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Letter to the Editor

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J Clin Pract Res 2026;48(1):VII

2025 Year in Review and Future Perspectives of Journal of Clinical Practice and Research

Dear Readers,

The year 2025 marked a significant milestone for the Journal of Clinical Practice and Research (J Clin Pract Res) in scientific advancement and institutional maturity. The indexing of our journal in PubMed stands out as one of our most important achievements, strengthening both our international visibility and academic credibility.

Throughout the past year, we have prioritized the publication of high-quality studies in both clinical and basic sciences that adopt evidence-based and multidisciplinary approaches. The increasing number of submissions and the expanding diversity of our authors clearly demonstrate the growing interest of the scientific community in J Clin Pract Res. At the same time, we have succeeded in optimizing review timelines while maintaining the rigor and quality of the peer-review process.

In 2026, we plan to publish articles aligned with the One Health approach, which has been prominently emphasized by the World Health Organization. This framework recognizes the close interconnection between human, animal, and environmental health. Human health is influenced by interactions with animals and the surrounding environment; animal health is critical due to its role in zoonotic diseases, food safety, and ecosystem balance; and environmental health encompasses ecological factors such as climate change, biodiversity, and pollution that shape disease patterns and health outcomes. By integrating these domains, the One Health approach promotes interdisciplinary collaboration to address emerging global health challenges.

On this occasion, I would like to express my sincere gratitude to our editorial board members, whose dedicated efforts have elevated the scientific quality of the journal; to our esteemed reviewers, whose meticulous evaluations have been essential to the editorial process; and to all authors who have chosen to share their work with J Clin Pract Res. The continued interest and constructive feedback from our readers remain our strongest sources of motivation.

Looking ahead to 2026, we aim to further increase the diversity of articles published in our journal, strengthen our publication policy in selected thematic areas, and expand international scientific collaborations.

Prof. Dr. Kürşat Gündoğan
Editor-in-Chief

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The Impact of Microplastics on Human Health: An Urgent Public Health Concern

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ABSTRACT

Microplastics (MPs) have rapidly become one of the most pervasive pollutants in the modern world. Far from being confined to oceans or waste sites, these tiny particles now circulate through the air we breathe, the water we drink, and the food we eat. As everyday plastic items break down over time, they form microscopic fragments that can travel long distances and enter the human body through inhalation or ingestion. Once inside the body, these particles may accumulate in organs and interact with biological systems in ways that scientists are only beginning to understand. This review synthesizes current scientific findings on the formation of MPs, their pathways into and persistence within the human body, and their potential health impacts. Although there is no standardized method for measuring MPs, the main analytical techniques used for biological samples are discussed. Furthermore, the toxicity and environmental impacts of plastic types to which humans are exposed are assessed using the EPI Suite™ program developed by the U.S. Environmental Protection Agency (USEPA). Certain MP types, such as polyethylene and polypropylene, appear more toxic due to their low biodegradability. Although evidence increasingly identifies MPs as a growing public health concern, many questions remain unanswered. Health effects related to gastrointestinal and respiratory health systems are examined, while long-term effects, behavior in human tissues, and associations with chronic diseases—including potential links to cancer—require further multidisciplinary investigation. As global plastic use continues to rise, understanding and mitigating MP exposure will be essential for protecting human health.

Keywords: Cancer, inhibitor, microplastics, migration, one health, toxicity

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INTRODUCTION

Formation and Environmental Occurrence of MPs

Plastics are versatile polymers characterized by their low density, malleability, and durability, leading to their ubiquity in modern life.[1] In addition to their use in household goods, vehicles, and industrial production processes, they are also produced extensively for single use in all areas of life.[2] Plastic production worldwide is increasing every year and has exceeded 400 million tons annually.[3] Due to problems in plastic waste management and the global recycling rate being around 10%,

the degradation and disintegration of plastic polymers lead to the formation of particles called microplastics (MPs), defined as particles ranging from 1 to 5,000 µm.[4]

Microplastics can be formed by the direct breakdown of plastics released into the environment, as well as through environmental factors, such as sunlight, wind, and long-term weathering and aging processes.[5] In addition to the widespread presence and use of plastics in all human environments (homes, offices, industrial parks, etc.), direct exposure also occurs. Fabrics made from plastics such as polyester or polyethylene terephthalate (PET) and shoes made from plastic derivatives such as ethylene-vinyl acetate (EVA) demonstrate that plastics are beginning to surround our bodies.[6,7] Empirical evidence has demonstrated that domestic laundry effluent is a significant source of synthetic microfibers.[8] During the recycling of these plastics, high amounts of MPs are released into receiving water bodies along with washing water.[9] Similarly, studies have identified domestic and industrial wastewater treatment plants as point sources of MPs.[10] Studies conducted in various water sources worldwide have revealed the presence of MPs at high concentrations, particularly in coastal areas.[11] Varying in morphology and composition, MPs can act as vectors for chemical pollutants due to their high surface adsorption capacity.[12]

Studies reported in the literature have demonstrated that the widespread presence of plastics across all environmental media results in the ubiquitous occurrence of MPs.[13] MPs have been detected in air (both indoor and outdoor), on land (including terrestrial environments and landfills), in marine, oceanic, and freshwater environments, and even in the Arctic.[14–17] Although MPs are not considered a conventional air pollution parameter, fiber MPs have been shown to be transported over distances of several kilometers.[18] Due to poor plastic waste management, particularly in rural regions, MP formation and plastic-related environmental impacts are more pronounced, leading to severe consequences such as open burning, open dumping, landfill burial, and waste imports.[19] Consequently, it has been reported that the transition to a circular economic perspective in plastic waste management is quite difficult, and there are significant barriers to achieving a sustainable waste management model.[20]

As a result, MPs are constantly transported across all environmental media (air, soil, and water) by environmental factors.[21] The presence of MPs is not limited to remote areas; they remain in continuous contact along long transport routes. MPs are in constant contact with humans in all areas of life, from seed and medicine packaging or compost used in agricultural fields to serums, syringes, and medications in hospitals.[22] Notably, hypertonic fluids are one of the sources of MPs that directly enter the human body via intravenous routes.[23] Therefore, it is necessary to investigate the pathways of MPs after they enter the human body. Crucial to this investigation is the standardization of analytical methodologies for detecting MPs in biological matrices.

While the prevalence of MPs is well documented, specific toxicological modeling of human exposure remains sparse. In preparing this review, research and review publications from the last 10 years were selected from the perspective of each author, including studies on MP formation and occurrence in environmental samples, MP analysis and toxicity in humans, and associated health effects. This review differentiates itself as a case study by applying EPI Suite™ to assess the biopersistence of common polymers. A focused analysis of the mechanisms of respiratory and gastrointestinal degradation is also provided. An interdisciplinary approach was employed to explore the relationship between environmental and medical sciences.

CLINICAL AND RESEARCH CONSEQUENCES

Calculation of Environmental Toxicity of MPs Using EPI Suite™

In this research, the twelve most frequently utilized MPs were analyzed. To gather insights into the toxicological impacts and biodegradability of MPs in the environment, the EPI Suite™ (Estimation Programs Interface) created by the U.S. Environmental Protection Agency (EPA) was employed to assess human toxicity.[24] Biodegradability parameters were evaluated using the BIOWIN module of the software, while toxicity metrics such as effective concentration (EC_{50}) and lethal concentration (LC_{50}) were determined using the ECOSAR module.[25] This program operates based on the principles of Quantitative Structure-Activity Relationships (QSAR), predicting the physical and chemical effects of substances on living organisms according to their molecular characteristics. Unlike basic toxicity screening tools, EPI Suite™ enables a comprehensive assessment of both environmental fate (e.g., partitioning and biodegradation) and toxicological endpoints based on the specific molecular geometry and functional groups of polymers. The integration of BIOWIN™ and ECOSAR™ models provides a standardized regulatory framework developed by the U.S. EPA, ensuring that the predictive data are aligned with internationally recognized environmental safety benchmarks.

Characterization and Detection of MPs in Biological Samples

Numerous studies have analyzed MPs using different methods. Variations in MP extraction processes, as well as in the instrumentation and analytical techniques employed,

Table 1. Comparison of microplastic (MP) analysis methods

Evaluation criteria	Spectroscopic methods			Thermo-analytical methods
Analysis methods	Stereomicroscopy	FTIR spectroscopy	Raman spectroscopy	Py-GC/MS
Chemical analysis	Not possible	Yes	Yes	Yes
Nanoplastic detection	Not possible	Limited	Possible	Possible (mass-based)
Operational complexity	Very low	Moderate	Expertise required	Moderate
Matrix effects	High	Low	Low	Very Low

FTIR: Fourier transform infrared spectroscopy; Py-GC/MS: Pyrolysis gas chromatography-mass spectrometry.

significantly increase the diversity of analyses.[26,27] The analysis of MPs in biological samples is complicated by the difficulty of isolating polymer particles from organic matrices. Because plastics are polymeric organic chemicals, selectively separating them from biological samples that also contain organic material is difficult and may lead to analytical errors.

Separation processes such as digestion, oxidation, or density gradient techniques used to remove the organic matrix from the analyte can result in analyte loss.[28] Therefore, it is essential to avoid the use of overly strong solvents or oxidants during sample separation. Recently, enzymatic digestion has emerged as a particularly suitable pre-separation method for biological samples. This approach has been reported to achieve high digestion efficiency using very low concentrations of trypsin or proteinase K, without analyte loss.[29]

Given the heterogeneity of sample matrices and polymer types, a universal "gold standard" for MP analysis remains elusive. Method selection should be strategically determined based on sample type (e.g., water, sediment, or tissue) and the targeted particle size range. Analytical methods can be broadly divided into two categories: spectroscopic and thermo-analytical approaches.[26,27]

The fundamental distinction in MP characterization lies between particle-based methods, such as Fourier Transform Infrared Spectroscopy (FTIR) or Raman spectroscopy, and mass-based methods, such as pyrolysis gas chromatography/mass spectroscopy (Py-GC/MS). FTIR and Raman analyses report data on a "pieces/liter" or "pieces/m^3" basis, quantifying pollution levels by particle number. Py-GC/MS, on the other hand, provides mass-based values such as µg/L. In the literature, it is debated whether mass-based data are more stable indicators than particle counts for determining ecotoxicological risks. Microscopic techniques are limited by the wavelength of light. FTIR generally experiences signal loss for particles smaller than 20 µm, whereas Raman spectroscopy can detect particles down to 1 µm. Py-GC/MS, in contrast, is size independent.[26,27] The advantages and disadvantages of each method are summarized in Table 1.[26–29]

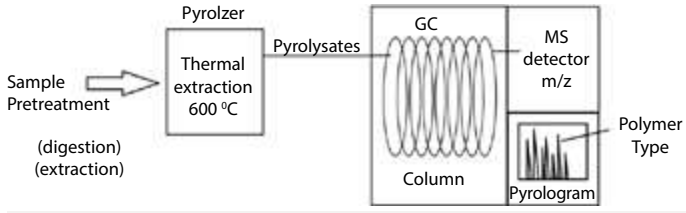

Figure 1. Gas chromatography-mass spectrometry (GC-MS) system equipped with a pyrolyzer.

For the quantitative and qualitative analysis of MPs in biological samples, the PY-GC/MS method is recommended, as it does not require pretreatment and minimizes analyte loss.[30] This method is based on the thermal treatment (pyrolysis) of the sample at high temperatures (600 °C) in an oxygen-free environment, followed by separation of the resulting pyrolysates in a GC column and detection using an MS detector. Qualitative and semi-quantitative analyses can be performed by comparison with GC-MS libraries.[30] Additionally, fully quantitative analyses can be achieved when analyte standards are prepared and appropriate calibrations are performed. The flowchart of the proposed method is shown in Figure 1.

Routes of Human Exposure

Recent studies have identified MPs such as polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polystyrene (PS), and polymethyl methacrylate (PMMA) in various human samples, including urine, breast milk, blood, semen, meconium, otitis media fluid, placenta, lung tissue, and heart tissue.[31–33] Therefore, the possible routes of entry into the human body are discussed with respect to ingestion and inhalation.

Ingestion

Microplastic ingestion represents a significant and prevalent pathway for human exposure to these ubiquitous environmental contaminants.[34,35] Humans routinely ingest MPs through various dietary sources, including packaged foods, bottled water, beverages, fruits, fish, sea salt, seafood,

Table 2. Toxicity properties of the most commonly used microplastics (MPs)

Polymer type	LC_{50}/test organism (mg/L)	EC_{50}/test organism (mg/L)	Biodegradability
Polyester (PS)	6.896/Mysid	8.691/green algae	No
Polyethylene (PE)	4.270×10^{-16}/Mysid	6.480×10^{-9}/green algae	No
Polypropylene (PP)	3.111×10^{-16}/Mysid	5.200×10^{-9}/green algae	No
Polyvinyl chloride (PVC)	9.925/Mysid	27.439 /green algae	No
Polyethylene terephthalate (PET)	1031.740/Daphnid	2567.640/green algae	Yes
Polycarbonate (PC)	1.041/Mysid	4.216/green algae	No
Polyurethane (PUR)	131.680/Fish	1.435/green algae	No
Nylon 6 (N-6)	16.420/Mysid	10.095/green algae	No
Nylon 66 (N-66)	10.426/Mysid	6.382/green algae	Yes
Polymethyl methacrylate (PMMA)	0.039/Daphnid	7.892/green algae	No
Styrene-butadiene rubber (SBR)	0.107/Mysid	0.911/green algae	No
Acrylonitrile-butadiene-styrene (ABS)	0.273/Fish	0.157/green algae	No

and agricultural crops.[34,36] Both aquatic ecosystems and agricultural activities serve as pathways through which MPs enter the human food chain.[36,37] Once ingested, MPs are absorbed primarily via transcytosis in enterocytes, with larger particles may be internalized through gaps in the intestinal lining.[38] Evidence indicates the widespread presence of MPs within the human body, as they have been detected in tissues and excreta such as stool, saliva, colon, and placenta.[39,40] Although ongoing research seeks to determine the full extent of health impacts, immediate concerns include long-term accumulation leading to intestinal damage, liver infection, microbial imbalance, and metabolic disorders.[34,35,41]

Inhalation

Humans are chronically exposed to airborne MPs, which are ubiquitous in both indoor and outdoor environments, with indoor settings representing a substantial source due to the amount of time individuals spend indoors.[34,42,43] The presence of MPs in human respiratory samples, including lung tissues, sputum, and the lower airways, has been confirmed in the literature.[44–46] For instance, polymeric particles and fibers have been observed in human lung tissue samples, with particles typically smaller than 5.5 µm and fibers ranging from 8.12 to 16.8 µm.[44] The synthetic fiber industry also represents a significant source of occupational inhalation exposure. The presence of MPs in the respiratory system raises concerns about potential adverse health effects, such as alterations in cellular metabolism, impacts on respiratory diseases, and inflammation.[44,45] Although ingestion was historically considered the primary exposure route, emerging evidence underscores the significance of airborne MP exposure in humans.[35]

CONCLUSIONS – HEALTH EFFECTS ASSOCIATED WITH MP EXPOSURE

Toxicity of Common Plastic Types

Polyethylene and polypropylene stand out for their high toxicity due to their very low LC_{50} and EC_{50} values (Table 2). Furthermore, polyethylene terephthalate and Nylon 66 (N-66) MPs are more biodegradable than other plastic types; therefore, prioritizing these materials may be more appropriate when considering alternatives. Additionally, MPs have the potential to adsorb other pollutants, allowing them to enter living organisms and exert synergistic toxic effects. Weathered plastics are continuously transported in the environment and act as vectors for pollutants due to the contaminants they adsorb or are exposed to.[47] Because these plastics are hydrophobic, they generally exhibit a high adsorption capacity and may accumulate in lipid-rich tissues. Studies have also explored the use of plastics or weathered MPs as adsorbents, as reported by Osman et al.[48] Consequently, when MPs adsorb pollutants, their potential to transport additional contaminants into the body, beyond the MPs themselves, is considerably high.

Gastrointestinal Health

Microplastic exposure to the human gastrointestinal system is a growing concern, as humans are estimated to ingest significant amounts of MPs weekly, with particles detected in various human biological samples, including feces, saliva, sputum, lungs, liver, and breast milk.[49] Once ingested, these MPs can enter the human body and translocate to the lymphatic and circulatory systems, accumulating in various organs.[50] Indeed, MPs have been detected in human colectomy specimens and have also been identified in human blood and

even brain tissue, with increasing concentrations reported over time.[51–54] Current evidence suggests that MPs are not easily excreted from the body after ingestion, leading to their accumulation in human tissues and organs, with particularly high concentrations observed in the colon and liver.[35,55] While the exact long-term impacts are still under investigation, these findings highlight the pervasive nature of MPs within the human body and their potential interactions with the gastrointestinal system.

Once ingested, MPs may accumulate in the gastrointestinal tract, leading to significant disruption of the gut microbiome.[56] This disruption often manifests as dysbiosis, characterized by alterations in the diversity and composition of beneficial bacteria and a potential increase in harmful bacterial population.[56,57] Studies indicate that MP exposure can negatively affect functional pathways and metabolic activity of the gut microbiota, contributing to oxidative stress, inflammation, and compromised intestinal barrier function.[57–59] Alterations to the gut microbiome may trigger a range of health problems, including digestive disorders and widespread inflammation. These changes can influence gut health, and, consequently, the rest of the body through its connections with the brain and the immune system.[56,60]

The liver and gastrointestinal tract serve as the primary sites for nutrient absorption and metabolic detoxification. MPs disrupt the gut-liver axis by triggering oxidative stress, inflammation, and programmed cell death (apoptosis).[61] These particles may further interfere with hepatic glucose and lipid regulation and exert indirect effects on the gut-brain axis by altering the intestinal microbiota.[61] Zhang et al.[62] reported in a meta-analysis that MPs can cause hepatocellular injury, oxidative stress, and elevated inflammatory markers, as well as increased liver enzyme levels and decreased antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, in animal models. Jin et al.[63] reported that MPs induce cellular toxicity in an in vitro human intestinal cell model. Ozsoy et al.[64] demonstrated the presence of MPs in stomach cells from 26 cadavers.

Respiratory Health

Microplastics have been detected in sputum, bronchoalveolar lavage fluid, and lung tissue. These findings highlight direct exposure routes and the accumulation of MPs within the human respiratory system.[31,45,46]

Evidence from both in vitro and in vivo models indicates that MPs may impair respiratory function. These effects are characterized by pulmonary inflammation, metabolic alterations at the cellular level, and dysregulation of proteins associated with apoptosis.[46] Airborne MPs are increasingly recognized as emerging contributors to respiratory diseases and may significantly influence their onset and progression.[65] Specifically, MP fibers can trigger alveolar macrophages and airway epithelial cells to produce pro-inflammatory cytokines, potentially resulting in chronic airway inflammation.[66] Collectively, these findings highlight the potential for broad respiratory health implications associated with MP exposure.[67]

According to Paplińska-Goryca et al.,[66] MP stimulation elicits distinct responses in the airway epithelial cells of patients with obstructive lung diseases compared to healthy controls. This differential response is associated with Th2-mediated inflammation, altered stress response pathways, and potential carcinogenic processes. Epithelial cells from patients with asthma and chronic obstructive pulmonary disease (COPD) are more susceptible to damage from MP fiber exposure. Anuar et al.[68] demonstrated that polyethylene MPs may adversely affect airway function by enhancing tissue contractile responses, mimicking pathophysiological features observed in asthma, chronic cough, and chronic obstructive pulmonary disease.

The main findings and conclusions can be summarized as follows:

- Polyethylene and polypropylene were identified as high-toxicity risks based on EPI Suite™ prediction (LC_{50}/EC_{50} values), whereas PET and Nylon 66 were highlighted as safer and more biodegradable alternatives.
- Hydrophobic MPs act as chemical vectors by adsorbing environmental pollutants and facilitating their transport into lipid-rich tissues.
- Ingested particles can translocate beyond the gastrointestinal tract into the lymphatic and circulatory systems, accumulating in organs such as the liver, blood, and brain.
- MP-induced gut dysbiosis disrupts the intestinal-hepatic axis, contributing to oxidative stress, liver injury, and metabolic disorders, including insulin resistance.
- Inhaled fibers stimulate pro-inflammatory cytokine production, with significantly higher risks of damage in patients with asthma and COPD.

Knowledge Gaps and Future Research Directions

Human exposure routes to MPs are not yet fully understood. Therefore, further studies are needed to elucidate their effects on various organs or tissues after entering the human body. Additionally, research focusing on the long-term effects of MPs, as well as their behavior and degradation within human tissues, is required. Although associations between certain plastic types or the presence of MPs and various cancers have been reported, more in-depth studies are necessary to establish causal relationships.

Microplastics are now known to be ubiquitous in the environment; therefore, it appears that they may have serious impacts on human health. Humans are in constant contact with plastics in daily lives, and because plastics eventually decompose into MPs, human exposure via air, water, and food is inevitable. In addition to affecting the respiratory and digestive systems, the effects of MPs on other systems of the human body, particularly vital organs such as the brain and heart via the bloodstream, need to be investigated. While some waste management strategies emphasize waste prevention and reduction through hierarchical approaches, it is hypothesized that legislative restrictions on plastic production could play a critical role in mitigating MP exposure.

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Diagnostic Accuracy of H_2FPEF and HFA-PEFF Algorithms for Heart Failure with Preserved Ejection Fraction (HFpEF): A Systematic Review and Meta-Analysis

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ABSTRACT

Objective: Heart failure with preserved ejection fraction (HFpEF) now accounts for the majority of heart failure cases worldwide, and its prevalence continues to rise. Despite this, diagnosis remains challenging because of substantial patient heterogeneity and the absence of universally accepted diagnostic standards. To address these challenges, the H_2FPEF (Heavy, Hypertensive, Atrial Fibrillation, Pulmonary Hypertension, Elderly, and Filling Pressure) and HFA-PEFF (Heart Failure Association–Pre-test Assessment, Echocardiography, and Functional Testing) scoring systems were developed. In this systematic review and meta-analysis, we evaluated the accuracy of these algorithms for identifying HFpEF and their utility in clinical practice.

Materials and Methods: A comprehensive literature search was conducted using PubMed, Embase, the Cochrane Library, and Web of Science to identify studies assessing the diagnostic accuracy of H_2FPEF and/or HFA-PEFF in adults with suspected HFpEF. Study quality was appraised using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies–2) tool. Diagnostic metrics were synthesized using bivariate random-effects models. The review adhered to PRISMA (the Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, and the certainty of evidence was assessed using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach.

Results: Ten studies met the inclusion criteria, representing diverse patient populations and clinical settings. The H_2FPEF algorithm demonstrated a pooled sensitivity of 0.76 (95% confidence interval [CI]: 0.56-0.87) and a specificity of 0.72 (95% CI: 0.59-0.82), with area under the curve (AUC) values ranging from 0.74 to 0.886. For the HFA-PEFF algorithm, pooled sensitivity was 0.70 (95% CI: 0.61-0.78), while specificity was substantially higher at 0.90 (95% CI: 0.85-0.94), with an AUC of 0.90. When both algorithms were applied to the same patient cohorts, 41% of cases yielded discordant diagnostic classifications.

