Ciydem E, Avci D. Effects of the psychiatric nursing course on students' beliefs toward mental illness and psychiatric nursing perceptions in Turkey. Perspect Psychiatr Care. 2022; 58(1): 348-54.
29. İnan FŞ, Günüşen N, Duman ZÇ, Ertem MY. The Impact of Mental Health Nursing Module, Clinical Practice and an Anti-Stigma Program on Nursing Students' Attitudes toward Mental Illness: A Quasi-Experimental Study. J Prof Nurs. 2019; 35(3): 201-8.
30. Giralt Palou R, Prat Vigué G, Torà Suarez N, Romeu-Labayen M, Tort-Nasarre G. The development of positive attitudes toward mental health among university nursing students: Countering the role of social desirability. Perspect Psychiatr Care. 2022; 58(4): 1680-90.

31. Sari SP, Yuliastuti E. Investigation of attitudes toward mental illness among nursing students in Indonesia. Int J Nurs Sci. 2018; 5(4): 414-8.

32. Stuhlmiller C, Tolchard B. Understanding the impact of mental health placements on student nurses' attitudes towards mental illness. Nurse Educ Pract. 2019; 34: 25-30.

33. Büyükbayram A, Baysan Arabacı L, Taş G, Kurt A. "The opinions of nursing students who have taken and not taken the psychiatric nursing courses towards psychiatric stigma." İzmir Katip Çelebi Univ Fac Health Sci J 2020; 5: 263-75.

34. Abd El-Gawad M, Ossman LH. Baccalaureate nursing students' attitudes toward patients with mental ıllness: ımpact of psychiatric nursing and mental health educational experience. Alexandria Scientific Nursing Journal, 2014; 16(2), 193-207.