Conclusion: Both scoring systems provide valuable diagnostic insights but exhibit unique strengths and limitations depending on the patient population and clinical context. These tools should be used to complement, rather than replace, comprehensive clinical evaluation. An effective strategy is to use the H_2FPEF score as an initial screening tool, followed by the HFA-PEFF algorithm for confirmation; in cases of discordance, further advanced diagnostic testing is recommended.

Keywords: Diagnostic accuracy, H_2FPEF score, Heart failure with preserved ejection fraction, heart failure, HFA-PEFF algorithm, non-invasive diagnostics.

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INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) represents a substantial public health challenge, accounting for more than half of all heart failure (HF) cases worldwide. Current estimates indicate that 64 million individuals globally are affected by HF, with approximately 69% residing in low- and middle-income countries.[1] Data from the Global Burden of Disease study demonstrate a 29.4% increase in HF prevalence between 2010 and 2019 (95% confidence interval [CI]: 27.5-34.2), underscoring its growing global impact.[2] HFpEF predominantly affects older adults, women, and individuals with multiple comorbidities, and poses significant diagnostic challenges due to heterogeneous pathophysiology and symptom overlap with non-cardiac conditions.

Unlike heart failure with reduced ejection fraction (HFrEF), which benefits from well-established diagnostic criteria and evidence-based therapies, HFpEF lacks a universally accepted diagnostic standard. This diagnostic uncertainty contributes to delayed recognition, suboptimal management, and the persistence of healthcare disparities.

Two non-invasive scoring approaches have emerged to address these diagnostic challenges:

H_2FPEF Score (Heavy, Hypertensive, Atrial Fibrillation, Pulmonary Hypertension, Elderly, and Filling Pressure): Developed by Reddy et al.[3] in 2018, this algorithm incorporates six clinical and echocardiographic parameters (hypertension, obesity, atrial fibrillation, pulmonary artery systolic pressure, age, and E/e' ratio) to estimate the likelihood of HFpEF.

HFA-PEFF Algorithm (Heart Failure Association–Pre-test Assessment, Echocardiography, and Functional Testing): Introduced by the Heart Failure Association of the European Society of Cardiology in 2019, this algorithm employs a stepwise approach encompassing functional, morphological, and biomarker assessments.[4]

Despite a shared diagnostic objective, emerging evidence indicates substantial discordance in patient classification. Published studies have reported disagreement rates of 28%-41% when both algorithms are applied to identical patient cohorts.[5,6] This inconsistency raises important questions regarding their interchangeability and clinical generalizability. The H_2FPEF score has undergone rigorous validation against invasive hemodynamic assessment—the diagnostic gold standard for HFpEF—and has been described as the first properly validated diagnostic instrument for this condition.[7] Its streamlined design, which relies on readily available clinical and echocardiographic parameters, facilitates practical implementation across a wide range of healthcare settings.

This systematic review and meta-analysis examine the diagnostic performance, clinical utility, and implementation considerations of the H_2FPEF and HFA-PEFF algorithms. Through systematic evidence synthesis, we aim to inform clinicians and researchers on the optimal use of these tools for diagnosing HFpEF and to identify priorities for future research.

MATERIALS AND METHODS

Protocol and Registration

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[8]

Eligibility Criteria

Studies were eligible for inclusion if they evaluated the diagnostic performance of the H_2FPEF and/or HFA-PEFF algorithms in adult patients (≥18 years) with suspected HFpEF. Acceptable study designs included diagnostic accuracy studies, prospective or retrospective cohort studies, and cross-sectional investigations. Eligible studies reported diagnostic outcomes such as sensitivity, specificity, predictive values, or the area under the receiver operating characteristic curve (ROC-AUC), using invasive hemodynamic testing or comprehensive expert clinical evaluation as reference standards.

Exclusion criteria included studies focused exclusively on HFrEF or other HF subtypes; non-primary research (reviews, editorials, case reports, or conference abstracts); and studies with insufficient data to reconstruct 2 × 2 contingency tables. Only English-language publications were considered, from database inception through the completion of the search.

Information Sources and Search Strategy

We systematically searched the PubMed/MEDLINE, Embase, Cochrane Library, and Web of Science databases. Search strategies, developed in consultation with a medical librarian, combined Medical Subject Headings (MeSH) and relevant keywords related to HFpEF, H_2FPEF, HFA-PEFF, and diagnostic accuracy. Reference lists of relevant reviews and included studies were manually screened to identify additional eligible articles.

Study Selection

Two independent reviewers screened all retrieved titles and abstracts. Full-text articles were subsequently assessed for eligibility based on predefined inclusion and exclusion criteria. Disagreements were resolved through discussion or consultation with a third reviewer. The study selection process is summarized in a PRISMA flow diagram.

Original full-text studies and research letters reporting primary diagnostic accuracy data for the H_2FPEF and/or HFA-PEFF

algorithms were included, provided they reported sufficient information to extract or calculate the AUC, sensitivity, or specificity, and employed recognized reference standards (invasive hemodynamics or expert clinical diagnosis). Research letters were included only if they met these criteria and were published in peer-reviewed journals.

Data Extraction

A standardized, pilot-tested data extraction form was used. Two independent reviewers (K.E.S. and M.W.) extracted data on study characteristics (author, year, country, and design), participant demographics (age, sex, and comorbidities), details of the index tests and reference standards, and diagnostic accuracy metrics, including sensitivity, specificity, true-positive and true-negative values, false-positive and false-negative values, and ROC-AUC. Additional information regarding clinical settings, population applicability, and potential sources of bias was also collected. Discrepancies were resolved by consensus or consultation with a third reviewer. Study authors were contacted when clarification or additional data were required.

Risk of Bias and Quality Assessment

We assessed methodological quality using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tools, which evaluates the risk of bias across four domains: patient selection, index test, reference standard, and flow and timing.[9] Two reviewers independently rated each domain as having low, high, or unclear risk of bias, with discrepancies resolved through discussion or consultation with a third reviewer (K.L.). Overall risk-of-bias judgments were determined based on domain-level assessments: a low overall risk required all domains to be rated as low risk, whereas a moderate overall risk was assigned when at least one domain was rated as high risk. A high overall risk reflected concerns or high risk in two or more domains.

Additionally, the certainty of evidence was evaluated using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach adapted for diagnostic accuracy studies.[10] This assessment encompassed five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias. Each outcome was assigned a certainty rating of high, moderate, low, or very low. Summary findings and corresponding GRADE ratings are presented in the Results section.

Statistical Analysis

Pooled diagnostic accuracy estimates were calculated using bivariate random-effects meta-analytic models that accounted for the correlation between sensitivity and specificity and addressed between-study heterogeneity.[11,12] Summary ROC curves were generated, and pooled sensitivity and specificity estimated with corresponding 95% confidence intervals were reported. Heterogeneity was quantified using the I^2 statistic, with values greater than 50% indicating substantial heterogeneity.[13] Subgroup analyses explored sources of heterogeneity based on reference-standard type, patient population characteristics, and study design. Publication bias was assessed using funnel plots and Deeks' test.[14] Substantial heterogeneity in H_2FPEF sensitivity (I^2=84%) was observed in the forest plot, with reported sensitivity estimates ranging from 0.527 to 0.996.[6,15]

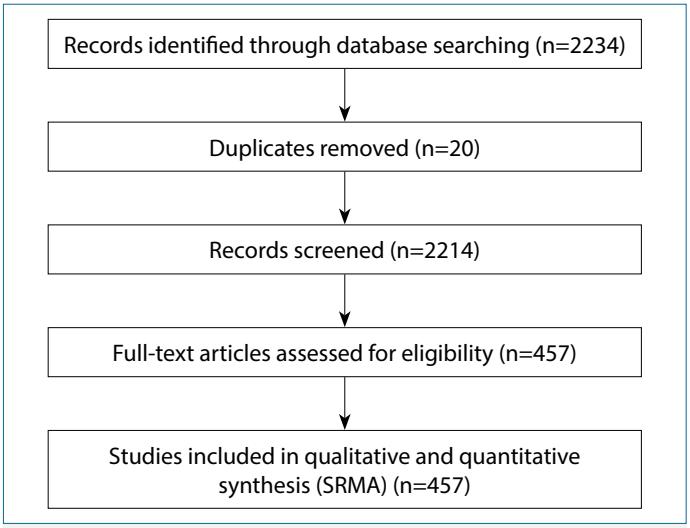

Figure 1. PRISMA flow diagram illustrating the study selection process for the systematic review and meta-analysis of H_2FPEF and HFA-PEFF diagnostic accuracy.

Meta-analyses were conducted using Stata version 18.0 with the midas and metandi commands,[16,17] and post hoc meta-regression analyses were performed using R version 4.5.2. Statistical significance was defined as $p<0.05$ for all analyses.

RESULTS

Study Selection

The systematic search identified 847 records. After removal of 215 duplicates, 632 titles and abstracts were screened. Of these, 45 full-text articles were assessed for eligibility. Ultimately, 10 studies met the inclusion criteria and were included in the meta-analysis (Fig. 1).

Study Characteristics

The 10 included studies encompassed diverse geographic regions and healthcare settings, including both single-center and multicenter designs. Sample sizes ranged from 75 to 414 participants (Table 1). Reference standards varied

Table 1. Characteristics of included studies assessing the diagnostic accuracy of the H_2FPEF and HFA-PEFF algorithms

Study	Country	Design	Sample size	Algorithms	Reference standard	Sensitivity
Parcha et al. [15]	USA, Netherlands	Prospective, cross-sectional	951	H_2FPEF, HFA-PEFF	Trial cohorts (TOPCAT/RELAX/ ARIC)	HFA-PEFF: 99.7%, H_2FPEF: 99.6%
Egashira et al.[18]	Japan	Prospective, single-center	502	HFA-PEFF	ESC task force guidelines	57.8%
Sanders-van Wijk et al.[19]*	Netherlands	Prospective cohort	363	H_2FPEF, HFA-PEFF	Expert clinical diagnosis	H_2FPEF: 52.7%, HFA-PEFF: 70.0%
Churchill et al.[20]*	USA	Retrospective cohort	156	H_2FPEF, HFA-PEFF	Invasive hemodynamic testing	HFA-PEFF: 72%, H_2FPEF: 31%
Reddy et al.[3]	USA	Retrospective	414 (derivation), 100 (validation)	H_2FPEF	Invasive hemodynamic exercise testing	NR
Suzuki et al.[21]	Japan	Prospective cohort	356	H_2FPEF	Clinical and echocardiographic assessment	47%
Tada et al.[22]	Japan	Retrospective/ prospective	372	H_2FPEF, HFA-PEFF	Expert clinical diagnosis	H_2FPEF: 97%, HFA-PEFF: 99%
Barandiarán Aizpurua et al.[23]	Netherlands, USA	Prospective cohort	729	HFA-PEFF	Expert clinical diagnosis	99% (rule-out)
Reddy et al.[24]	Netherlands, Denmark, Australia	Multicenter cohort	736	H_2FPEF, HFA-PEFF	Clinical assessment	NR
Amanai et al.[25]	Japan	Retrospective, cross-sectional	187	H_2FPEF, HFA-PEFF	Invasive catheterization; ASE/EACVI criteria	NR

Not reported (NR) indicates data not provided in the original publication. Empty cells (–) indicate that the study did not evaluate the corresponding algorithm or did not report AUC values. Statistical significance is denoted as follows: P<0.05; P<0.01; P<0.001. *: Research letter. NR: Not reported; ESC: European Society of Cardiology; ASE: American Society of Echocardiography; EACVI: European Association of Cardiovascular Imaging; H_2FPEF: Heavy, Hypertensive, Atrial Fibrillation, Pulmonary hypertension, Elderly, Filling pressure score; HFA-PEFF: Heart Failure Association Pre-test assessment, Echocardiography and Natriuretic peptide, Functional testing, Final etiology algorithm.

across studies and included invasive hemodynamic testing and comprehensive clinical evaluation by heart failure specialists. Patient populations differed with respect to age, sex distribution, and comorbidity profiles.

Quality Assessment

Assessment using the QUADAS-2 tool demonstrated an overall low risk of bias across the included studies (Fig. 2). All studies were judged to have a low risk of bias in the domains of patient selection, index test, and flow and timing. One study raised concerns in the reference standard domain, whereas the remaining studies were rated as low risk in this domain. Overall, the methodological quality of the included evidence was considered robust.

Diagnostic Accuracy of the H_2FPEF Algorithm

Seven studies evaluated the diagnostic performance of the H_2FPEF algorithm. The pooled sensitivity was 0.76 (95% CI: 0.56-0.87), and the pooled specificity was 0.72 (95% CI: 0.59-0.82). Reported AUC values ranged from 0.74 to 0.886 across

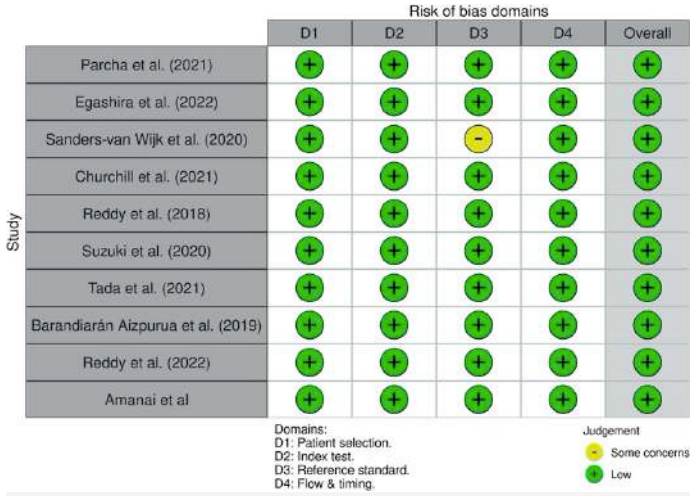

Figure 2. QUADAS-2 quality assessment summary depicting risk of bias and applicability concerns across the included studies.

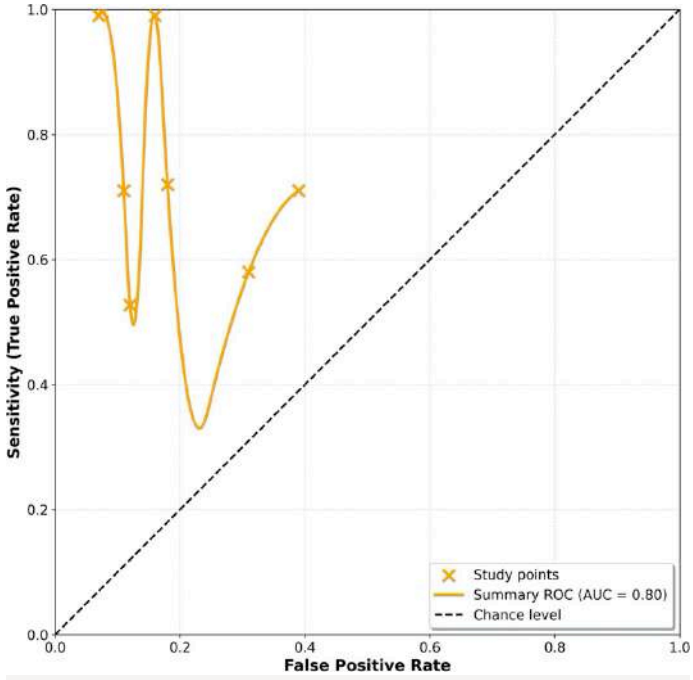

Figure 3. Summary receiver operating characteristic (SROC) curve for the H_2FPEF algorithm, showing pooled sensitivity and specificity estimates with 95% confidence regions.

different populations (Table 2). Substantial heterogeneity was observed for both sensitivity (I^2=84%) and specificity (I^2=78%). The summary ROC curve demonstrated good overall diagnostic accuracy, with optimal performance observed in populations with clearly defined HFpEF phenotypes (Fig. 3).

Table 2. AUC values for the H_2FPEF and HFA-PEFF algorithms by study

Study	H_2FPEF AUC	HFA-PEFF AUC
Egashira et al.[18]	--	0.633*
Sanders-van Wijk et al.[19]	0.77***	0.88***
Churchill et al.[20]	0.74**	0.73**
Reddy et al.[3]	0.841***	--
Suzuki et al.[21]	0.77***	--
Amanai et al.[25]	0.71**	0.61*
Tada et al.[22]	0.89***	0.82***
Barandiarán Aizpurua et al.[23]	--	0.90***
Reddy et al.[24]	0.845***	0.71**

Dashes (--) indicate that the study did not evaluate the corresponding algorithm or did not report AUC values. Statistical significance is denoted as follows: *: P<0.05; **: P<0.01; ***: P<0.001. In Tada et al. (2021), the difference in algorithm performance was statistically significant (p=0.004), favoring H_2FPEF. In Sanders-van Wijk et al. (2020), the difference was statistically significant (p<0.009), favoring HFA-PEFF. AUC: Area under the curve.

Diagnostic Accuracy of the HFA-PEFF Algorithm

Six studies assessed the diagnostic performance of the HFA-PEFF. The pooled sensitivity was 0.70 (95% CI: 0.61-0.78), and the pooled specificity was 0.90 (95% CI: 0.85-0.94). AUC values reached 0.90 in select populations (Table 2). Moderate heterogeneity was observed for sensitivity (I^2=62%), whereas low heterogeneity was noted for specificity (I^2=28%). The summary ROC curve indicated excellent specificity and moderate sensitivity, supporting the utility of the HFA-PEFF algorithm for confirming a diagnosis of HFpEF when results are positive (Fig. 4).

Comparative Performance

Three studies directly compared both algorithms within identical patient cohorts. Classification discordance was observed in 41% of patients. The H_2FPEF algorithm demonstrated higher sensitivity but lower specificity compared to the HFA-PEFF algorithm. When stratified by reference standard type, studies using invasive hemodynamic testing exhibited higher diagnostic accuracy for both algorithms than those relying on clinical assessment alone.

Subgroup Analyses

Subgroup analysis based on reference standard type revealed improved performance when invasive hemodynamics were employed (H_2FPEF AUC: 0.85 vs. 0.76; HFA-PEFF AUC: 0.89 vs. 0.82). Geographic region and healthcare setting did not significantly influence algorithm performance.

Publication Bias

Deeks' funnel plot asymmetry test demonstrated no significant publication bias for either algorithm (H_2FPEF p=0.34; HFA-PEFF p=0.41) (Fig. 5).

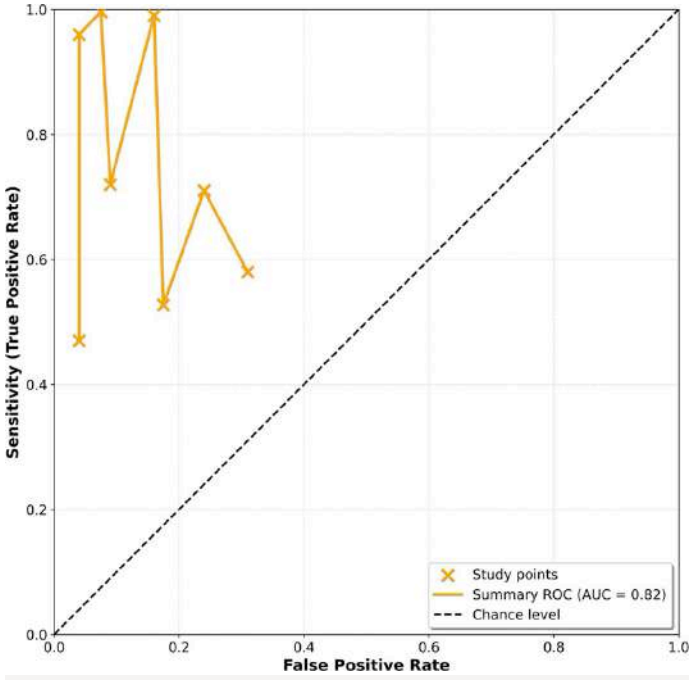

Figure 4. Summary receiver operating characteristic (SROC) curve for the HFA-PEFF algorithm, showing pooled sensitivity and specificity estimates with 95% confidence regions.

Post Hoc Heterogeneity Analysis

To further investigate the substantial heterogeneity observed in H_2FPEF sensitivity (I^2=84%), we conducted additional subgroup analyses. Studies with an atrial fibrillation prevalence greater than 30% demonstrated higher H_2FPEF sensitivity (0.82; 95% CI: 0.74-0.88) compared to studies in which atrial fibrillation (AF) prevalence was 30% or lower (0.71; 95% CI: 0.58-0.81; p=0.03 for subgroup difference). This finding aligns with AF being a heavily weighted component (3 points) within the H_2FPEF score.

Meta-regression analysis revealed that AF prevalence explained 89.3% of the between-study variance in H_2FPEF sensitivity (R^2=0.893, p<0.001). Other contributors to heterogeneity included:

- Reference standard variation (invasive vs. clinical diagnosis): 28% of variance
- Mean age of the study population: 15% of variance
- Prevalence of obesity (body mass index [BMI] ≥30): 12% of variance.

For the HFA-PEFF algorithm, heterogeneity was lower (I^2=62% for sensitivity) and was primarily attributable to differences in natriuretic peptide cutoff values across studies and the availability of advanced echocardiographic parameters.

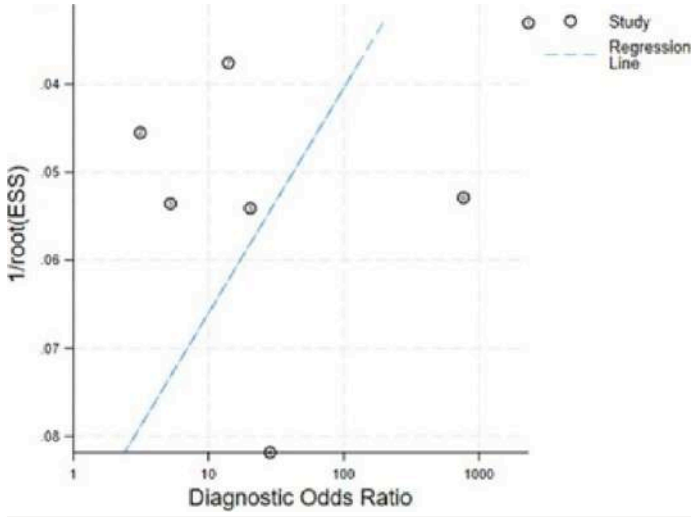

Figure 5. Deeks' funnel plot assessing publication bias in studies evaluating the H_2FPEF and HFA-PEFF algorithms.

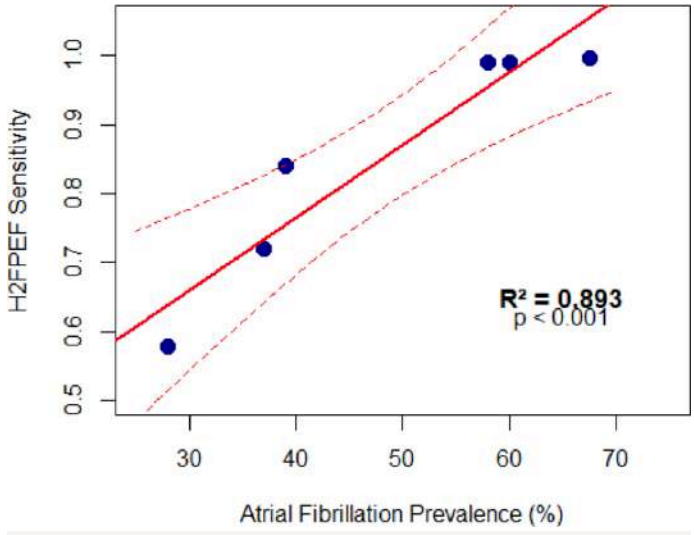

Figure 6. Association between atrial fibrillation prevalence and H_2FPEF sensitivity, shown using linear regression with 95% confidence intervals (R^2=0.893, p<0.001).

Post hoc meta-regression demonstrated a strong association between atrial fibrillation prevalence and H_2FPEF sensitivity (R^2=0.893, p<0.001) (Fig. 6). Studies with AF prevalence below 40% reported sensitivity values ranging from 57.8% to 84.1%, whereas all studies with AF prevalence exceeding 50% demonstrated sensitivity greater than 99%. Meta-regression revealed an intriguing pattern: despite high heterogeneity in absolute performance (I^2=84.8%), the comparative performance between the two algorithms remained stable across studies (I^2=0% for AUC differences). This finding

indicates that factors such as AF prevalence influence both algorithms similarly, thereby preserving their complementary diagnostic roles across diverse populations.

GRADE Evidence Certainty

The GRADE assessment rated the certainty of evidence as moderate for both algorithms. Concerns related to heterogeneity and variability in reference standards precluded high-certainty ratings; however, no serious issues regarding imprecision or indirectness were identified.

DISCUSSION

This systematic review and meta-analysis demonstrate that both H_2FPEF and HFA-PEFF algorithms provide meaningful diagnostic value for HFpEF, albeit with distinct performance profiles. The H_2FPEF score exhibits higher sensitivity (0.76), making it more suitable for screening and ruling out HFpEF, whereas the HFA-PEFF algorithm demonstrates exceptional specificity (0.90), making it better suited for diagnostic confirmation. The 41% classification discordance observed when both algorithms are applied to identical populations underscores their differing diagnostic philosophies and highlights that they should not be considered interchangeable tools.

These findings have important clinical implications. In primary care or screening settings, where sensitivity is prioritized, the H_2FPEF score may serve as an appropriate initial evaluation tool to identify patients who require further assessment. Conversely, in specialty care settings where diagnostic certainty is essential before initiating HFpEF-specific therapies, the superior specificity of the HFA-PEFF algorithm may be advantageous. The substantial discordance rate suggests that sequential application of both algorithms, or selective use based on clinical context, may optimize diagnostic accuracy.

Our analysis revealed considerable heterogeneity across studies, particularly for the H_2FPEF algorithm (I^2=84% for sensitivity). This heterogeneity likely reflects differences in patient populations, reference standard application, and healthcare settings. Studies using invasive hemodynamics as the reference standard demonstrated superior diagnostic performance compared with those relying solely on clinical assessment, underscoring the importance of rigorous reference standards in diagnostic accuracy research.

The observed differences in algorithm performance may stem from their fundamentally distinct design philosophies. The H_2FPEF score was specifically validated against invasive hemodynamic measurements and emphasizes readily available clinical parameters, thereby enhancing practical applicability.[7] In contrast, the HFA-PEFF algorithm employs a more comprehensive, stepwise framework incorporating biomarkers and functional testing, which may improve specificity at the expense of increased complexity and resource utilization.[4]

The substantial heterogeneity observed, particularly for the H_2FPEF algorithm (I^2=84%), warrants careful interpretation. Our post hoc analyses identified atrial fibrillation prevalence as a major contributor to this heterogeneity. Because AF contributes 3 points to the H_2FPEF score (out of a total of 9), populations with higher AF prevalence may mechanically achieve higher scores, potentially inflating apparent sensitivity in these cohorts. This observation raises important questions regarding the algorithm's performance across populations with differing AF burdens and suggests that local calibration may be necessary. This variability underscores the population-dependent nature of algorithm performance, as illustrated by the atrial fibrillation regression analysis shown in Figure 6.

The lower heterogeneity observed for HFA-PEFF specificity (I^2=28%) likely reflects its more standardized, stepwise approach and incorporation of objective biomarkers. However, this advantage comes at the cost of increased complexity and greater resource requirements.

Our finding that AF prevalence explains 89.3% of the heterogeneity in H_2FPEF sensitivity has important implications. Given that AF accounts for 3 of the 9 possible points in the H_2FPEF score, populations with a higher AF burden may systematically achieve higher scores, potentially leading to inflated sensitivity estimates. These findings support the need to calibrate H_2FPEF thresholds to population-specific values.

Comparison with Existing Literature

Our findings are consistent with prior observational studies reporting discordance between the two algorithms. Previous research has shown that approximately one-third of patients receive differing classifications depending on the algorithm applied.[5,6] Our meta-analysis extends these observations by providing pooled estimates across multiple populations and confirming that this discordance represents a consistent pattern rather than an isolated finding. Recent guideline updates have acknowledged the complexity of diagnosing HFpEF and recommend incorporating multiple diagnostic modalities. Our findings support this multimodal approach, suggesting that reliance on a single algorithm may be insufficient for certain patients, particularly those with borderline findings or atypical presentations.

Management of Intermediate Scores

A significant clinical challenge arises in patients who receive intermediate scores on both algorithms. The intermediate range of the H_2FPEF score (2-5 points) and the intermediate category of the HFA-PEFF algorithm (2-4 points) reflect diagnostic

uncertainty that requires further evaluation. Based on our analysis, several strategies may be considered for these patients:

1. Comprehensive echocardiographic assessment, including strain imaging and diastolic stress testing
2. Exercise testing with evaluation of hemodynamic responses
3. Cardiac magnetic resonance imaging (MRI) to assess myocardial fibrosis and structural abnormalities
4. Consideration of invasive hemodynamic testing, particularly when therapeutic decisions depend on diagnostic certainty
5. Serial reassessment over time, as features of HFpEF may become more apparent with disease progression.

The high rate of diagnostic discordance (41%) observed in our analysis frequently involved patients with intermediate scores on one or both algorithms, underscoring that these tools should inform (rather than dictate) clinical decision-making. Interestingly, although we observed substantial heterogeneity in individual algorithm performance (I^2=84.8%), the difference between the H_2FPEF and HFA-PEFF algorithms showed no heterogeneity (I^2=0%, p=0.835). This observation suggests that population characteristics influence both algorithms in a similar manner, thereby preserving their relative performance across diverse settings. Future research should specifically address optimal diagnostic strategies for patients with intermediate scores, as this subgroup may benefit most from emerging diagnostic modalities and novel biomarkers.

Strengths and Limitations

The strengths of this review include comprehensive database searches, independent duplicate screening and data extraction, rigorous quality assessment using the QUADAS-2 tool, and the application of appropriate statistical methods that account for correlation between diagnostic test measures. Additionally, we employed the GRADE methodology to evaluate the certainty of the evidence, thereby enhancing transparency regarding confidence in the findings. Several limitations merit consideration. First, substantial heterogeneity across included studies limits the ability to provide definitive recommendations for specific clinical contexts. Second, variability in reference standards affects result interpretation, as studies relying on clinical assessment may have incorporated elements of the index tests under evaluation, potentially introducing incorporation bias. Third, the majority of included studies originated from high-income countries, which may limit generalizability to resource-limited settings. Fourth, we were unable to perform extensive subgroup analyses stratified by specific patient characteristics due to inconsistent reporting across studies. Fifth, we did not extract or analyze specific B-type natriuretic peptide (BNP) or proBNP levels across studies, despite their established importance in HFpEF diagnosis. Heterogeneity in natriuretic peptide reporting, variable cutoff values, and incomplete data precluded meaningful meta-analysis of these biomarkers. This limitation is particularly relevant given that the HFA-PEFF algorithm incorporates natriuretic peptides as a diagnostic criterion, which may contribute to observed differences in algorithm performance.

Clinical Implications

Clinicians should recognize that the H_2FPEF and HFA-PEFF algorithms serve complementary rather than redundant roles. Based on our findings, a sequential diagnostic strategy may optimize accuracy, with initial screening using the H_2FPEF score (leveraging its higher sensitivity) followed by confirmation with the HFA-PEFF algorithm (capitalizing on its superior specificity). When the algorithms yield discordant results—which occurred in 41% of cases—advanced testing, such as invasive hemodynamic assessment or cardiac MRI, should be considered. Clinical context and available resources should guide algorithm selection, particularly depending on whether the priority is to rule out HFpEF (favoring H_2FPEF) or to rule in HFpEF (favoring HFA-PEFF). Both algorithms should be used to augment, rather than replace, comprehensive clinical evaluation. They are most valuable when integrated with clinical judgment, patient history, physical examination findings, and additional diagnostic testing.

Future Research Directions

Future research should focus on prospective, head-to-head validation studies of the two algorithms using invasive hemodynamics as the reference standard. Investigation of optimal sequential application strategies may help identify approaches that maximize the strengths of each algorithm while mitigating their limitations. Studies examining diagnostic performance in diverse populations, particularly in low- and middle-income countries and across different ethnic groups, would enhance the generalizability of these findings. Additionally, research evaluating whether algorithm-guided diagnostic strategies improve patient outcomes and cost-effectiveness would provide valuable clinical guidance.

CONCLUSION

Both the H_2FPEF and HFA-PEFF algorithms demonstrate moderate-to-good diagnostic accuracy for HFpEF but exhibit distinct performance profiles. The H_2FPEF score offers higher sensitivity, making it well suited for screening contexts, whereas the HFA-PEFF algorithm provides superior specificity for diagnostic confirmation. The substantial classification discordance observed when both algorithms are applied to the same population underscores the importance of avoiding their

interchangeable use. Instead, algorithm selection should be guided by clinical context, resources, and diagnostic objectives. Both tools are best used as adjuncts to comprehensive clinical assessment rather than as standalone diagnostic instruments; accordingly, an effective approach is to screen with H_2FPEF, confirm with HFA-PEFF, and, in cases of discordance, proceed with advanced testing. Future research should focus on prospective head-to-head comparisons and the development of optimal implementation strategies to maximize clinical utility.

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Investigation of Hair Diseases Accompanying Bitemporal Alopecia: An Observational Cross-Sectional Study

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ABSTRACT

Objective: Bitemporal alopecia may occur in association with various hair diseases. However, it has not yet been officially classified as a distinct type of alopecia. This study aimed to identify hair diseases that commonly accompany bitemporal hair loss and to evaluate the clinicodemographic characteristics of affected patients.

Materials and Methods: This study included 86 patients aged ≥18 years with bitemporal alopecia. Clinicodemographic characteristics and concomitant hair diseases were recorded. The severity of bitemporal alopecia was classified into three categories: mild, moderate, and severe.

Results: The mean age of the patients was 36.2±15.7 years. Of the participants, 58.1% were female and 41.9% were male. Most patients were in the 18-24 (29.1%) and 25-34 (25.6%) age groups. A positive hair pull test was observed in 45 patients. Bitemporal alopecia was classified as mild in 22 patients (25.6%), moderate in 40 patients (46.5%), and severe in 24 patients (27.9%). Hair diseases accompanying bitemporal alopecia included androgenetic alopecia (n=57, 66.3%), seborrheic dermatitis (n=38, 44.2%), telogen effluvium (n=29, 33.7%), traction alopecia (n=11, 12.8%), lichen planopilaris (n=5, 5.8%), alopecia areata (n=3, 3.5%), frontal fibrosing alopecia (n=2, 2.3%), and anagen effluvium (n=1, 1.2%). The most frequent coexisting conditions were androgenetic alopecia (25.6%), androgenetic alopecia with seborrheic dermatitis (25.6%), seborrheic dermatitis with telogen effluvium (9.3%), androgenetic alopecia with telogen effluvium (8.1%), seborrheic dermatitis with telogen effluvium and traction alopecia (5.8%), and other combinations.

Conclusion: Bitemporal alopecia may serve as a potential predictive indicator for the diagnosis and follow-up of associated hair diseases.

Keywords: Alopecia, bitemporal alopecia, hair diseases, hair loss, hair.

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INTRODUCTION

Bitemporal alopecia is a clinical finding that may be observed in various hair disorders and is not typically recognized as a distinct disease entity.[1] It can pose a challenge for dermatologists due to uncertainties in the nomenclature and classification of alopecia.[1] Severe bitemporal alopecia may significantly affect a patient's aesthetic appearance, potentially leading to social stigma and feelings of shame.[1]

Numerous conditions can affect the frontal hairline to varying degrees.[2] When involvement of the temporal region is not a typical feature of a disease, it is considered uncommon,[2] which may lead to delayed diagnosis and treatment. Disorders in which bitemporal hair loss may be observed include female and male pattern androgenetic alopecia, frontal fibrosing alopecia, trichotillomania, traction alopecia, alopecia areata, congenital triangular alopecia, chemotherapy-induced alopecia, central centrifugal cicatricial alopecia, seborrheic dermatitis, alopecia areata incognito, and chronic telogen effluvium.[2–5] A patient history and comprehensive dermatological, dermoscopic, and histopathological evaluations are essential for distinguishing conditions that present solely with bitemporal alopecia and for enabling early disease control.[2] It is important to inquire about the presence of systemic diseases, medication or hormone use, dietary habits, emotional stressors, weight loss, hair care practices (such as the use of hair straighteners or stylers, chemical treatments, and perms), smoking, and sun protection habits.[4] Additionally, a comprehensive approach is required, as some patients may have systemic comorbidities alongside the diagnosed hair disorder.[4]

This study aimed to investigate hair diseases associated with bitemporal alopecia, as well as the clinical and demographic characteristics of affected patients.

MATERIALS AND METHODS

Study Setting and Design

This observational cross-sectional study included patients aged ≥18 years who were diagnosed with bitemporal alopecia during examinations at dermatology outpatient clinics between July 2024 and May 2025.

Ethical Approval

The study was approved by Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (Approval Number: AEŞH-BADEK-2024-564, Date: 26.06.2024). Written informed consent was obtained from all participants. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Patients and Data Collection

For all patients with bitemporal alopecia, detailed medical histories, dermatological and dermoscopic examinations, and, when indicated, laboratory and histopathological findings were recorded, along with the identified diagnoses, to evaluate accompanying hair diseases.

Diagnostic Criteria

The severity of bitemporal alopecia was categorized into three groups: mild, moderate, and severe (Fig. 1).

KEY MESSAGES

- Bitemporal alopecia frequently occurs alongside other hair conditions, most commonly androgenetic alopecia, seborrheic dermatitis, and telogen effluvium, and more than half of the patients demonstrated a positive hair pull test, indicating ongoing hair shedding.
- The severity of bitemporal alopecia varies, with most cases classified as mild to moderate.
- Early recognition of bitemporal alopecia may facilitate prompt diagnosis and monitoring of associated hair disorders.

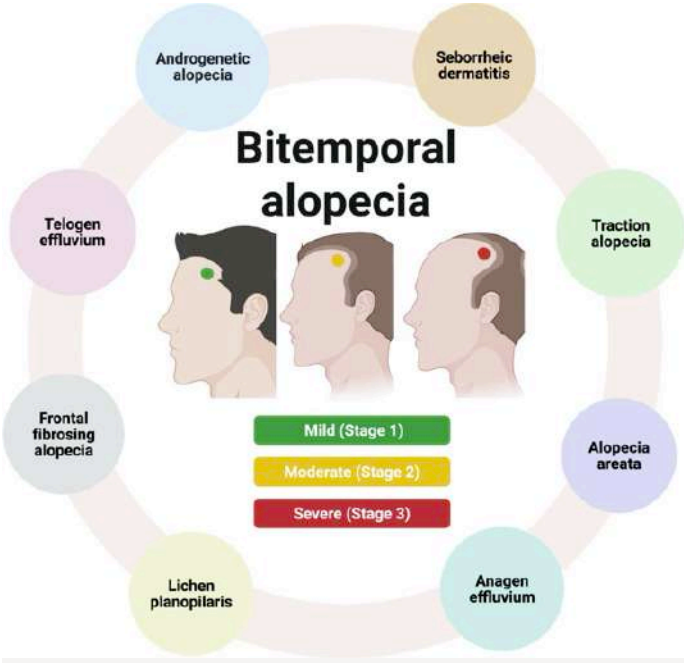

Figure 1. Hair diseases associated with bitemporal alopecia and severity grading of bitemporal alopecia. Created with BioRender.com.

Inclusion Criteria

Patients with clinically detected bitemporal alopecia were enrolled in the study.

Exclusion Criteria

Patients who had undergone hair transplantation or who were pregnant were excluded from the study.

Clinical, Surgical, and Laboratory Investigations

In this study, no laboratory tests specific to the diagnosis of bitemporal alopecia were requested.

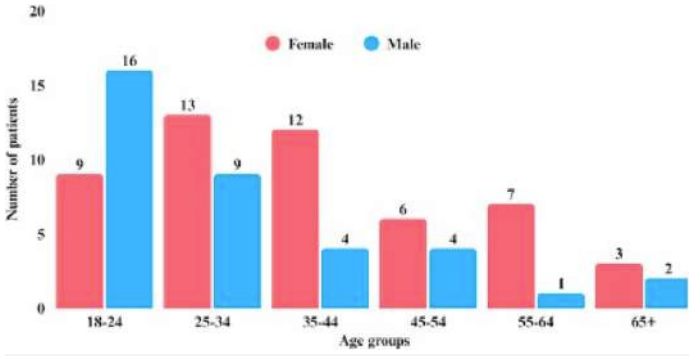

Figure 2. Distribution of patients by age group.

Statistical Analysis

Statistical analyses were performed using Jamovi (version 2.3.28; computer software; Sydney, Australia). Descriptive statistics were calculated as frequencies and percentages for categorical data and as means with standard deviations for continuous data. The chi-square (χ^2) test was used to compare differences among categorical variables. The level of statistical significance was set at $p<0.05$.

RESULTS

A total of 86 patients were included in the study; 50 (58.1%) were female and 36 (41.9%) were male. Patient ages ranged from 18 to 87 years, with a mean age of 36.2±15.7 years. The majority of patients were in the 18-24 (29.1%) and 25-34 (25.6%) age groups (Fig. 2). Twenty-two patients (25.6%) were smokers, seven (8.1%) had hypothyroidism, six (7.0%) had hypertension, and three (3.5%) had type 2 diabetes mellitus. A positive hair pull test was observed in 45 patients. Bitemporal alopecia was classified as mild in 22 patients (25.6%), moderate in 40 patients (46.5%), and severe in 24 patients (27.9%) (Table 1).

Hair diseases accompanying bitemporal alopecia included androgenetic alopecia (n=57, 66.3%), seborrheic dermatitis (n=38, 44.2%), telogen effluvium (n=29, 33.7%), traction alopecia (n=11, 12.8%), lichen planopilaris (n=5, 5.8%), alopecia areata (n=3, 3.5%), frontal fibrosing alopecia (n=2, 2.3%), and anagen effluvium (n=1, 1.2%) (Table 2, Fig. 3).

Based on the distribution of hair diseases, isolated androgenetic alopecia was the most common diagnosis among the 86 patients (25.6%). This was followed by androgenetic alopecia with seborrheic dermatitis (25.6%), seborrheic dermatitis with telogen effluvium (9.3%), androgenetic alopecia with telogen effluvium (8.1%), seborrheic dermatitis with telogen effluvium and traction alopecia (5.8%), and other disease combinations (Table 3). Data regarding the severity of bitemporal alopecia in relation to the accompanying hair diseases are presented in Table 4.

Table 1. Demographic characteristics and general data of all patients

Variables	N, Mean±SD	%, Min–Max
Age	36.2±15.7	18–87
Gender		
Female	50	58.1
Male	36	41.9
Skin type		
Type II	22	25.6
Type III	34	39.5
Type IV	28	32.6
Type V	2	2.3
Hypertension	6	7.0
Hypothyroidism	7	8.1
Type 2 diabetes mellitus	3	3.5
Smoking	22	25.6
Positive pull test	45	52.3
Severity of bitemporal alopecia		
Mild (stage 1)	22	25.6
Moderate (stage 2)	40	46.5
Severe (stage 3)	24	27.9
Family history of bitemporal alopecia	44	51.2
Use of sunscreen	28	32.6
History of sunburn	12	14.0
Use of hair dye	29	33.7
Hair breakage	42	48.8

N: Number; %: Percentage; SD: Standard deviation; Min: Minimum; Max: Maximum.

Patterns of Hair Diseases

- Androgenetic alopecia: A total of 57 patients were diagnosed with androgenetic alopecia (AA). The mean age at onset was 31.0±14.0 years, and the mean disease duration was 68.5 months. Pathological examination was performed in two patients, while the remaining patients were diagnosed clinically and by dermoscopic evaluation. Among male patients, five (5.8%) had Hamilton–Norwood Type I, seven (8.1%) Type II, 13 (15.1%) Type III, five (5.8%) Type IV, four (4.7%) Type V, and two (2.3%) Type VI disease. Among female patients, seven (8.1%) were classified as Ludwig Type I and 14 (16.3%) as Ludwig Type II.
- Seborrheic dermatitis: Among the 38 patients with seborrheic dermatitis (SD), the mean age at disease onset was 27.4±11.5 years, and the mean disease duration was 50.6 months. The severity of scalp SD at the time of

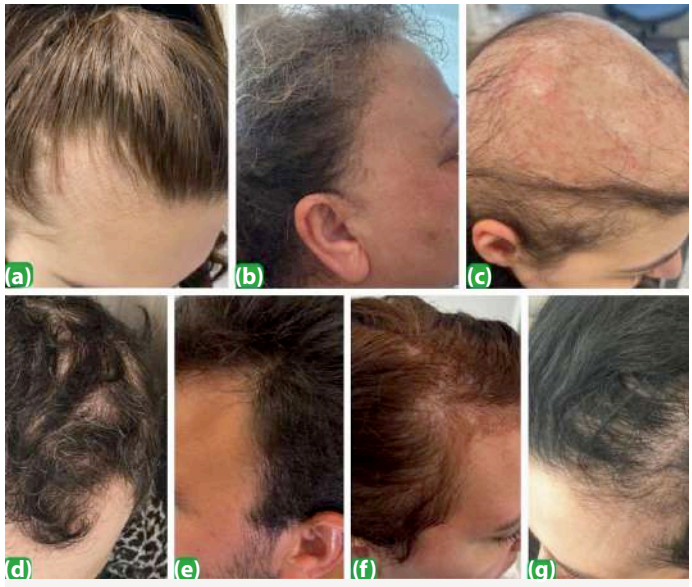

Figure 3. Clinical manifestations of bitemporal alopecia: **(a)** traction alopecia; **(b)** frontal fibrosing alopecia; **(c)** lichen planopilaris; **(d)** alopecia areata; **(e)** male androgenetic alopecia; **(f)** telogen effluvium; **(g)** female pattern androgenetic alopecia.

examination was evaluated using the Clinical Severity Score Criteria (CSSC), with a mean score of 4.29±1.80.

- Telogen effluvium: The mean disease duration among the 29 patients with telogen effluvium was 19.3 months. Histopathological examination was performed in four patients, while the remaining patients were diagnosed clinically and by dermoscopic evaluation.
- Traction alopecia: The mean age at disease onset among the 11 patients with traction alopecia was 31.8±11.1 years, with a mean disease duration of 28.4 months. Pathological examination was performed in three patients, and the remaining cases were diagnosed clinically. Additionally, four patients reported a history of using hair straighteners or hairstyling products.
- Lichen planopilaris: The mean age at disease onset among the five patients with lichen planopilaris was 40.4±12.8 years, with a mean disease duration of 67.4 months. One patient had eyebrow involvement, while two patients had skin or mucosal involvement of lichen planus. All patients reported regular sunscreen use.
- Alopecia areata: The mean age at disease onset among the three patients with alopecia areata was 35.7±2.52 years, with a mean disease duration of 15.3 months. Diagnosis was confirmed histopathologically in one patient and clinically with dermoscopic evaluation in the remaining two patients.
- Frontal fibrosing alopecia: The mean age at disease onset among the two patients with frontal fibrosing alopecia was 48.0±17.0 years, with a mean disease duration of 18.0 months. Both patients underwent pathological examination, which revealed eyebrow involvement, and both reported sunscreen use.
- Anagen effluvium: One patient diagnosed with anagen effluvium was a 63-year-old woman who had received chemotherapy for breast cancer.

DISCUSSION

Bitemporal alopecia may result from a wide range of scarring and non-scarring hair disorders, which can also occur in combination.[5] Despite its relevance, there is a lack of studies investigating bitemporal alopecia.

Table 2. Hair diseases accompanying bitemporal alopecia according to gender

Hair diseases	Male n (%)	Female n (%)	Total n (%)	p*
Androgenetic alopecia	36 (41.9)	21 (24.4)	57 (66.3)	<0.001
Seborrheic dermatitis	19 (22.1)	19 (22.1)	38 (44.2)	0.17
Telogen effluvium	3 (3.5)	26 (30.2)	29 (33.7)	<0.001
Traction alopecia	0 (0.0)	11 (12.8)	11 (12.8)	0.002
Lichen planopilaris	0 (0.0)	5 (5.8)	5 (5.8)	0.051
Alopecia areata	0 (0.0)	3 (3.5)	3 (3.5)	0.13
Frontal fibrosing alopecia	0 (0.0)	2 (2.3)	2 (2.3)	0.22
Anagen effluvium	0 (0.0)	1 (1.2)	1 (1.2)	0.39

*: Chi-Square (χ^2) test; n: Number; %: Percentage.

Table 3. Co-occurrence of hair diseases accompanying bitemporal alopecia

Hair diseases	Male n (%)	Female n (%)	Total n (%)	p*
Androgenetic alopecia	14 (16.3)	8 (9.3)	22 (25.6)	
Androgenetic alopecia + seborrheic dermatitis	19 (22.1)	3 (3.5)	22 (25.6)	
Seborrheic dermatitis + telogen effluvium	0 (0.0)	8 (9.3)	8 (9.3)	
Androgenetic alopecia + telogen effluvium	3 (3.5)	4 (4.7)	7 (8.1)	
Seborrheic dermatitis + telogen effluvium + traction alopecia	0 (0.0)	5 (5.8)	5 (5.8)	
Lichen planopilaris	0 (0.0)	4 (4.7)	4 (4.7)	
Telogen effluvium + traction alopecia	0 (0.0)	3 (3.5)	3 (3.5)	
Telogen effluvium	0 (0.0)	2 (2.3)	2 (2.3)	
Androgenetic alopecia + seborrheic dermatitis + telogen effluvium	0 (0.0)	1 (1.2)	1 (1.2)	
Androgenetic alopecia + seborrheic dermatitis + traction alopecia	0 (0.0)	1 (1.2)	1 (1.2)	
Androgenetic alopecia + telogen effluvium + traction alopecia	0 (0.0)	1 (1.2)	1 (1.2)	<0.001
Androgenetic alopecia + traction alopecia	0 (0.0)	1 (1.2)	1 (1.2)	
Androgenetic alopecia + frontal fibrosing alopecia	0 (0.0)	1 (1.2)	1 (1.2)	
Androgenetic alopecia + alopecia areata	0 (0.0)	1 (1.2)	1 (1.2)	
Seborrheic dermatitis	0 (0.0)	1 (1.2)	1 (1.2)	
Telogen effluvium + alopecia areata	0 (0.0)	1 (1.2)	1 (1.2)	
Telogen effluvium + lichen planopilaris	0 (0.0)	1 (1.2)	1 (1.2)	
Traction alopecia	0 (0.0)	1 (1.2)	1 (1.2)	
Frontal fibrosing alopecia	0 (0.0)	1 (1.2)	1 (1.2)	
Alopecia areata	0 (0.0)	1 (1.2)	1 (1.2)	
Anagen effluvium	0 (0.0)	1 (1.2)	1 (1.2)	

*: Chi-Square (χ^2) test; n: Number; %: Percentage.

In 2021, a review analyzed 94 studies published between 1957 and 2019 using keywords related to hair diseases that may cause bitemporal alopecia.[5] Reported combinations of hair disorders included female pattern androgenetic alopecia with telogen effluvium, female androgenetic alopecia with telogen effluvium, lichen planopilaris/frontal fibrosing alopecia, female pattern androgenetic alopecia with central centrifugal cicatricial alopecia, central centrifugal cicatricial alopecia with lichen planopilaris/frontal fibrosing alopecia, central centrifugal cicatricial alopecia with traction alopecia, central centrifugal cicatricial alopecia with traction alopecia and seborrheic dermatitis, and female androgenetic alopecia with seborrheic dermatitis.[5] The review emphasized the importance of recognizing the causes of temporal alopecia.[5] In our study, the most common coexisting hair disorders observed in patients with bitemporal alopecia were androgenetic alopecia and seborrheic dermatitis. In contrast to the findings of the aforementioned review, androgenetic alopecia was more frequently observed in men in our cohort. However, among patients with other combined conditions, including androgenetic alopecia alone; androgenetic alopecia with telogen effluvium; androgenetic alopecia with seborrheic dermatitis and telogen effluvium; androgenetic alopecia with seborrheic dermatitis and traction alopecia; androgenetic alopecia with telogen effluvium and traction alopecia; androgenetic alopecia with traction alopecia; androgenetic alopecia with frontal fibrosing alopecia; and androgenetic alopecia with alopecia areata, androgenetic alopecia were more prevalent in women than in men. However, as most combinations involved only a single patient, it would be inappropriate to draw definitive conclusions.

Bitemporal alopecia has also been reported as a potential complication following thread-lift procedures, which are non-surgical facial rejuvenation treatments.[6,7] In one report, a 61-year-old woman who developed swelling and pain in the temporal region after a thread-lift procedure subsequently experienced severe bilateral bitemporal alopecia three days

Table 4. Severity of bitemporal alopecia and accompanying hair diseases

Hair diseases	Mild BTA n (%)	Moderate BTA n (%)	Severe BTA n (%)	Total BTA n (%)	p*
Androgenetic alopecia	3 (3.5)	11 (12.8)	8 (9.3)	22 (25.6)	
Androgenetic alopecia + seborrheic dermatitis	5 (5.8)	13 (15.1)	4 (4.7)	22 (25.6)	
Seborrheic dermatitis + telogen effluvium	3 (3.5)	4 (4.7)	1 (1.2)	8 (9.3)	
Androgenetic alopecia + telogen effluvium	2 (2.3)	3 (3.5)	2 (2.3)	7 (8.1)	
Seborrheic dermatitis + telogen effluvium + traction alopecia	2 (2.3)	3 (3.5)	0 (0.0)	5 (5.8)	
Lichen planopilaris	1 (1.2)	1 (1.2)	2 (2.3)	4 (4.7)	
Telogen effluvium + traction alopecia	1 (1.2)	0 (0.0)	2 (2.3)	3 (3.5)	
Telogen effluvium	2 (2.3)	0 (0.0)	0 (0.0)	2 (2.3)	
Androgenetic alopecia + seborrheic dermatitis + telogen effluvium	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	
Androgenetic alopecia + seborrheic dermatitis + traction alopecia	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	
Androgenetic alopecia + telogen effluvium + traction alopecia	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)	0.30
Androgenetic alopecia + traction alopecia	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	
Androgenetic alopecia + frontal fibrosing alopecia	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	
Androgenetic alopecia + alopecia areata	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	
Seborrheic dermatitis	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)	
Telogen effluvium + alopecia areata	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	
Telogen effluvium + lichen planopilaris	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	
Traction alopecia	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	
Frontal fibrosing alopecia	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	
Alopecia areata	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)	
Anagen effluvium	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	

*: Chi-Square (χ^2) test; n: Number; %: Percentage; BTA: Bitemporal alopecia.

later. The hair pull test was negative, and histopathological examination revealed no evidence of inflammation or hair shaft abnormalities.[6] The condition was thought to have developed secondary to pressure effects in areas of maximal tension, and the patient responded favorably to topical minoxidil therapy.[6] In another case, a 52-year-old woman who underwent a facelift developed bitemporal alopecia nine weeks after the procedure.[7] Clinical examination revealed thinning of hair in the bilateral bitemporal regions, a negative hair pull test, and histopathological findings demonstrating an increased proportion of hair follicles in the catagen and telogen phases. This presentation was interpreted as a form of acute, transient, localized telogen effluvium.[7] No treatment was initiated, and the patient's hair returned to its original state within one year.[7] It is thought that the histopathological findings and the lack of established guidelines for the management of bitemporal alopecia account for the differences in treatment approaches between the two patients.

In the present study, telogen effluvium was one of the most common conditions observed in association with bitemporal alopecia and was significantly more prevalent in women than in men ($p<0.001$). Most affected female patients were in the middle adult age group (25–34 years), similar to patients with chronic telogen effluvium.[8] Telogen effluvium (TE) is one of the most common causes of hair loss and may present in either acute or chronic forms.[9] In acute TE, an identifiable triggering factor is typically present within the preceding three months.[9] In most patients with acute TE, improvement occurs within a few months.[9] However, in a small proportion of cases, improvement may not occur. In such patients, underlying androgenetic alopecia or diffuse alopecia areata should be considered.[9] Defining chronic telogen effluvium is challenging due to the lack of consensus.[10] Studies on chronic telogen effluvium have not definitively established whether it represents a form of premature female pattern androgenetic alopecia or is triggered by an unknown secondary factor.[10] Furthermore, it remains unclear whether factors such as hair length or stress contribute

to the development of this condition.[10] Chronic telogen effluvium can persist for years and is characterized by periods of increased hair shedding, known as attacks, and periods of remission marked by a spontaneous decrease in hair loss.[11] It is crucial to perform histopathological examination during the active phase of the disease to ensure accurate diagnosis and appropriate treatment.[11] As supported by the findings of our study, bitemporal alopecia may result from TE; therefore, this condition warrants comprehensive investigation.

In a study conducted in 2021 on fibrosing alopecia in a pattern distribution (FAPD), a new type of alopecia exhibiting features of both androgenetic alopecia and lichen planopilaris, 15 studies examining FAPD concluded that the condition is triggered by androgenetic alopecia (AGA) and that inflammatory responses to damaged hair follicles contribute to its pathogenesis.[12] The importance of dermoscopy and biopsy in guiding diagnosis has been emphasized, as FAPD is frequently misdiagnosed as androgenetic alopecia with seborrheic dermatitis.[12] In the present study, a substantial proportion of patients had concomitant AGA and seborrheic dermatitis (n=22; 25.6%). This raises the possibility that scarring may not be a prominent feature, particularly in the early stages, suggesting that these patients may fall within the FAPD spectrum over time. Therefore, patients presenting with AGA and seborrheic dermatitis in addition to bitemporal alopecia should be closely monitored, and histopathological examination should be performed when necessary to avoid overlooking FAPD.

Another study reported that seborrheic dermatitis may potentially trigger central centrifugal cicatricial alopecia (CCCA), a condition of unknown etiology, and was the most common accompanying hair disorder in patients with CCCA.[13] Similarly, our study found that seborrheic dermatitis, along with telogen effluvium, was the most common hair disease, suggesting that it may contribute to the development of bitemporal alopecia. It is believed that treating or controlling seborrheic dermatitis could positively influence the prognosis of bitemporal alopecia.

In a review published in 2022, androgenetic alopecia (approximately 40%) and seborrheic dermatitis were among the most common accompanying hair disorders in patients with frontal fibrosing alopecia (FFA).[14] Frontal fibrosing alopecia is a variant of lichen planopilaris and progresses through three clinical stages with differing prognoses.[14] Our findings suggest that the coexistence of FFA and androgenetic alopecia raises the possibility that bitemporal alopecia may also play a role in patient staging and long-term management. However, studies comparing larger cohorts of patients with and without bitemporal alopecia are needed to clarify this relationship.

In female patients, similar to males with Hamilton-type hair loss, hair loss or thinning is typically observed in the front and top regions of the scalp.[4] Previous studies have shown that, particularly during menopause, although the Ludwig pattern of hair loss is often observed initially, a Hamilton-type pattern may develop later.[4] In our study, more than half of the women with androgenetic alopecia had an additional concomitant hair disorder, highlighting the need to further investigate whether early-onset or progressive bitemporal alopecia is triggered by accompanying conditions (e.g., telogen effluvium and seborrheic dermatitis).

Limitations

The main limitation of this study is that it was conducted at a single center with a relatively small number of patients, which may limit the generalizability of the findings. Therefore, larger multicenter studies are required to validate and extend these results. Additionally, the mild severity of bitemporal alopecia observed in some patients may be attributable to individual hairline characteristics or genetic factors, which should not be overlooked.

CONCLUSION

Bitemporal alopecia is a pattern of hair loss that may occur in various hair disorders, particularly androgenetic alopecia, seborrheic dermatitis, and telogen effluvium. The coexistence of multiple hair conditions may exacerbate bitemporal alopecia, significantly affecting a patient's appearance and potentially leading to permanent hair loss. Bitemporal alopecia may be considered a distinct clinical feature, as it could serve as a predictive indicator of associated hair disorders and should be incorporated into a comprehensive diagnostic approach that accounts for multiple disease associations.

Ethics Committee Approval: The Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee granted approval for this study (date: 26.06.2024, number: AEŞH-BADEK-2024-564).

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Can Time to Blood Culture Positivity Influence the Treatment Strategy in Candidemia Management?

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ABSTRACT

Objective: The primary objective of this study was to investigate whether time to blood culture positivity (TTP) can reliably differentiate *Candida* species and to evaluate their antifungal susceptibility profiles to support more effective clinical decision-making.

Materials and Methods: This retrospective study was conducted at Sivas Numune Hospital between January 1, 2022 and February 28, 2025. Adult patients (>18 years) with positive blood cultures for *Candida* species were included.

Results: A total of 101 blood culture bottles from 70 patients were evaluated. *C. albicans* was the most common isolate (51.5%), followed by *C. glabrata* (21.5%). The shortest TTP was observed for *C. tropicalis* (19 hours), and the longest for *C. glabrata* (42 hours). When *C. albicans* and *C. tropicalis* were classified as early TTP species and *C. glabrata* and *C. parapsilosis* as late TTP species, the median TTP values were 21 and 35 hours, respectively. Receiver operating characteristic (ROC) analysis identified a TTP cut-off value of 28 hours as optimal for predicting the early TTP group (area under the curve: 0.751), indicating moderate discriminatory ability. Early TTP species were generally susceptible to fluconazole, whereas resistance was more frequent among late TTP species.

Conclusion: Blood culture positivity times differ among *Candida* species. Early TTP species, such as *C. albicans* and *C. tropicalis*, were more likely to be fluconazole-susceptible, suggesting that fluconazole may be an appropriate option for empirical therapy in such infections.

Keywords: Antifungal susceptibility, candidemia, empirical treatment, fluconazole, signal time.

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INTRODUCTION

Microorganisms of the genus *Candida* are the most common cause of fungal infections. *Candida* species reproduce by budding and may form true or pseudohyphae. They can cause invasive infections associated with high mortality rates despite antifungal therapy.[1] Because certain *Candida* species naturally colonize the skin and the genitourinary and gastrointestinal tracts, most invasive infections arise endogenously. According to the World Health Organization (WHO) fungal pathogens list (2022), *Candida auris* and *Candida albicans* are categorized as critical priority pathogens, whereas *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*

are classified as high-priority pathogens.[2,3] Since *Candida auris* was first identified in 2009 in the external ear canal of a patient, antifungal resistance rates in *C. auris* as well as in other *Candida* species have shown a steady and concerning increase, underscoring the growing challenges in managing infections caused by these opportunistic pathogens.[4–6] Given the rising resistance rates, it is essential for each medical center to determine its local resistance patterns to guide empirical antifungal therapy.

Early targeted treatment of fungal infections is of vital importance; however, delays in initiating therapy are frequently encountered.[7] One of the initial strategies to facilitate early treatment is the determination of blood culture time to positivity (TTP). A limited number of studies suggest that this parameter may aid in the identification of *Candida* species. Moreover, assessment of TTP may contribute to the development of additional strategies to assist in selecting appropriate antifungal therapy.[8]

This study aimed to investigate variations in blood culture positivity times among *Candida* species and to evaluate whether this parameter could serve as a diagnostic marker. Additionally, in light of increasing resistance rates, we sought to assess the antifungal susceptibility profiles of *Candida* spp. in our center. Through this approach, the study aims to contribute to optimizing antifungal therapy selection and improving early treatment strategies.

KEY MESSAGES

- Signal durations in fungal cultures differ significantly among *Candida* species. Species such as *C. albicans* and *C. tropicalis* exhibit early signals, whereas *C. glabrata* and *C. parapsilosis* tend to signal later.
- Early-signaling *Candida* spp. are susceptible to fluconazole, while fluconazole resistance is more frequently observed in later-signaling species.
- Signal duration of *Candida* spp. may help guide empirical antifungal treatment decisions. Fluconazole may be considered an appropriate option for species that signal early.

MATERIALS AND METHODS

Study Design and Data Collection

Prior to the initiation of this retrospective cohort study, formal ethical approval was obtained from Sivas Cumhuriyet University Health Sciences Research Ethics Committee (approval number: 2025-04/04, date: 24.04.2025), ensuring that all study procedures adhered to established ethical standards and guidelines. The study included all hospitalized patients aged over 18 years at Sivas Numune Hospital who had blood cultures positive for *Candida* spp. between January 1, 2022 and February 28, 2025. Blood culture bottles from patients under 18 years of age, those contaminated with skin flora, or those inoculated with non-blood specimens (e.g., pleural fluid or synovial fluid) were excluded. For each patient, the time to positivity of each individual blood culture bottle collected simultaneously was recorded separately. Antifungal susceptibility results, however, were documented as a single consolidated result per patient. Bottles inoculated with non-blood specimens (e.g., pleural fluid or synovial fluid) and patients under 18 years of age were excluded from the study. Patient demographic data (age and sex), the species and strain of the isolated pathogen, and the time to positivity (in hours) were retrieved from the hospital information system and recorded in Microsoft Excel (Microsoft Office Version 365, USA; URL: https://www.office.com/).

Laboratory Procedures

Blood samples were aseptically collected at the patient's bedside and inoculated into BACTEC Plus Aerobic Medium bottles (Becton Dickinson, USA). Following inoculation, the bottles were incubated for up to five days in an automated BACTEC blood culture system (Becton Dickinson, USA) to ensure optimal conditions for microbial growth and detection. Bottles signaling positive growth were subjected to Gram staining, and samples were subcultured onto blood agar and Sabouraud dextrose agar. Colonies grown on agar media were isolated and identified, and antifungal susceptibility testing was performed using a fully automated identification and susceptibility testing system. TTP was defined as the duration (in hours) between placement of the inoculated bottle into the automated culture system and detection of microbial growth, which triggered an alert.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics version 23 (IBM Corp., USA). The normality of continuous variables was assessed using the Shapiro-Wilk or Kolmogorov-Smirnov tests. For descriptive statistics, normally distributed variables were expressed as mean±standard deviation (SD), whereas non-normally distributed variables were presented as median and interquartile range (IQR).

Comparisons between study groups were performed using parametric or non-parametric tests according to data distribution. Continuous variables with normal distribution were analyzed using the independent samples Student's t-test, while not meeting normality assumptions were evaluated using the Mann–Whitney U test. Categorical variables were summarized as absolute numbers and corresponding percentages. The discriminatory performance of selected

Table 1. Distribution of yeast isolates in blood cultures

Species	No. of Isolates (%)
C. albicans	36 (51.5)
C. glabrata	15 (21.5)
C. parapsilosis	8 (11.5)
C. tropicalis	6 (8.5)
C. melibiosica	2 (2.8)
C. kefyr	1 (1.4)
C. lipolytica	1 (1.4)
C. dubliniensis	1 (1.4)
Total	70 (100)

Table 2. Comparison of time to positivity (TTP) among *Candida* isolates

	Signal Time Median (hours, IQR)	p
C. albicans	21 (16-32)	**<0.001**
C. glabrata	42 (35-48)	
C. albicans	21 (16-32)	0.082
C. tropicalis	19 (8-22)	
C. albicans	21 (16-32)	0.116
C. parapsilosis	29 (21-44)	
C. glabrata	42 (35-48)	**<0.001**
C. tropicalis	19 (8-22)	
C. glabrata	42 (35-48)	0.068
C. parapsilosis	29 (21-44)	
C. tropicalis	19 (8-22)	**0.004**
C. parapsilosis	29 (21-44)	

IQR: Interquartile range.

laboratory and clinical parameters was further evaluated using receiver operating characteristic (ROC) curve analysis. Optimal threshold values were determined using the Youden index, which maximizes the combined sensitivity and specificity of a test. All analyses were conducted using two-tailed statistical tests, and statistical significance was defined as a p-value <0.05.

Given the retrospective nature of the study, no prospective sample size calculation was performed prior to data collection. However, a post hoc power analysis was conducted based on the observed intergroup differences in blood culture time to positivity among *Candida* species. Assuming an alpha level of 0.05, the resulting statistical power was estimated to be approximately 85%, indicating an adequate ability to detect clinically meaningful differences.

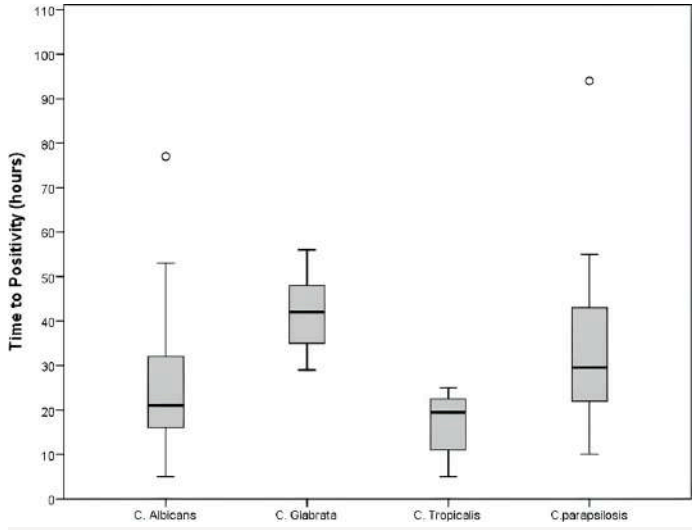

Figure 1. Time to blood culture positivity (TTP) among *Candida* isolates.

RESULTS

A total of 101 blood culture specimens obtained from 70 individual patients with *Candida* species growth were included in the analysis. The cohort consisted of 31 males and 39 females, with a mean age of 73.2±12.0 years (±SD). Antifungal susceptibility results were available for 48 of the 70 patients included in the study. Among the 70 patients, *C. albicans* was isolated in 36 cases, *C. glabrata* in 15 cases, *C. parapsilosis* in eight cases, *C. tropicalis* in six cases, and other *Candida* species in five cases (Table 1). Time to positivity data were available for 82 blood culture bottles. *C. glabrata* demonstrated a significantly longer TTP compared to both *C. albicans* and *C. tropicalis*. Although TTP was also longer compared to *C. parapsilosis*, this difference did not reach statistical significance (Table 2). Among all *Candida* species, *C. tropicalis* exhibited the shortest time to positivity; this difference was statistically significant when compared to *C. glabrata* and *C. parapsilosis* (Table 2). The distribution of TTP values for all *Candida* isolates is presented in Figure 1.

Antifungal susceptibility results were available for 48 patients included in the study. All *C. albicans* isolates were susceptible to fluconazole and amphotericin B, while susceptibility rates to voriconazole, anidulafungin, and micafungin exceeded 95%. *C. glabrata* isolates demonstrated 100% susceptibility to amphotericin B and anidulafungin, and 66% susceptibility to fluconazole. All *C. tropicalis* isolates included in the study exhibited complete susceptibility (100%) to all antifungal agents tested. Detailed antifungal susceptibility profiles of the *Candida* isolates are summarized in Table 3.

Table 3. Susceptibility of *Candida* isolates to antifungal agents

Susceptibility (n)	*C. albicans* (%)	*C. glabrata* (%)	*C. tropicalis* (%)	*C. parapsilosis* (%)
Fluconazole (48)	22 (100)	6 (50)	4 (100)	6 (85.7)
Voriconazole (38)	21 (95.4)	4 (80)	4 (100)	5 (71.4)
Amphotericin B (42)	22 (100)	11 (100)	2 (100)	7 (100)
Anidulafungin (43)	20 (95.2)	12 (100)	3 (100)	5 (71.4)
Micafungin (43)	21 (95.4)	10 (83.3)	2 (100)	7 (100)

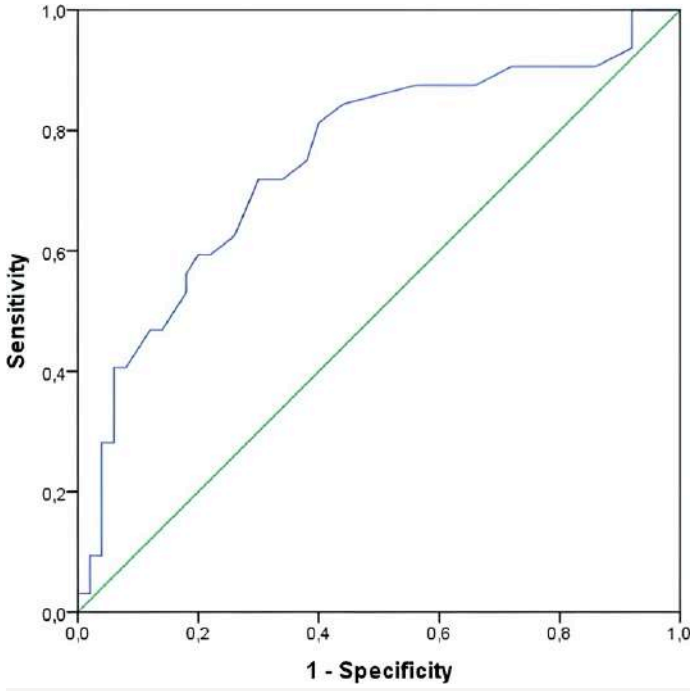

Figure 2. Receiver operating characteristic curve of time to blood culture positivity (TTP) for predicting the *C. albicans–C. tropicalis* group.

In our study, as all *C. albicans* and *C. tropicalis* isolates demonstrated complete susceptibility to fluconazole and their TTP values did not differ significantly, these two species were combined into a single group. In contrast, *C. glabrata* and *C. parapsilosis* were categorized into a separate group for TTP comparison. The median TTP for the *C. albicans–C. tropicalis* group was 21 hours (IQR: 16–30), whereas it was 35 hours (IQR: 23–47) for the *C. glabrata–C. parapsilosis* group; this difference was statistically significant ($p<0.001$). The discriminatory ability of TTP to identify the *C. albicans–C. tropicalis* group and the corresponding threshold values were evaluated using ROC analysis, enabling assessment of both sensitivity and specificity (Fig. 2). According to the ROC analysis, a cut-off value of 28 hours provided the optimal balance between sensitivity (72%) and specificity (70%) (area under the curve: 0.751; 95% confidence interval: 0.638–0.863).

DISCUSSION

Infections caused by *Candida* species may involve various organs, including the lungs (pneumonia), the heart (endocarditis), and the central nervous system, and are associated with high mortality rates. Established risk factors for infections caused by *Candida* spp. include the use of broad-spectrum antibiotics, receipt of blood transfusions, prior *Candida* colonization, and administration of total parenteral nutrition.[9,10] The World Health Organization classifies *Candida* species within the critical and high-priority fungal pathogen groups.[2] Given the clinical significance of these microorganisms, early and effective treatment is essential.

Although recent studies have highlighted the increasing prevalence of non-albicans *Candida* species in *Candida* infections,[11,12] *C. albicans* accounted for the majority of isolates in our study (51.5%) (Table 1). A total of eight distinct *Candida* species were identified among the isolates analyzed during the present investigation. Species identification was performed using the BD Phoenix 100 system, which has been reported to reliably and accurately identify the most commonly encountered *Candida* species, such as *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*, with an overall accuracy exceeding 95%, thereby providing a robust tool for clinical and laboratory diagnosis.[13] In our study, 92.8% of the isolates were common *Candida* species (Table 1). Based on these findings, the likelihood of species misidentification was considered to be low.

The study evaluated variations in TTP among different *Candida* isolates, including *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*, using blood culture specimens. Consistent with the findings of Sachu et al.[8] and Yang et al.,[14] *C. tropicalis* demonstrated the shortest TTP among the species analyzed (Fig. 1). However, although *C. tropicalis* had the shortest median TTP, the difference compared with *C. albicans* did not reach statistical significance ($p=0.082$) (Table 2). This may be attributable to the limited number of blood culture samples positive for *C. tropicalis* in our study. In line with previous reports,[8,14] *C. glabrata* exhibited the longest TTP.

Candida species are conventionally classified as albicans and non-albicans groups.[11] However, in the present study, grouping

was based primarily on similarity in TTP values. *C. albicans* and *C. tropicalis* isolates exhibited similar growth kinetics, with no statistically significant difference in their TTP values. In contrast, *C. glabrata* and *C. parapsilosis* demonstrated longer TTP values. Therefore, grouping species with similar growth dynamics was considered appropriate for TTP-based analyses. When classified according to TTP, *C. tropicalis* and *C. albicans* constituted the early-detection group, whereas *C. glabrata* and *C. parapsilosis* comprised the late-detection group, with median TTPs of 21 hours and 35 hours, respectively ($p<0.001$). Based on ROC analysis, the optimal threshold value was determined to be 28 hours. When TTP was less than 28 hours, the sensitivity and specificity for identifying the *C. albicans*–*C. tropicalis* group were 72% and 70%, respectively, indicating that this threshold provides moderate discriminatory ability for correctly classifying these species in blood culture specimens (Fig. 2). While Sachu et al.[8] reported a cut-off value of 24 hours for *C. tropicalis*, Yang et al.[14] reported 25.5 hours. The higher cut-off value identified in our study (28 hours) may be attributable to the inclusion of *C. albicans* alongside *C. tropicalis*. In addition, another study reported a cut-off value of 33.1 hours for *C. tropicalis* and found that antifungal susceptibility was not associated with TTP.[15] Taken together, these findings suggest that TTP may serve as a useful indicator for species identification.

A total of 48 *Candida* isolates underwent antifungal susceptibility testing. Fluconazole susceptibility was observed in all *C. albicans* and *C. tropicalis* isolates. These findings suggest that empirical fluconazole therapy could be considered for early-detecting *Candida* species. A recent systematic review including 89 studies reported fluconazole susceptibility rates of 93.25% for *C. parapsilosis*, 91.6% for *C. albicans*, 79.4% for *C. glabrata*, 77.95% for *C. tropicalis*, 76% for C. guilliermondii, 50% for C. pelliculosa, and 0% for *C. auris*.[16] In another recent study evaluating antifungal susceptibility patterns of *Candida* species, *Candida albicans* isolates demonstrated 13.3% resistance to fluconazole, 2.2% resistance to amphotericin B, and no resistance to anidulafungin. *Candida glabrata* isolates showed 40% resistance and 60% intermediate susceptibility to fluconazole, while *Candida* parapsilosis isolates were 83% susceptible to fluconazole.[15] The lower fluconazole susceptibility rates observed for *C. glabrata* and *C. parapsilosis* in our study may be attributable to the relatively small number of isolates included in this investigation.

Given that all *C. albicans* and *C. tropicalis* isolates were susceptible to fluconazole, this agent may represent an appropriate option for empirical therapy in patients with early yeast detection. Additionally, all *Candida* isolates in our study were susceptible to amphotericin B, whereas echinocandin resistance was observed among the late-detecting species (*C. glabrata* and *C. parapsilosis*) (Table 3). Based on these findings, amphotericin B may be considered in cases of late yeast detection. Each center should determine the distribution of *Candida* species, antifungal resistance patterns, and TTP values using their own systems to guide the management of fungal infections, which are associated with substantial mortality and serious clinical complications.

Although it has been suggested that candidemia concentration may influence TTP, Yang et al.[14] demonstrated that TTP is independent of inoculum concentration by inoculating blood culture bottles with various concentrations and species of *Candida* isolates.

The relatively small sample size of our study, its single-center design, and its restriction to a single city limit our ability to establish definitive antifungal resistance rates and TTP thresholds. Moreover, the impact of TTP on mortality was not evaluated, which represents an additional limitation of our study.

CONCLUSION

In conclusion, although TTP alone may not definitively predict *Candida* species, it may serve as a useful supportive indicator. When combined with local antifungal resistance data, these findings may assist clinicians in selecting appropriate empirical therapy. As no previous studies from Türkiye have addressed this topic, this research aims to fill an existing knowledge gap and provide an original and valuable contribution to the scientific literature in Türkiye.

Ethics Committee Approval: Ethics committee approval was obtained from Sivas Cumhuriyet University Health Sciences Research Ethics Committee (date: 24.04.2025, number: 2025-04/04).

Informed Consent: Written consent was obtained from all participants (or their legal representatives/guardians). The consent form provided detailed information about the study's purpose, methodology, potential risks, expected benefits, and participant rights. Additionally, participants were informed about the confidentiality of their personal data and the protection of their anonymity.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Clinical and Anthropometric Correlates of Polysomnography-Defined Severity in Obstructive Sleep Apnea Syndrome

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ABSTRACT

Objective: Obstructive sleep apnea syndrome (OSAS) is a common sleep-related breathing disorder frequently associated with cardiometabolic morbidity. In this retrospective study of 339 adults who underwent polysomnography (PSG) between January 2020 and December 2024, we investigated the relationship between routinely obtained clinical and anthropometric measures—body mass index (BMI), neck circumference (NC), waist circumference (WC), and the Epworth Sleepiness Scale (ESS)—and PSG-defined OSAS severity.

Materials and Methods: Adults (≥18 years) who underwent overnight PSG and had complete clinical and anthropometric data were included. OSAS severity was defined using the apnea–hypopnea index (AHI): no OSA (<5 events/h), mild (5–14.9), moderate (15–29.9), and severe (≥30). Associations between AHI and clinical or anthropometric measures were assessed using Spearman's correlation analysis.

Results: A total of 339 patients were included (mean age 46.1±12.0 years; 73.2% male; mean BMI 33.2±6.2 kg/m^2). The median ESS score was 4 (interquartile range [IQR]: 2–9), and the median AHI was 10.1 (IQR: 6.3–32.2). AHI showed statistically significant but weak correlations with age (r=0.207), weight (r=0.136), NC (r=0.273), and WC (r=0.184), whereas the association with ESS was stronger (r=0.649; all p<0.05). No significant correlations were observed with height or BMI. Patients with moderate-to-severe OSAS had higher NC, WC, ESS scores, symptom burden, and cardiometabolic comorbidities compared to those with normal-to-mild OSAS.

Conclusion: NC, WC, and ESS were associated with PSG-defined OSAS severity. However, correlations with age, weight, NC, and WC were weak in magnitude despite statistical significance, while BMI showed limited association. These routinely obtained measures may aid clinical assessment in sleep laboratory populations.

Keywords: Anthropometry, apnea–hypopnea index, Epworth Sleepiness Scale, neck circumference, Obstructive sleep apnea syndrome, polysomnography, waist circumference.

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INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a prevalent sleep-related respiratory disorder characterized by recurrent episodes of airflow limitation or cessation due to upper airway obstruction during sleep. The condition is widely recognized as a contributor to several major health problems, including cardiovascular disease, metabolic syndrome, and type 2 diabetes. Polysomnography (PSG), the gold standard for diagnosing OSAS and determining its severity, enables the measurement of brain activity, muscle tone, respiratory patterns, blood oxygen levels, and other physiological parameters during sleep.[1] Clinical and anthropometric characteristics are among the key factors influencing susceptibility to OSAS. Excess body weight, along with increased neck and waist circumference and elevated body mass index (BMI), has consistently been associated with a higher risk of upper airway obstruction during sleep. BMI is directly related to OSAS severity; as BMI increases, so does the propensity for upper airway collapse. Excess adipose tissue in the neck area contributes to airway obstruction, thereby increasing the likelihood of apnea events.[2]

When PSG findings are analyzed, individuals with elevated BMI, neck circumference (NC), and waist circumference (WC) generally exhibit higher apnea–hypopnea index (AHI) values. A positive correlation has been demonstrated between increases in BMI and NC and AHI, as these factors contribute to narrowing of the upper airway and increase the frequency of apnea–hypopnea episodes.[3] Data from the United States suggest that moderate-to-severe obstructive sleep apnea (defined as AHI ≥15 events per hour) affects approximately 13% of men and 6% of women aged 30–70 years.[4] The seriousness of comorbidities associated with OSAS—which is common yet often underdiagnosed—underscores the need for reliable screening programs, particularly for asymptomatic individuals.[5]

However, the strength of the associations between anthropometric variables and PSG-defined severity varies across populations, suggesting that further characterization of these relationships may provide additional clinical insight, particularly in referred sleep laboratory cohorts.

Many individuals at high risk for OSAS face barriers to accessing sleep clinics due to immobility, transportation limitations, or residence in remote areas. Excess body weight is a prominent clinical feature often used to identify at-risk individuals. However, beyond BMI or witnessed apneas, there is a need for practical screening tools that account for diverse body types and can rapidly identify those at risk. Although screening tools exist, understanding how simple bedside measurements relate to objective PSG-defined severity within clinical populations remains clinically relevant.[6]

KEY MESSAGES

- Neck circumference and waist circumference showed statistically significant but weak correlations with polysomnography-defined OSAS severity.
- The Epworth Sleepiness Scale demonstrated a stronger association with the apnea–hypopnea index compared with anthropometric measures, reflecting its relevance as a complementary clinical parameter.
- Patients with moderate-to-severe OSAS had a higher symptom burden and a greater prevalence of cardiometabolic comorbidities, highlighting the need for comprehensive clinical evaluation in sleep clinic populations.

Given that OSAS can lead to significant health complications and that PSG-derived AHI values are critical for determining disease severity, exploring the associations between routinely obtained clinical and anthropometric parameters and PSG outcomes may assist clinicians in contextualizing disease burden. Therefore, careful assessment of anthropometric measurements—such as BMI, NC, and WC—should be an integral component of OSAS evaluation.

This study sought to evaluate how various clinical and anthropometric characteristics relate to polysomnographic outcomes in patients with a confirmed diagnosis of OSAS. Rather than proposing a predictive model, the study focuses on describing the extent to which commonly collected clinical measures correlate with PSG-defined severity. By providing population-specific data, this work may support more individualized diagnostic considerations within sleep laboratory settings. We hypothesized that significant correlations exist between clinical variables, such as age, sex, and subjective sleep quality, and anthropometric measures, such as BMI, NC, and WC, in relation to PSG parameters—particularly the AHI.

MATERIALS AND METHODS

Study Protocol and Design

This study was designed as a retrospective descriptive investigation. Patients aged ≥18 years who presented to the pulmonology outpatient clinic of our hospital between January 2020 and December 2024 with complaints of sleep disturbances, were indicated for PSG, and subsequently underwent overnight PSG in the sleep laboratory were included.

Ethics Approval/Informed Consent

This research was conducted in accordance with national and institutional guidelines governing human research and adhered

to the ethical principles of the Declaration of Helsinki. Approval for the study was granted by Recep Tayyip Erdogan University Non-Interventional Clinical Research Ethics Committee (Approval Number: E-40465587-050.01.04-1367, 2025/50, Date: 12.02.2025). The requirement for informed consent was waived due to the retrospective design and the use of anonymized patient data.

Patients and Data Collection

All clinical and anthropometric assessments were performed during the routine outpatient sleep clinic evaluation prior to polysomnography. Polysomnography was conducted on the same night or within one week of the outpatient assessment.

Neck circumference was measured with the participant standing upright and the head positioned in the Frankfort horizontal plane, with the measuring tape placed at the level of the cricothyroid cartilage. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest during quiet expiration. Body weight and height were obtained using calibrated clinical devices, and body mass index was calculated as weight (kg) divided by height squared (m^2).

Epworth Sleepiness Scale (ESS) scores were retrieved retrospectively from routine sleep clinic evaluation records. The ESS consists of eight questions, each rated on a scale from 0 to 3, yielding a total score ranging from 0 to 24, with higher scores indicating greater self-reported daytime sleepiness.

All respiratory events recorded during overnight polysomnography were evaluated in accordance with the 2012 guidelines of the American Academy of Sleep Medicine (AASM). Apnea was defined as a reduction in airflow of at least 90% lasting for a minimum of 10 seconds, whereas hypopnea was defined as a decrease in airflow of at least 30% lasting at least 10 seconds and associated with either a ≥3% oxygen desaturation or an electroencephalographic arousal.

The apnea–hypopnea index was defined as the average number of apneic and hypopneic respiratory events per hour of recorded sleep. Based on AHI values, OSAS severity was categorized into four groups:

- ***No OSA:*** AHI <5 events/h
- ***Mild OSA:*** AHI 5–14.9 events/h
- ***Moderate OSA:*** AHI 15–29.9 events/h
- ***Severe OSA:*** AHI ≥30 events/h

Inclusion Criteria: Participants were eligible for inclusion if they were aged 18 years or older; had been referred for overnight polysomnography due to suspected sleep-disordered breathing; had complete PSG recordings available; and had comprehensive clinical and anthropometric data documented, including age, sex, height, body weight, neck circumference, waist circumference, and Epworth Sleepiness Scale scores.

Exclusion Criteria: Patients were excluded if they had a prior history of upper airway surgery for obstructive sleep apnea, documented neuromuscular disease, craniofacial abnormalities, central sleep apnea, chronic respiratory failure, pregnancy, or incomplete clinical, anthropometric, or polysomnographic records. Only individuals who met all eligibility criteria were included in the final analysis.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software (version 21; SPSS Inc., Chicago, IL, USA). The distribution of continuous variables was assessed using the Kolmogorov–Smirnov normality test. Normally distributed data were expressed as mean±standard deviation, whereas non-normally distributed variables were reported as median (interquartile range). Categorical variables were summarized as counts and percentages.

For group comparisons, the Student's t-test was applied to normally distributed variables, while the Mann–Whitney U test was used for non-normally distributed variables. Categorical data were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. Fisher's exact test was applied when expected cell frequencies were less than five.

Because the apnea–hypopnea index did not follow a normal distribution and the Epworth Sleepiness Scale is an ordinal variable, associations between AHI and clinical or anthropometric parameters were examined using Spearman's rank correlation analysis. Statistical significance was defined as $p<0.05$.

A post hoc power analysis evaluating differences in neck circumference between the moderate-to-severe and normal-to-mild OSA groups demonstrated sufficient statistical power (Cohen's d=0.50; power=0.99).

Given the descriptive and retrospective nature of the study and the strong collinearity among anthropometric variables, multivariable regression modeling was not performed. Accordingly, the analysis was restricted to correlation-based evaluation of associations between clinical parameters and AHI.

The primary endpoints of the study were the apnea–hypopnea index and the Epworth Sleepiness Scale. Secondary outcomes included anthropometric parameters (BMI, neck circumference, and waist circumference), symptom profiles, and cardiometabolic comorbidities.

Confidence intervals for Spearman correlation coefficients were not routinely generated by the statistical software used in this retrospective analysis. Future prospective studies should incorporate confidence interval estimation to improve precision and clinical interpretability.

RESULTS

A total of 339 patients were included in the analysis. The mean age was 46.10±12.00 years, and 73.20% were male. Mean anthropometric values were as follows: height 171±9 cm, weight 97±19 kg, BMI 33.20±6.20 kg/m^2, NC 41±4 cm, and WC 110±17 cm. The median ESS score was 4 (interquartile range [IQR]: 2–9), and the median AHI was 10.10 (IQR: 6.30–32.20). Snoring (52.20%), witnessed apneas (41.30%), morning fatigue (33.90%), nocturia (30.70%), and morning dry mouth (33.60%) were the most frequently reported symptoms. Based on AHI values, 10.1% of patients had no obstructive sleep apnea (OSA), 51.30% had mild OSA, 6.50% had moderate OSA, and 32.20% had severe OSA. Hypertension (36.90%) and type 2 diabetes (17.40%) were the most common comorbidities (Table 1).

Spearman's correlation analysis demonstrated significant positive associations between AHI and age (r=0.207, p<0.001), weight (r=0.136, p=0.012), NC (r=0.273, p<0.001), WC (r=0.184, p=0.001), and ESS (r=0.649, p<0.001).

The correlations with age, weight, NC, and WC were weak, whereas the association between ESS and AHI was strong.

No statistically significant correlations were observed between AHI and height (r=0.094, p=0.082) or BMI (r=0.086, p=0.114) (Table 2).

A scatter plot illustrating the association between ESS and AHI is presented in Figure 1.

When grouped by disease severity, patients with moderate-to-severe OSA were older (49±10 vs. 45±13 years, p=0.003; Cohen's d=0.35, 95% confidence interval [CI]: 0.12–0.58) and predominantly male (p<0.001). NC and WC were also significantly higher in this group (NC: 42±4 vs. 40±4 cm; p<0.001; Cohen's d=0.50, 95% CI: 0.27–0.73; WC: 113±17 vs. 108±17 cm, p<0.001; Cohen's d=0.29, 95% CI: 0.07–0.51). ESS scores were substantially higher in moderate-to-severe OSA (median 9 [IQR: 7–12]) compared with normal-to-mild cases (median 2 [IQR: 0–4], p<0.001).

Symptom frequency increased markedly with disease severity: snoring (96% vs. 25%), witnessed apneas (95% vs. 7%), morning fatigue (79% vs. 4%), nocturia (75% vs. 3%), and dry mouth (77% vs. 6%) (all p<0.001).

Table 1. Demographic and clinical characteristics of the study population (n=339)

Parameter	Value
Age (years)	46.1±12.0
Sex, n (%)	
Female	91 (26.8)
Male	248 (73.2)
Height (cm)	171±9
Weight (kg)	97±19
BMI (kg/m^2)	33.2±6.2
NC (cm)	41±4
WC (cm)	110±17
ESS (points)	4 (IQR: 2–9)
AHI (events/h)	10.1 (IQR: 6.3–32.2)
OSA-related symptoms, n (%)	
Snoring	177 (52.2)
Witnessed apnea	140 (41.3)
Morning fatigue	115 (33.9)
Nocturia	104 (30.7)
Dry mouth	114 (33.6)
OSA severity classification, n (%)	
No OSA (AHI <5)	34 (10.0)
Mild OSA (AHI: 5–15)	174 (51.3)
Moderate OSA (AHI: 16–30)	22 (6.5)
Severe OSA (AHI ≥30)	109 (32.2)
Comorbidities, n (%)	
Hypertension	125 (36.9)
Type 2 diabetes mellitus	59 (17.4)
Coronary artery disease	24 (7.1)
Chronic obstructive pulmonary disease	8 (2.4)
Asthma	18 (5.3)
Allergy	79 (23.3)
Arrhythmia	22 (6.5)

Table 1 (Cont). Demographic and clinical characteristics of the study population (n=339)

Parameter	Value
Hyperlipidemia	42 (12.4)
Gastritis	45 (13.3)
Restless legs syndrome	24 (7.1)

Data are presented as mean±standard deviation, median (interquartile range), or number (percentage), as appropriate. Primary outcomes were apnea–hypopnea index (AHI) and the Epworth Sleepiness Scale (ESS). Secondary outcomes included anthropometric parameters, symptom profiles, and comorbidities.

Table 2. Correlation between apnea–hypopnea index (AHI) and clinical/anthropometric variables (Spearman's rank correlation analysis)

Parameter	Correlation Coefficient (r)	p
Age (years)	0.207	<0.001*
Height (cm)	0.094	0.082
Weight (kg)	0.136	0.012*
BMI (kg/m^2)	0.086	0.114
NC (cm)	0.273	<0.001*
WC (cm)	0.184	0.001*
ESS (points)	0.649	<0.001*

Apnea–hypopnea index (AHI) was defined as the primary outcome variable. All other variables were treated as secondary explanatory variables. *p<0.05 indicates statistical significance.

Similarly, cardiometabolic comorbidities were more prevalent in moderate-to-severe OSA, including hypertension (53% vs. 27%), type 2 diabetes (25% vs. 13%), coronary artery disease (11% vs. 5%), arrhythmia (15% vs. 1%), hyperlipidemia (20% vs. 8%), and restless legs syndrome (14% vs. 3%) (all p<0.05). These findings indicate a progressive increase in both symptom burden and comorbidity frequency with advancing OSA severity (Table 3).

DISCUSSION

The findings of this study highlight four key points:

a) Significant associations between clinical and anthropometric parameters—namely NC, WC, and the ESS—and the AHI.

b) A strong positive correlation between ESS and AHI, suggesting that subjective daytime sleepiness may serve as a useful marker of disease severity.

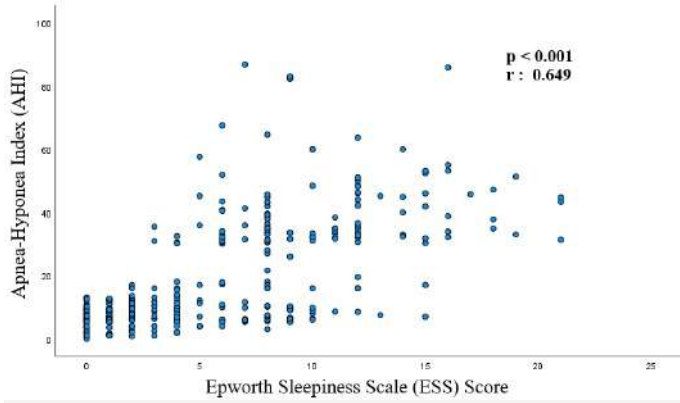

Figure 1. Scatter plot illustrating the monotonic association between the Epworth Sleepiness Scale (ESS) score and the apnea–hypopnea index (AHI). The plot provides a descriptive visualization of the relationship based on Spearman's correlation analysis and does not represent a predictive regression model.

c) A notable increase in age, central obesity indicators, and symptom burden among individuals with moderate-to-severe OSAS.

d) A significant rise in the prevalence of cardiometabolic comorbidities with increasing OSA severity.

This study provides a comprehensive evaluation of the relationships between clinical and anthropometric parameters and PSG findings in patients diagnosed with OSAS. Consistent with previous research, the results demonstrate meaningful correlations between AHI and specific anthropometric indicators, particularly NC and WC. Rather than implying novelty, these findings reaffirm the clinical relevance of routinely obtained anthropometric markers in OSAS assessment, especially in settings where access to PSG may be limited.

The predominance of male patients in our cohort (73.2%) aligns with existing epidemiological data indicating a higher prevalence of OSAS among men. The mean BMI of 33.2 kg/m^2 coupled with mean WC and NC measurements of 110 cm and 41 cm, respectively, reflects the strong association between OSAS and central obesity.[7] The distribution of OSAS severity in our cohort—with 51.3% classified as mild and 32.2% as severe— likely reflects referral bias and the selective nature of patients undergoing PSG, underscoring the heterogeneity of OSAS in clinical practice. Symptomatically, the most frequently reported complaints were snoring (52.2%), morning fatigue (33.9%), and nocturia (30.7%), reflecting both upper airway obstruction and systemic manifestations of the disorder. Hypertension (36.9%) and type 2 diabetes mellitus (17.4%) were the most prevalent comorbidities, consistent with literature linking OSAS to increased cardiometabolic

Table 3. Comparison of patients with normal-to-mild and moderate-to-severe obstructive sleep apnea

Variable	Normal-to-Mild OSA (AHI ≤15) (n=208)	Moderate-to-Severe OSA (AHI ≥16) (n=131)	p
Age (years)	45±13	49±10	0.003*
Sex (F/M)	71/137	20/111	<0.001*
Height (cm)	170±9	172±8	0.090
Weight (kg)	96±19	100±19	0.076
BMI (kg/m^2)	32.9±6.1	33.7±6.5	0.256
NC (cm)	40±4	42±4	<0.001*
WC (cm)	108±17	113±17	0.010*
ESS (points)	2 (0–4)	9 (7–12)	<0.001*
Snoring (%)	51 (25)	126 (96)	<0.001*
Witnessed apnea (%)	15 (7)	125 (95)	<0.001*
Morning fatigue (%)	8 (4)	107 (79)	<0.001*
Nocturia (%)	6 (3)	98 (75)	<0.001*
Dry mouth (%)	13 (6)	101 (77)	<0.001*
Hypertension (%)	56 (27)	69 (53)	<0.001*
Type 2 diabetes mellitus (%)	26 (13)	33 (25)	0.003*
Coronary artery disease (%)	10 (5)	14 (11)	0.040*
Chronic obstructive pulmonary disease (%)	4 (2)	4 (3)	0.504†
Asthma (%)	12 (6)	6 (5)	0.634
Arrhythmia (%)	3 (1)	19 (15)	<0.001*
Allergy (%)	54 (26)	25 (19)	0.145
Hyperlipidemia (%)	16 (8)	26 (20)	<0.001*
Restless legs syndrome (%)	6 (3)	18 (14)	<0.001*

Data are presented as mean±standard deviation, median (interquartile range), or number (percentage), as appropriate. Primary outcomes were apnea–hypopnea index (AHI) and the Epworth Sleepiness Scale (ESS). Secondary outcomes included anthropometric parameters, symptom profiles, and comorbidities. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Fisher's exact test was applied when expected cell counts were <5. *Statistically significant difference between groups (p<0.05). † Fisher's exact test.

risk through mechanisms such as systemic inflammation, oxidative stress, and insulin resistance.[8,9] These observations further support the concept of OSAS as a multisystem disorder rather than an isolated respiratory condition.

Although the observed associations are largely consistent with previous literature, the present findings provide clinically relevant insight derived from a real-world tertiary sleep clinic population characterized by high rates of obesity, cardiometabolic comorbidity, and symptomatic sleep-disordered breathing. Our cohort represents a referral-based clinical population characterized with a high prevalence of central obesity, cardiometabolic comorbidity, and a broad spectrum of OSAS severity, reflecting contemporary clinical practice in a regional healthcare setting. In contrast to population-based screening cohorts, our study captures patients with symptomatic sleep-disordered breathing who were referred for diagnostic polysomnography, thereby

offering practical information applicable to daily clinical decision-making. Furthermore, the regional referral patterns and healthcare access characteristics of our center contribute to a distinct patient profile, with a predominance of male patients, advanced obesity, and high cardiometabolic risk. These features underscore the clinical relevance of our findings for sleep clinics serving similar populations and healthcare systems.

Apnea–hypopnea index showed statistically significant positive associations with age, body weight, neck circumference, waist circumference, and ESS scores. However, correlations with age, weight, NC, and WC were weak in magnitude, indicating small effect sizes despite statistical significance. Among these variables, ESS exhibited the strongest association with AHI ($r=0.649$, $p<0.001$), suggesting a moderate-to-strong monotonic relationship between subjective daytime sleepiness and respiratory disturbance severity.[10] However, given the ordinal nature of ESS and the skewed distribution of AHI, this association should be interpreted as reflecting a monotonic relationship rather than serving as a predictive metric. The associations between AHI and both NC and WC—indicators of central adiposity—further support the role of fat distribution, particularly in the upper body, in the pathophysiology of OSAS.[7] Conversely, the absence of significant associations between AHI and either height or BMI underscores the limited predictive value of BMI alone. This observation is consistent with evidence that BMI does not adequately capture regional fat deposition, particularly in the upper body, which may explain discrepancies across studies. In line with these findings, Yetkin et al.[11] recently demonstrated that alternative anthropometric indices reflecting body fat distribution, such as the triponderal mass index, are significantly associated with obstructive sleep apnea severity and may provide complementary information beyond conventional BMI measurements.

The robust positive correlation between ESS and AHI ($r=0.649$, $p<0.001$) indicates that subjective daytime sleepiness generally parallels objective respiratory disturbance. Although this finding supports the clinical relevance of ESS, it should not be interpreted as a predictive model, as the present study did not include multivariable or prognostic analyses. Nevertheless, the strength of this association aligns with previous evidence suggesting that ESS may serve as a practical clinical indicator of OSAS severity.[10] Such a relationship may be useful for prioritizing patients for diagnostic evaluation; however, validation in larger and more heterogeneous populations is required, and the influence of coexisting comorbidities should be evaluated in adjusted models.

In this study, significant differences in both demographic and anthropometric characteristics were observed across OSAS severity levels. The older age observed in the moderate-to-severe OSAS group reflects the cumulative risk associated with aging and the progressive nature of the disorder. The predominance of male patients in this group—along with higher AHI values—is likely attributable to narrower upper airway anatomy and differences in fat distribution patterns between sexes.[12] The notably higher neck and waist circumference measurements among individuals with more severe OSAS further reinforce the role of central obesity as a major structural risk factor predisposing to upper airway collapse. Regarding symptomatology, the near-universal prevalence of snoring, witnessed apneas, morning fatigue, nocturia, and dry mouth in the moderate-to-severe OSAS group demonstrates a close relationship between disease severity and symptom burden. The significantly elevated ESS scores in this group further confirm the clinical alignment between subjective sleepiness and objective respiratory disturbance.

With respect to accompanying medical conditions, cardiometabolic disorders—such as hypertension, type 2 diabetes, coronary artery disease, arrhythmias, and lipid abnormalities—were observed far more frequently among individuals with moderate-to-severe OSAS compared with those with milder disease. This pattern suggests that increasing AHI levels are associated with multisystem involvement, potentially mediated by mechanisms such as systemic inflammation, endothelial dysfunction, and autonomic imbalance.[13] Although these findings are consistent with previous reports, causal inferences cannot be drawn due to the cross-sectional design. Furthermore, considering the heterogeneous nature of OSAS phenotypes, risk stratification based on clinical and anthropometric indicators should be complemented by phenotype-specific therapeutic strategies. Recent literature has highlighted that, particularly in positional OSAS, individualized treatment approaches may enhance therapeutic effectiveness.[14]

Taken together, these findings further support the concept that OSAS is a complex, multisystemic disorder extending beyond isolated nocturnal respiratory disturbances. Rather than extending existing knowledge, the present study consolidates current evidence within a real-world clinical context. The integration of anthropometric profiling, symptom assessment, and comorbidity screening into routine clinical evaluation remains essential. Early identification of nonmodifiable risk factors, such as central obesity, advanced age, and male sex, is critical for developing targeted diagnostic strategies and tailored treatment plans. Given the progressive nature of OSAS and its strong links to cardiometabolic morbidity, optimal patient care requires a multidisciplinary approach and individualized follow-up.

This study has several limitations. First, its retrospective design and reliance on data from a single center may limit

the applicability of the findings to broader or more diverse populations. As a result, the observed associations may primarily reflect the characteristics of patients referred to a tertiary sleep clinic rather than those of community-based or asymptomatic populations. Second, because the study population consisted of patients referred for suspected sleep disorders, selection bias is inevitable; therefore, the results may not be fully applicable to asymptomatic individuals or community-based cohorts. This referral-based sampling may have led to an overrepresentation of individuals with more advanced disease and greater symptom burden. Third, although anthropometric measurements such as neck and waist circumferences were extracted from standard medical records, the possibility of interobserver variability in these assessments cannot be entirely excluded. Such variability may have introduced random error, potentially attenuating the strength of the observed associations. Fourth, due to the cross-sectional nature of the study, causal relationships between the investigated parameters and OSAS severity cannot be inferred. Accordingly, the findings should be interpreted as associative rather than indicative of causal pathways. Finally, the study did not include assessments of craniofacial structure, upper airway imaging, or relevant biochemical markers, all of which could have contributed to a more comprehensive understanding of the pathophysiological mechanisms linking anthropometric features to OSAS. The absence of these variables limits our ability to explore mechanistic pathways underlying the observed clinical associations. Future prospective, multicenter studies addressing these limitations are needed to enhance the validity and generalizability of our findings.

CONCLUSION

This study demonstrates that clinical and anthropometric measures—particularly neck circumference, waist circumference, and the Epworth Sleepiness Scale—are associated with polysomnography-defined OSAS severity. These findings support the relevance of simple, routinely collected clinical parameters in the assessment of patients undergoing evaluation for suspected OSAS. However, as the analyses were correlational, these measures should not be interpreted as independent predictors of disease severity without further multivariable or prognostic modeling.

Obstructive sleep apnea syndrome remains a multifaceted condition with systemic consequences that extend beyond disordered breathing during sleep. Even in patients with mild symptoms or low subjective sleepiness, incorporating anthropometric evaluation and comorbidity screening may help clinicians better characterize overall disease burden. Early identification of key risk factors—such as central obesity, older age, and male sex—may assist in guiding diagnostic prioritization and clinical decision-making.

Given the progressive and cardiometabolic nature of OSAS, patient care should rely on comprehensive assessment and multidisciplinary management. Future prospective studies incorporating adjusted analyses and predictive modeling are needed to clarify the independent contribution of these clinical parameters and to determine their potential role in diagnostic pathways.

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Anticancer Activity and Molecular Docking Studies of Selected Benzoxazole Derivatives as Apoptosis Inducers in Non-Small Cell Lung Cancer

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ABSTRACT

Objective: Non-small cell lung cancer (NSCLC), the most prevalent type of lung cancer, remains the leading cause of cancer-related deaths worldwide. Late-stage diagnosis and resistance to conventional treatments highlight the need for further research into its molecular mechanisms. This study aimed to evaluate the anticancer effects of several benzoxazole derivatives (2-(4-tert-butylphenyl)-5-nitrobenzoxazole (1a), 2-(4-tert-butylphenyl)-6-nitrobenzoxazole (1b), 2-(2,3-dimethylphenyl)-5-nitrobenzoxazole (2a), and 2-(2,3-dimethylphenyl)-6-nitrobenzoxazole (2b)) on the viability of A549 cells.

Materials and Methods: Cell viability was assessed using the MTT assay. We also performed a molecular docking study to investigate the interactions between the benzoxazole derivatives and caspase-3, a key executioner caspase involved in apoptosis.

Results: The benzoxazole derivatives coded 1a, 1b, 2a, and 2b exhibited anticancer activity against A549 cells, with half-maximal inhibitory concentration (IC_{50}) values of 17.41±0.16, 20.50±0.08, 32.17±0.08, and 31.13±0.07 μM, respectively. Among the tested benzoxazoles, 1a and 1b showed activity comparable to cisplatin (IC_{50}=19.65±0.09 μM). According to the docking results, all compounds demonstrated satisfactory docking scores ranging from -4.339 to -5.202 kcal/mol.

Conclusion: Our results demonstrate that the benzoxazole derivatives 1a and 1b exhibit significant anticancer effects by inhibiting lung cancer cell proliferation at low concentrations, similar to cisplatin. The structure-activity relationship suggests that substitution of a phenyl group at the 2-position of the benzoxazole ring with a tert-butyl group at the para position enhances anticancer activity against A549 cells. This preliminary study indicates that these benzoxazole derivatives have promising potential as cytotoxic agents for the treatment of NSCLC.

Keywords: Apoptosis, benzoxazole, cancer, molecular docking, non-small cell lung cancer.

INTRODUCTION

Lung cancer remains a major global health issue and the leading cause of cancer-related deaths, despite advances in prevention and treatment strategies. Non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer cases, with adenocarcinoma being the most prevalent subtype.[1,2] The five-year relative survival rate varies depending on the stage at diagnosis. However, a considerable proportion of NSCLC cases are diagnosed at an advanced stage, with 30%–40% identified at stage IV. Given its poor prognosis and limited response to conventional treatments such as radiation therapy and chemotherapy, further investigation into the molecular mechanisms underlying NSCLC is essential for the development of more effective therapeutic strategies.[2]

Cancer arises from the dysregulation of cell cycle progression and apoptotic mechanisms. Apoptosis is mediated by a series of cysteine-dependent aspartate-specific proteases known as caspases. Caspases are attractive therapeutic targets in several diseases, and the induction of caspase activity may offer new treatment strategies for cancer, which is characterized by uncontrolled cell proliferation.[3] Caspase-3, a key executioner caspase, plays an essential role in apoptosis; however, its specific involvement in the pathogenesis of lung cancer remains inadequately understood.[4]

A hallmark of cancer is its ability to evade apoptosis through modulation of anti-apoptotic and pro-apoptotic gene expression. Although chemotherapy aims to induce apoptosis and eliminate cancer cells, it has not achieved complete clinical success.[4] The identification of novel anticancer agents with high efficacy and low toxicity is a major priority in cancer drug development. Given that drug resistance is a significant challenge in cancer treatment, the development of alternative chemotherapeutic agents is essential.[5]

Heterocyclic compounds play a fundamental role in drug discovery and development. Numerous approved drugs and promising therapeutic agents are based on heterocyclic scaffolds.[6] Benzoxazole is one of the most extensively studied heterocycles in medicinal chemistry, with both naturally occurring and synthetic derivatives exhibiting diverse biological activities.[7] A broad spectrum of pharmacological effects, including anti-inflammatory,[8] antimicrobial,[9] antifungal,[10] and antitumor[5,11,12] activities, has been attributed to benzoxazole derivatives.

In 2004, a series of benzoxazole derivatives and their analogs were evaluated for inhibitory activity against eukaryotic DNA topoisomerase II (Topo II). Among these compounds, 2-(2-methoxyphenyl)-6-nitrobenzoxazole and 6-methyl-2-(2-nitrophenyl)benzoxazole demonstrated greater potency than etoposide, a clinically used reference drug.[13] Subsequent three-dimensional quantitative structure–activity relationship (3D QSAR) studies, employing comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA), were conducted on the same compound series in 2005[14] and 2006[15] to elucidate the structural features contributing to Topo II inhibitory activity. These investigations revealed that hydrophilic substitution at positions 5 or 6 of the heterocyclic core significantly enhanced enzyme inhibition compared to hydrophobic groups. Additionally, the presence of hydrophobic substituents at the ortho and/or para positions of the phenyl ring attached at the 2-position of the heterocyclic core was identified as a critical factor contributing to increased activity. As a continuation of these investigations, we reported the syntheses and hTopo I and hTopo IIα enzyme inhibition activities of a series of 2-(substituted-phenyl)benzoxazole derivatives bearing a nitro group at the 5- or 6-position in 2021.[5]

KEY MESSAGES

- Several 2-(substituted phenyl)-5(6)-nitrobenzoxazole derivatives were evaluated for cytotoxicity against A549 cells.
- Compounds 2-(4-tert-butylphenyl)-5-nitrobenzoxazole (1a) and 2-(4-tert-butylphenyl)-6-nitrobenzoxazole (1b) showed cytotoxic activity comparable to cisplatin.
- Molecular docking studies suggested possible interactions between the compounds and caspase-3 (PDB ID: 3GJQ).

In the present study, as a continuation of this research, we aimed to investigate the anticancer effects of selected benzoxazole derivatives (2-(4-tert-butylphenyl)-5-nitrobenzoxazole (1a), 2-(4-tert-butylphenyl)-6-nitrobenzoxazole (1b), 2-(2,3-dimethylphenyl)-5-nitrobenzoxazole (2a), and 2-(2,3-dimethylphenyl)-6-nitrobenzoxazole (2b)) whose syntheses and hTopo I and hTopo IIα enzyme inhibition activities were previously reported by our group in 2021, on the viability of A549 human NSCLC cells. Additionally, due to the insufficient hTopo I and hTopo IIα inhibitory activities of these compounds,[5] we performed a molecular docking study to examine the interactions between these benzoxazole derivatives and caspase-3, an executioner caspase involved in apoptosis. Moreover, the ADME/Tox (absorption, distribution, metabolism, excretion, and toxicity) properties of these derivatives were evaluated using Schrödinger software suite to identify the most promising candidates as antitumor agents.

MATERIALS AND METHODS

Chemicals

This study was conducted between August 2024 and February 2025. The in silico and *in vitro* investigations of molecules 1a,

1b, 2a, and 2b, whose syntheses were previously published by our group, were carried out.[5] As this study consisted solely of *in vitro* experiments and molecular docking analyses, ethical approval was not required.

In Vitro Studies

Cell Culture

Human NSCLC A549 cells (CCL-185™, ATCC, Rockville, MD, USA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM-HG) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO_2.

Cell Viability Assay

The viability of A549 cells was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells were seeded at a density of 7×10^3 cells/mL and cultured until 80% confluence was reached. The cells were then treated with various concentrations (0–100 µM) of compounds 1a, 1b, 2a, and 2b, as well as cisplatin (2.5, 5, 10, 20, and 50 µM), for 48 hours. Following treatment, 10 µL of MTT reagent (5 mg/mL) was added to each well and incubated for four hours. After removal of the medium, 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using an Epoch 2 BioTek ELISA (enzyme-linked immunosorbent assay) reader. The half maximal inhibitory concentration (IC_{50}) values, defined as the concentration required to inhibit 50% of cell growth, were calculated by nonlinear regression analysis using a variable slope model in GraphPad Prism 9.5.1 (GraphPad Software, USA). Each experiment was performed in triplicate (n=3), based on previously established optimization studies and similar *in vitro* experiments.[12]

Statistical Analysis

Data were analyzed using GraphPad Prism 9.5.1 (GraphPad Software, USA). Data normality was assessed using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was performed to assess overall group differences, followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was defined as follows: ***$p<0.001$, **$p<0.01$, *$p<0.05$. Data are presented as mean±standard error of the mean (SEM).

Molecular Docking Studies

Molecular docking studies were performed using the Schrödinger Maestro 2022-4 Glide package[16] to calculate the binding energies of protein-ligand complexes and to visualize their interactions.

Protein Preparation

The protein preparation process aims to optimize the protein structure prior to molecular docking.[17] The X-ray crystal structure of caspase-3 enzyme complexed with a peptide inhibitor was retrieved from the RCSB Protein Data Bank (PDB ID: 3GJQ) at a resolution of 2.60 Å, and the peptide inhibitor was used as the reference ligand.[18] The Protein Preparation Wizard module in Schrödinger Maestro was used to prepare the protein for optimization, hydrogen bond addition, elimination of atomic clashes, and removal of water molecules from protein crystal structures prior to docking. The protein was cleaned, bond orders and charges were corrected, missing hydrogens were added, and the protein structure was optimized.[19]

Ligand Preparation

All ligands were drawn using the 2D Sketcher module in Schrödinger Maestro. Further minimization of all ligands was performed using the Schrödinger Maestro "Minimize Selected Atoms" tool. Subsequently, all ligands were prepared using the "LigPrep" module by converting each ligand into 3D conformers, neutralizing charged structures, and ionizing the entire structure at neutral pH 7±2.0.[20] We set 32 stereoisomers as the maximum limit per ligand.

Grid Box Preparation

After opening the "Receptor Grid Generation" module, the grid box was generated by selecting the reference ligand complexed with the protein. The grid box was defined within a 20 Å region around the reference ligand.[21]

Docking Studies

Molecular docking of compounds 1a, 1b, 2a, 2b, and the original ligand was performed to evaluate their binding to the target caspase-3 enzyme using the Schrödinger Maestro "Ligand Docking" module.[22] Following the docking procedure, the Schrödinger Maestro Grid-Based Ligand Docking with Energetics (GLIDE) tool calculated the docking score, Glide score, Glide Emodel, and Glide energy to assess protein-ligand interaction energies. The Glide scores of compounds 1a, 1b, 2a, and 2b were compared with that of the peptide inhibitor used as the reference ligand.[23]

RESULTS

In Vitro Anticancer Activities of Benzoxazole Derivatives Against A549 Cells

The anticancer effects of several previously synthesized benzoxazole derivatives (1a, 1b, 2a, and 2b) were evaluated by treating A549 lung cancer cells with varying concentrations of these compounds. All derivatives exhibited anticancer activity against A549 cells (Fig. 1, Appendix 1). The IC_{50} values for compounds 1a, 1b, 2a, and 2b were 17.41±0.16, 20.50±0.08, 32.17±0.08, and 31.13±0.07 µM, respectively (Table 1). The IC_{50} value of cisplatin was determined to be 19.65±0.09 µM. Comparison of IC_{50} values revealed that compounds 1a and

1b exhibited activity similar to cisplatin, whereas compounds 2a and 2b showed higher IC_{50} values than both cisplatin and compounds 1a and 1b ($p<0.01$). In addition to differences in IC_{50} values, treatment with compounds 2a and 2b at concentrations of 40, 60, 80, and 100 µM resulted in a significant reduction in cell viability compared with 20 µM (Appendix 1).

Results of Docking Studies

3D molecular docking of all benzoxazole derivatives (1a, 1b, 2a, and 2b), along with the original ligand, into the crystal structure of the caspase-3 enzyme (PDB ID: 3GJQ) (Figs. 2-6) was conducted for the first time using Schrödinger Release 2022-4 molecular modeling software (Schrödinger Release 2022–4, Glide, LLC, New York, NY, USA).[22,24,25] The study aimed to evaluate binding energies and investigate the interaction modes within the enzyme's active site. To estimate binding affinities and optimal alignment of the benzoxazole derivatives within the active site, different types of noncovalent interactions with surrounding amino acids, along with Glide scores, were analyzed. Based on the docking analysis, all compounds exhibited satisfactory docking scores, ranging from -4.339 to -5.202 kcal/mol (Table 2). The interaction diagrams of the original ligand and compounds 1a, 1b, 2a, and 2b are shown in Figures 2-6, respectively. As illustrated in Figures 3-6, the benzoxazole derivatives interacted with amino acid residues such as ASN208, ALA162, CYS163, GLN161, SER209, TRP206, TRP214, HIS121, ARG64, ARG207, TRP206, PHE252, PHE256, and SER249 through hydrogen bonds, π-π stacking, π-cation interactions, salt bridges, and hydrophobic interactions.

DISCUSSION

Lung cancer has the highest incidence and mortality rates among all malignant neoplasms, and 80%–85% of cases are

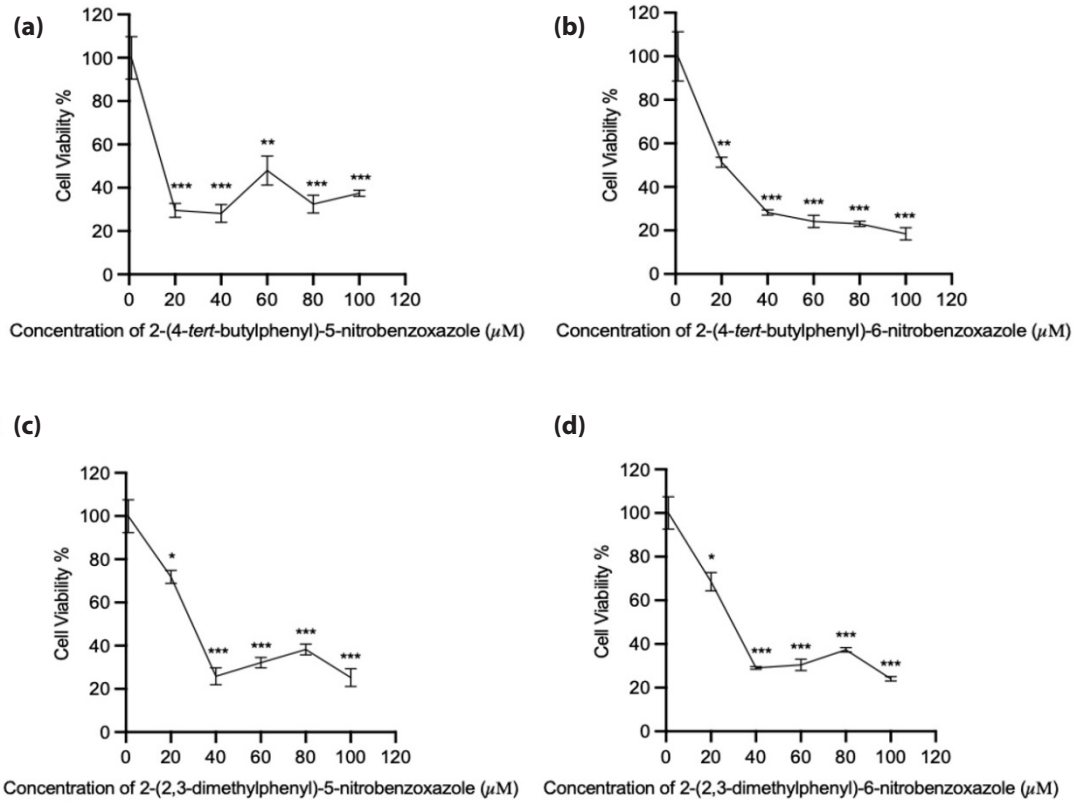

Figure 1. Effects of benzoxazole derivatives at different concentrations on the cell viability of A549 cells after 48 hours. The graphs demonstrate the percentage of cell viability after treatment with **(a)** 2-(4-tert-butylphenyl)-5-nitrobenzoxazole (1a), **(b)** 2-(4-tert-butylphenyl)-6-nitrobenzoxazole (1b), **(c)** 2-(2,3-dimethylphenyl)-5-nitrobenzoxazole (2a), and **(d)** 2-(2,3-dimethylphenyl)-6-nitrobenzoxazole (2b). Data are presented as mean±standard error of the mean (SEM) from three independent experiments (n=3). Statistical analyses were performed using one-way analysis of variance (ANOVA) for multiple-group comparisons and unpaired t-tests for comparisons between two groups. Significance levels are indicated as follows: ***p<0.001, **p<0.01, *p<0.05 versus the untreated group.

Table 1. IC_{50} values of benzoxazole derivatives and reference compound against A549 cells

Compound	IC_{50} (μM)±SEM
1a	17.41±0.16
1b	20.50±0.08
2a	32.17±0.08
2b	31.13±0.07
Cisplatin	19.65±0.09

IC_{50}: Concentration required to cause 50% inhibition of cell growth; μM: Micromolar; SEM: Standard error of the mean; 1a: 2-(4-tert-butylphenyl)-5-nitrobenzoxazole; 1b: 2-(4-tert-butylphenyl)-6-nitrobenzoxazole; 2a: 2-(2,3-dimethylphenyl)-5-nitrobenzoxazole; 2b: 2-(2,3-dimethylphenyl)-6-nitrobenzoxazole. Data represent the mean±standard error of the mean (SEM) of three independent experiments (n=3).

classified as NSCLC. Although treatment modalities such as radiotherapy, targeted therapy, immunotherapy, and chemotherapy are available, they are often associated with severe side effects and the development of drug resistance, which significantly affect patients' quality of life. As a result, the overall cure and survival rates for NSCLC remain low.[26]

Benzoxazole is a combination of a benzene ring and an oxazole ring. This heterocyclic compound is widely utilized as a core scaffold structure in drug research and development, significantly contributing to the discovery of new therapeutic agents.[27] Several benzoxazoles are already used in the treatment of various diseases, and some are currently in clinical trials. Additionally, an increasing number of benzoxazole derivatives are being explored in the early stages of drug discovery as potential hit or lead compounds.[7] Benzoxazole is an important heterocyclic compound due to its diverse pharmacological properties, including anticancer activity. Both synthetic and naturally occurring benzoxazole derivatives have demonstrated strong anticancer effects against various human cancer cell lines.[28] The anticancer activities of benzothiazoles and their bioisosteres (benzimidazoles and benzoxazoles), have been extensively documented.[29-31] In particular, a series of benzothiazole and benzoxazole derivatives were evaluated for antitumor activity against human breast cancer cells (MCF-7 and MDA-MB-231), with N-methylpiperazinyl derivatives demonstrating exhibiting notably strong inhibitory effects. Docking studies have revealed potential interactions with epidermal growth factor receptors (EGFR), indicating therapeutic potential in cancer treatment.[12] Moreno-Rodríguez et al.[32] reported that 2-[(5-chlorobenzoxazol-2-yl)thio]-N-(3-fluorophenyl)-2-phenylacetamide, a benzoxazole-based amide/

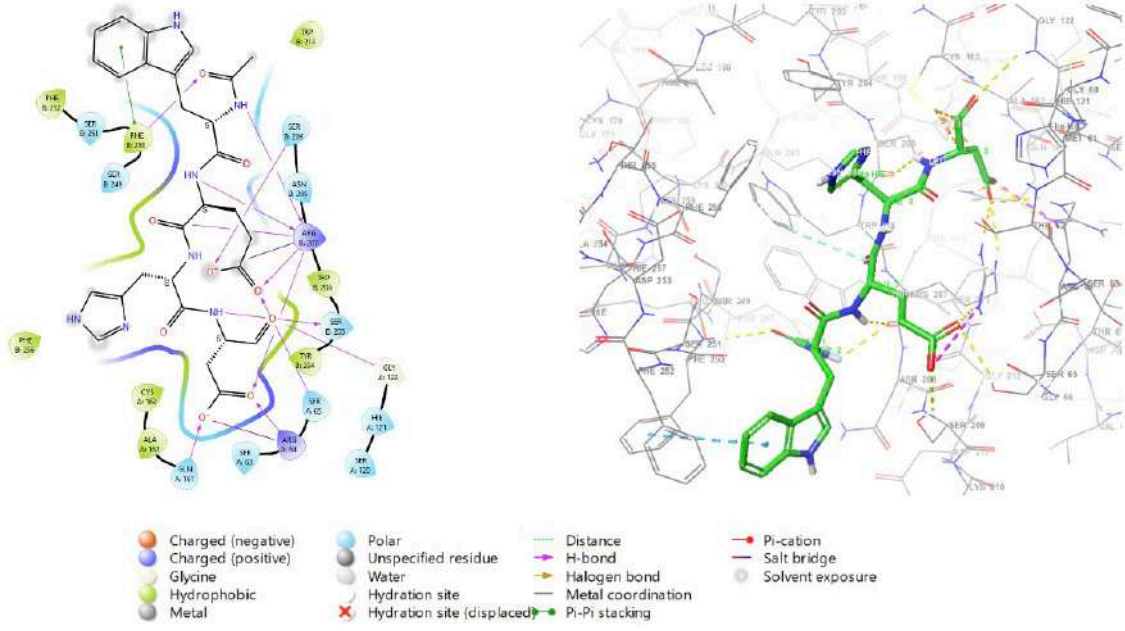

Figure 2. 2D and 3D interaction diagrams of the original ligand with the caspase-3 enzyme (PDB ID: 3GJQ). The 2D diagram shows the ligand's interactions with active-site amino acids: hydrogen bonds (purple arrows), π–π stacking (green arrows), and salt bridge interactions (two-colored lines).

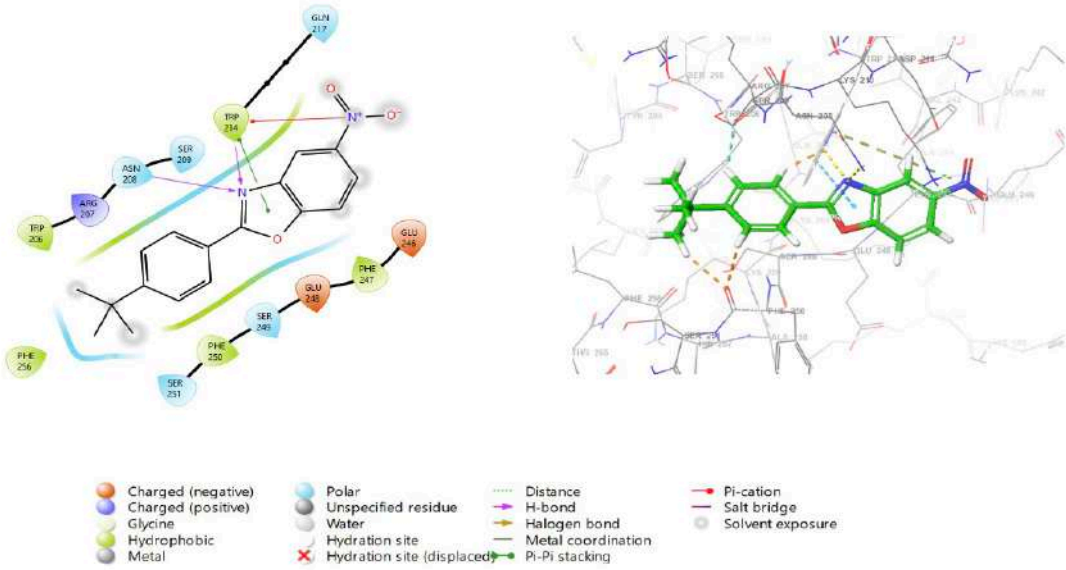

Figure 3. 2D and 3D interaction diagrams of compound 1a with the caspase-3 enzyme (PDB ID: 3GJQ). The 2D diagram shows the interactions of compound 1a with active-site amino acids: hydrogen bonds (purple arrows), π–π stacking (green arrows), and π-cation interactions (red line).

sulfonamide derivative, was the most cytotoxic compound in their series, demonstrating antiproliferative activity and caspase activation in colorectal cancer cells HT-29 and HCT116. Three benzoxazole derivatives were shown to exhibit strong cell growth inhibition in the HCT-116 cell line, accompanied by increased caspase-3 levels.[33] Tricyclic decylbenzoxazole has also been reported to inhibit proliferation and induce apoptosis in liver cancer cells (SMMC-7721).[34] In another study, several benzoxazole derivatives were synthesized as potential anticancer agents targeting sirtuin 1 (SIRT1) in NSCLC. Two of these compounds emerged as promising orally bioavailable SIRT1 modulators for targeted NSCLC therapy.[35] In a previous

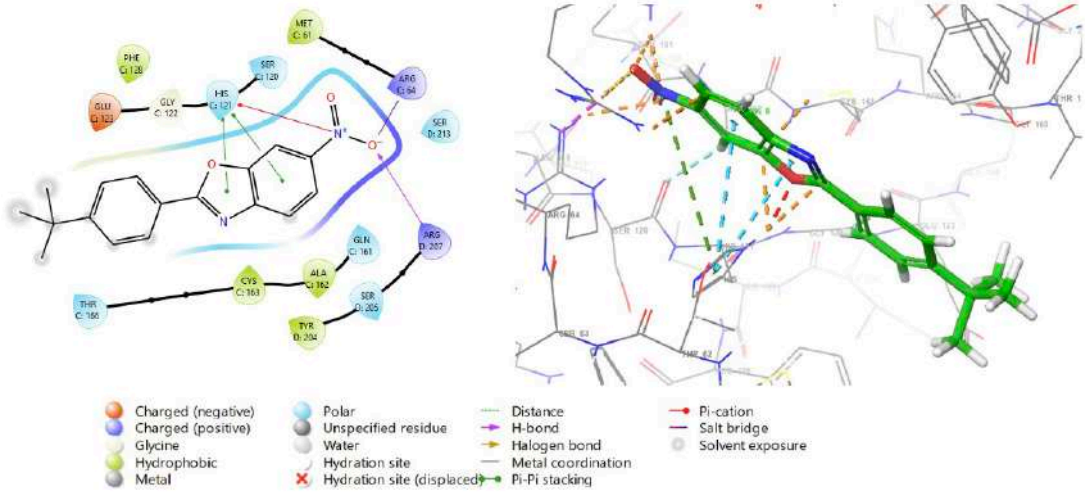

Figure 4. 2D and 3D interaction diagrams of compound 1b with the caspase-3 enzyme (PDB ID: 3GJQ). The 2D diagram shows the interactions of compound 1b with active-site amino acids: hydrogen bonds (purple arrows), π–π stacking (green arrows), π-cation interactions (red line), and salt bridge interactions (two-colored lines).

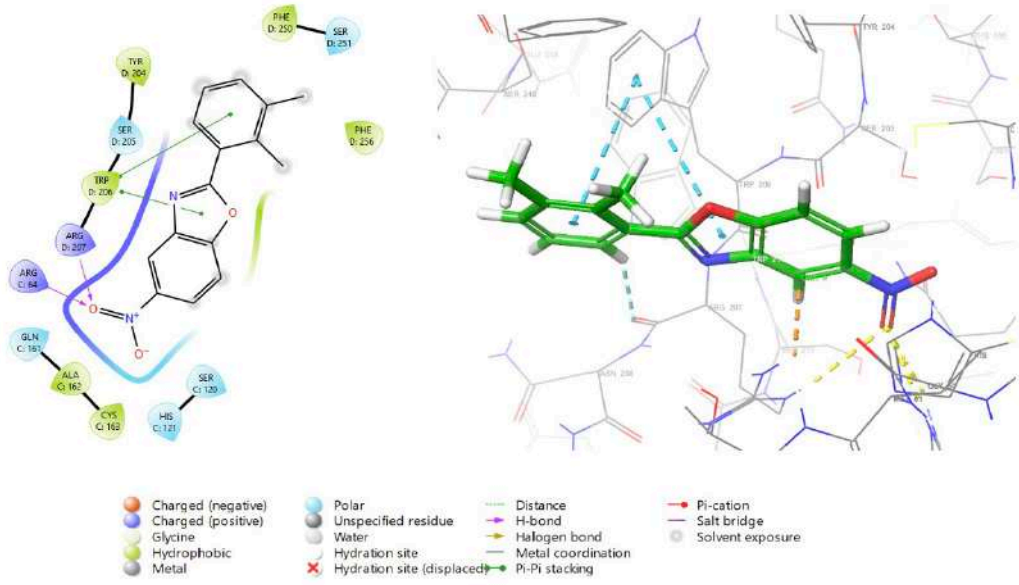

Figure 5. 2D and 3D interaction diagrams of compound 2a with the caspase-3 enzyme (PDB ID: 3GJQ). The 2D diagram shows the interactions of compound 2a with active-site amino acids: hydrogen bonds (purple arrows) and π–π stacking (green arrows).

investigation, a 2-aminobenzothiazole derivative demonstrated the strongest activity against NSCLC HOP-92 cells. Molecular docking studies supported that the synthesized compounds bind in a manner similar to EGFR inhibitors, highlighting key interactions that may guide the design of more potent inhibitors.[30] Furthermore, novel benzoxazole-hydrazone and benzoxazole-1,3,4-oxadiazole derivatives have been reported to induce apoptosis in human A549 lung cancer cells, consistent with their cytotoxic activity against these cells.[36]

In the present study, previously synthesized benzoxazole derivatives (1a, 1b, 2a, and 2b) were evaluated for their

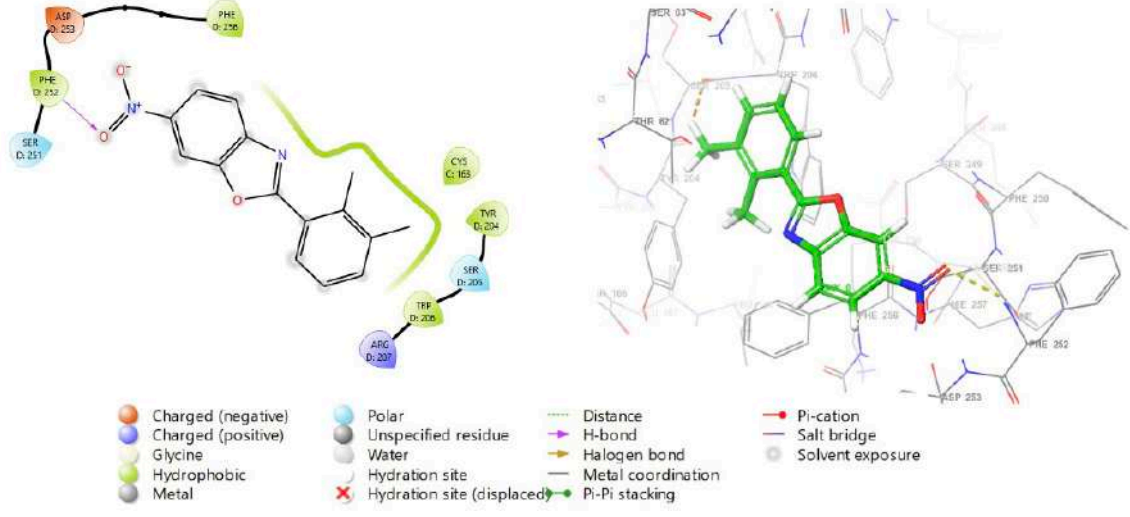

Figure 6. 2D and 3D interaction diagrams of compound 2b with the caspase-3 enzyme (PDB ID: 3GJQ). The 2D diagram shows the interaction of compound 2b with an active-site amino acid: hydrogen bond (purple arrow).

Table 2. Docking interactions of compounds 1a, 1b, 2a, 2b, and the original ligand with the caspase-3 enzyme (PDB ID: 3GJQ) predicted by Glide docking simulations

Compound	Docking score (kcal/mol)	Glide score (kcal/mol)	Glide energy (kcal/mol)	Glide evdw (kcal/mol)	Glide emodel (kcal/mol)
1a	-5.202	-5.202	-30.874	-27.424	-39.451
1b	-4.373	-4.373	-34.514	-32.767	-43.593
2a	-4.339	-4.339	-31.524	-28.572	-40.109
2b	-4.640	-4.640	-27.456	-24.857	-35.161
Original ligand	-12.490	-13.053	-77.411	-47.695	-175.847

Docking score, Glide score, Glide energy, Glide evdw, and Glide emodel are expressed in kcal/mol. More negative docking or Glide scores indicate stronger predicted binding affinity.

anticancer activities against A549 cells using the MTT cell viability assay, with cisplatin serving as the reference drug. Compounds 1a, 1b, 2a, and 2b exhibited potent anticancer activity and produced significant cytotoxic effects on A549 lung cancer cells by suppressing cell proliferation in a dose-dependent manner. The IC_{50} values of the benzoxazole derivatives ranged from 17.41 µM to 32.17 µM. Compounds 1a and 1b demonstrated greater antiproliferative activity than compounds 2a and 2b, with IC_{50} values comparable to that of cisplatin. These findings suggest that compounds 1a and 1b possess potential anticancer activity similar to that of cisplatin.

To investigate the relationship between these benzoxazole derivatives and caspase-3 enzyme activity, molecular docking studies were performed. To our knowledge, this is the first study to evaluate the interactions of these benzoxazole derivatives with caspase-3 using molecular docking analysis. According to the docking results, the tested benzoxazoles (1a, 1b, 2a, and 2b) may interact favorably within the binding site of caspase-3 (PDB ID: 3GJQ). Our compounds were observed to interact with amino acid residues similar to those of the original ligand of caspase-3, including ARG64, ARG207, TRP214, TRP206, GLY122, ASN208, TRP206, GLN161, ALA162, CYS163, PHE256, and SER249. The nitro group appeared to play an important role in mediating interactions with caspase-3. The NO_2 group at the 5-position of the benzoxazole ring in compound 1a exhibited a π-cation interaction with TRP214. In contrast, the same group at the 6-position of heterocyclic structure in compound 1b formed a π-cation interaction with HIS121 and a hydrogen bond with ARG207. While the nitrogen atom of the benzoxazole ring in compound 1a formed H-bonds with ASN208 and TRP214, no such interaction was observed for compound 1b. In compound 1a, a π–π interaction was observed with TRP214 only at the oxazole section of the benzoxazole structure, whereas in compound 1b, π–π interactions were observed at both the oxazole and benzene sections of the bicyclic structure with the HIS121 residue. Furthermore, compound 1a demonstrated a relatively lower docking score and IC_{50} value compared with the other derivatives, suggesting a preliminary indication of cytotoxic potential that warrants further investigation. Evaluation of the structure-activity relationship suggests that substitution of the phenyl group at the 2-position of the benzoxazole ring with a para-tert-butyl group contributes to increased antiproliferative activity against A549 cells.

It was observed that the nitro group played an important role in the interaction with the caspase-3 enzyme in both compounds 2a and 2b, regardless of whether it was located at the 5- or 6-position. In compound 2a, the nitro group formed H-bonds with ARG207 and ARG64, whereas in compound 2b, it formed an H-bond only with PHE252. No π–π interactions with the enzyme were observed in compound 2b, either within the heterocyclic structure or the phenyl ring. In contrast, compound 2a exhibited interactions with the TRP206 residue involving both the oxazole part of the benzoxazole structure and the phenyl ring at the 2-position. It is also suggested that substitution at the para position of the phenyl ring attached to the 2-position of the benzoxazole ring is important for hydrophobic interactions. This is supported by the observation that the tert-butyl group of the phenyl at the para position of compound 1a forms a hydrophobic interaction with the PHE256 residue.

Considering both the *in vitro* activity and molecular docking results, these compounds demonstrate preliminary cytotoxic potential, which may be associated with caspase-3 activation. Although the compounds exhibited limited activity against the hTopo IIα enzyme,[5] they may exert their effects on A549 cells through caspase-3. A limitation of the present study is that the cytotoxic effects of the benzoxazole derivatives were evaluated only in the A549 lung cancer cell line. Comparative analysis using normal lung epithelial cells would provide additional insight into the selectivity and potential safety profile of these compounds. Future studies are planned to include mechanistic assays, such as Western blotting and

Annexin V/PI staining, as well as *in vivo* efficacy and toxicity studies in NSCLC models. These investigations will provide a clearer understanding of the cytotoxic potential and underlying mechanisms of benzoxazole derivatives and guide the design of new analogs based on these compounds.

CONCLUSION

Our findings indicate that the previously synthesized benzoxazole derivatives, 1a and 1b, exhibit preliminary cytotoxic potential by inhibiting lung cancer cell proliferation at low concentrations, similar to cisplatin. Based on the structure-activity relationship within this series, substitution of the phenyl at the 2-position of the benzoxazole ring with a para-tert-butyl group may enhance anticancer activity against A549 cells. Moreover, molecular docking studies demonstrate that all benzoxazole derivatives may interact with the active site of caspase-3, suggesting a possible involvement in caspase-3–mediated mechanisms. Therefore, the anticancer effects of these compounds in A549 cells may be associated with caspase-3 activation. This preliminary study suggests that these benzoxazole derivatives have potential as cytotoxic agents for the treatment of NSCLC. Future research will focus on optimizing the potency of these compounds, elucidating their mechanisms of action through mechanistic assays, and evaluating their *in vivo* efficacy and toxicity in murine NSCLC models.

Ethics Committee Approval: As this study consisted solely of *in vitro* experiments and molecular docking analyses, ethical approval was not required.

Informed Consent: This study reports the results of experimental investigations that did not involve human or animal subjects; therefore, informed consent is not required.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Evaluation of the Effect of Different Mesenchymal Stem Cell Microvesicles on Diabetic Wound Healing

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ABSTRACT

Objective: Mesenchymal stem cell (MSC)–derived microvesicles play a pivotal role in the regenerative cascade of wound healing. This study aimed to comparatively evaluate the therapeutic potential of microvesicles isolated from different MSC sources in the healing of diabetic cutaneous wounds.

Materials and Methods: Forty Wistar albino rats were randomly assigned to four experimental groups (n=10 per group). Following the induction of diabetes and the creation of full-thickness circular dorsal skin defects, each group received a distinct treatment: Group 1 received saline (control), Group 2 received adipose-derived stem cell microvesicles (ADSC-MVs), Group 3 received umbilical cord-derived stem cell microvesicles (UCDSC-MVs), and Group 4 received bone marrow-derived stem cell microvesicles (BMDSC-MVs). Outcomes related to re-epithelialization, neovascularization, and collagen matrix formation were evaluated both macroscopically and histologically.

Results: The microvesicle-treated groups demonstrated faster wound closure and improved collagen fiber alignment compared with the control group. Angiogenic activity was increased in all treatment groups, with the most pronounced effects observed in the UCDSC-MVs group. Notably, the UCDSC-MVs group exhibited a significantly greater epithelial tongue length than the control group on postoperative days 3 and 14 ($p=0.008$ and $p<0.001$, respectively).

Conclusion: These findings indicate that UCDSC–MVs possess a superior capacity to promote re-epithelialization and may represent an effective cell-free therapeutic approach for diabetic wound repair.

Keywords: Diabetes wound, mesenchymal stem cells, microvesicles, regeneration, wound healing.

INTRODUCTION

A wound represents a discontinuity or disruption in the integrity of living tissue. Effective repair of such injuries is essential to maintain tissue function and prevent systemic infection. The wound-healing process progresses through several overlapping yet coordinated phases: hemostasis, inflammation, proliferation, and remodeling.[1] These phases may be impaired by various systemic conditions, notably diabetes mellitus (DM), hypertension, obesity, and certain rheumatologic or inflammatory disorders.[2–4]

Diabetes mellitus is a chronic metabolic disorder that significantly delays the wound-healing cascade due to persistent hyperglycemia, microvascular dysfunction, peripheral neuropathy, and chronic inflammation.[5,6] It is estimated that 15–25% of individuals with diabetes will develop diabetic foot ulcers (DFUs) during their lifetime.[7] Despite advances in medical care, the clinical management of DFUs is largely dependent on conservative approaches, and a fully effective treatment strategy has yet to be established. Therefore, the development of new therapeutic strategies aimed at accelerating diabetic wound repair remains an urgent clinical priority.

Mesenchymal stem cells (MSCs) have emerged as a potential regenerative therapy for impaired wound healing, primarily through their paracrine secretion of bioactive molecules, including cytokines and growth factors.[8–11] These multipotent, fibroblast-like, non-hematopoietic cells can be harvested from multiple sources, including bone marrow, adipose tissue, and umbilical cord. Increasing evidence suggests that the therapeutic activity of MSCs is largely mediated by small extracellular vesicles known as microvesicles (MSC-MVs), rather than by direct cell replacement.

Microvesicles are membrane-bound nanoparticles measuring 100–1000 nm and carry diverse cargo, including proteins, lipids, DNA, and regulatory RNAs.[12] Numerous investigations have confirmed that MSC-derived microvesicles from different tissue origins contribute to wound repair through distinct molecular constituents and signaling effects.[13] Nevertheless, comparative in vivo evaluations of microvesicles derived from various MSC sources in the context of wound healing remain limited. The present study was therefore designed to assess and compare the wound-healing efficacy of microvesicles isolated from adipose-derived stem cells (ADSC), umbilical-cord-derived stem cells (UCDSC), and bone-marrow-derived stem cells (BMDSC) in a diabetic rat model. Determining which MSC-microvesicle source yields the strongest regenerative response may help refine future cell-free therapeutic strategies for DFU management.

KEY MESSAGES

- Mesenchymal stem cells provide new therapeutic tools in the management of diabetic wounds; however, challenges related to storage, potential immune rejection, and ethical concerns continue to limit their clinical use.
- Mesenchymal stem cell-derived microvesicles offer similar biologic advantages in wound healing without the drawbacks associated with stem cell-based therapies.
- Umbilical cord-derived stem cell microvesicles demonstrate a superior capacity for re-epithelization, which may play an important role in cell-free diabetic wound management.

MATERIALS AND METHODS

Microvesicle Isolation and Characterization

Mesenchymal stem cells were supplied by the Good Manufacturing Practice (GMP) laboratory of the Erciyes University Genome and Stem Cell Center. Human ADSCs and BMDSCs were cultured in Dulbecco's Modified Eagle Medium (low glucose; Biological Industries, USA), whereas UCDSCs were maintained in Alpha Minimum Essential Medium (α-MEM) supplemented with 10% serum, 1% antibiotics, and 1% stable L-glutamine (Biological Industries, USA), as previously described.[14,15]

The immunophenotypic profiles of MSCs were confirmed by flow cytometry (Beckman Coulter, USA) using antibodies against CD44, CD90, CD105, CD11b, CD19, and CD34 (BD Stem Flow hMSC Kit, USA).

When thawed MSCs reached approximately 90% confluence, the culture medium was replaced with serum-free medium, and the cells were incubated for an additional 24 hours to obtain conditioned medium (secretome). The collected secretomes were subjected to microvesicle isolation using a commercial precipitation-based isolation kit (ExoQuick-TC, System Biosciences, USA) in accordance with the manufacturer's instructions.[16] Briefly, the secretomes were centrifuged at 3000 × g for 15 minutes to remove cellular debris, and the resulting supernatants were mixed with the precipitation reagent at a 1:5 ratio. Following overnight incubation at 4°C, the mixtures were centrifuged at 1500 × g for 5 minutes to pellet the microvesicles.

The morphological features and size characteristics of the isolated microvesicles were evaluated using a scanning electron microscope (SEM) (Zeiss GEMINI 500, Germany). Size distribution and particle concentration were analyzed by nanoparticle

tracking analysis (NTA) using a NanoSight NS300 system (Malvern, UK), according to the manufacturer's protocol. Total protein concentration was determined by the Bradford assay (Bio-Rad, USA). Based on previous reports,[17] microvesicles were prepared at a final concentration of 200 µg/mL for administration to experimental animals using a concentrator device (Eppendorf Concentrator Plus, Thermo Fisher Scientific, UK).

The term "microvesicle" is used throughout the manuscript to describe extracellular vesicles isolated by a precipitation-based method. Given the isolation technique employed, the obtained vesicles likely represent a heterogeneous population of extracellular vesicles rather than a single vesicle subtype defined by biogenesis.

Animals, Experimental Design, and Surgical Procedure

All animal experiments were conducted following approval from the Erciyes University Animal Experiments Ethics Committee (approval number: 22/265, date: 07.12.2022), and in accordance with international guidelines for the care and use of laboratory animals.

A priori power analysis was performed to determine the appropriate sample size, based on previously reported differences in wound closure outcomes in diabetic wound models. Accordingly, 40 male Wistar albino rats (weighing 213–330 g) were included in the study. DM was induced by intraperitoneal injection of streptozotocin (55 mg/kg; Sigma-Aldrich, USA). Blood glucose levels were measured from tail vein samples 48 hours after injection, and animals with glucose levels ≥250 mg/dL were considered diabetic.

The rats were randomly allocated into four experimental groups (n=10 per group):

- Group 1: Saline (control)
- Group 2: Bone-marrow-derived stem cell microvesicles (BMDSC-MVs)
- Group 3: Umbilical-cord-derived stem cell microvesicles (UCDSC-MVs)
- Group 4: Adipose-derived stem cell microvesicles (ADSC-MVs).

General anesthesia was achieved via intraperitoneal administration of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg). The dorsal skin was shaved and disinfected with povidone–iodine solution. Two circular, full-thickness excisional wounds (2 cm in diameter and spaced 2 cm apart) were created on the dorsal surface of each rat under sterile conditions. The experimental protocol was terminated on postoperative day 14, after which all animals were euthanized for tissue harvesting (Fig. 1).

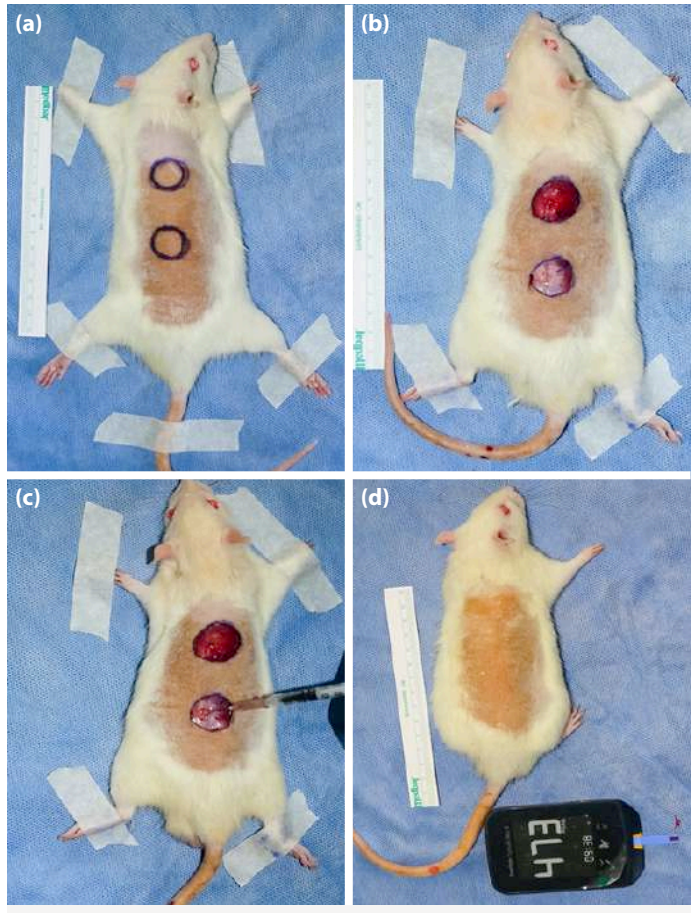

Figure 1. (a) Preparation of the surgical area and marking of full-thickness wounds. **(b)** Creation of wounds. **(c)** Injection of microvesicles into the wound sites. **(d)** Measurement of glucose levels from tail vein samples.

Analysis of Wound Closure Rate

Digital images of the wounds were captured at a standardized distance of 50 cm using a Canon EOS 250D camera on postoperative days 0, 3, 5, 7, 10, and 14. Wound area measurements were performed using ImageJ software (NIH, USA) by an investigator blinded to the experimental groups.

To enable early histopathological evaluation, the cranial wound created in each animal was designated for biopsy sampling on postoperative day 3. As the biopsy procedure constitutes an additional surgical intervention that may influence the wound-healing process, the cranial wound was excluded from wound closure analysis. Accordingly, wound closure rates were assessed exclusively using the caudal wound, which was not subjected to any additional surgical manipulation. ImageJ-based wound area measurements were therefore performed solely on the caudal wound.

The wound closure rate was calculated using the following formula:

$$\text{Healing rate (\%)} = \frac{S_0 - S_A}{S_0} \times 100$$

where S_0 represents the initial wound area on day 0, and S_A represents the wound area at the indicated postoperative time point.

Histopathological Assessment

To avoid sampling-related interference between early and late healing phases, histopathological specimens were obtained from different wound sites at predefined time points. In each animal, the cranial wound was designated for early-stage histopathological evaluation, and a 3-mm full-thickness tissue specimen was harvested on postoperative day 3. For late-stage evaluation, a 3-mm full-thickness tissue specimen was obtained from the caudal wound on postoperative day 14.

Harvested tissues were fixed in 10% formalin for 24 hours, dehydrated through a graded ethanol series, cleared with xylene, and embedded in paraffin following incubation in a molten paraffin bath for two hours. Paraffin-embedded tissues were sectioned at a thickness of 5 µm and stained with hematoxylin–eosin (H&E) and Masson's trichrome.

Light microscopic evaluation was performed using an Olympus BX-51 light microscope. Histological images were captured with a digital microscope camera (Zeiss, Germany) and analyzed using ImageJ software (NIH, USA) by an investigator blinded to the experimental groups.

Microscopic evaluation was performed using a light microscope. Epithelial tongue length (µm) was measured as an objective indicator of re-epithelialization and was quantified using ImageJ software (NIH, USA) on images captured with a digital microscope camera (Zeiss, Germany). In addition, wound healing was evaluated semi-quantitatively according to the scoring system described by Galeano et al.[18] Within this system, epidermal regeneration and angiogenesis (granulation tissue formation) were scored on a 4-point scale, with higher scores indicating more advanced wound healing. Collagen deposition was assessed semi-quantitatively on Masson's trichrome–stained sections using a 4-point scoring system.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean ± standard deviation (SD) for normally distributed variables and as median (interquartile range) for non-normally distributed or ordinal variables. Normality of data distribution was assessed using the Shapiro–Wilk test. Wound closure analysis was performed using a single wound per animal to avoid clustering bias. Repeated wound area measurements obtained on postoperative days 0, 3, 5, 7, 10, and 14 were analyzed using repeated-measures analysis of variance (ANOVA) to account for within-animal correlations and to evaluate the effects of group, time, and group × time interaction. The assumption of sphericity was assessed using Mauchly's test, and when violated, the Greenhouse–Geisser correction was applied. Accordingly, corrected degrees of freedom are reported.

Epithelial tongue length, which did not show a normal distribution, and histopathological scoring parameters (epidermal regeneration, angiogenesis, and fibrosis), which were ordinal variables, were compared among groups using the Kruskal–Wallis test, followed by Dunn's post hoc test for multiple comparisons. A p value <0.05 was considered statistically significant.

RESULTS

All animals successfully met the predefined criteria for diabetes induction prior to wound creation and were included in the subsequent analyses.

Two rats in the ADSC-MVs group and one rat in the BMDSC-MVs group died due to anesthesia-related complications. Accordingly, data analysis included eight animals in the ADSC-MVs group, nine in the BMDSC-MVs group, and 10 animals in each of the remaining two groups.

Characterization of MSCs and MSC-Derived Microvesicles

All MSC cultures retained the typical spindle-shaped, fibroblast-like morphology. Flow cytometric immunophenotyping confirmed their mesenchymal phenotype. ADSCs expressed CD90 (99.87%), CD44 (98.1%), and CD105 (99.55%), while hematopoietic markers CD11b, CD19, and CD34 were nearly absent (0.84%). Similarly, BMDSCs expressed CD90 (99.84%), CD44 (99.55%), and CD105 (99.05%), with minimal expression of CD11b, CD19, and CD34 (0.25%). UCDSCs showed high expression of CD90 (99.8%), CD44 (95.72%), and CD105 (99.37%), with low positivity for CD11b, CD19, and CD34 (1.41%).

Scanning electron microscopy and nanoparticle tracking analysis confirmed vesicular morphology and particle size consistent with microvesicles (Fig. 2).

Wound-Healing Rate

Wound area measurements derived from serial macroscopic images were compared across postoperative time points using repeated-measures ANOVA. The analysis demonstrated a significant main effect of time (F(2.90,

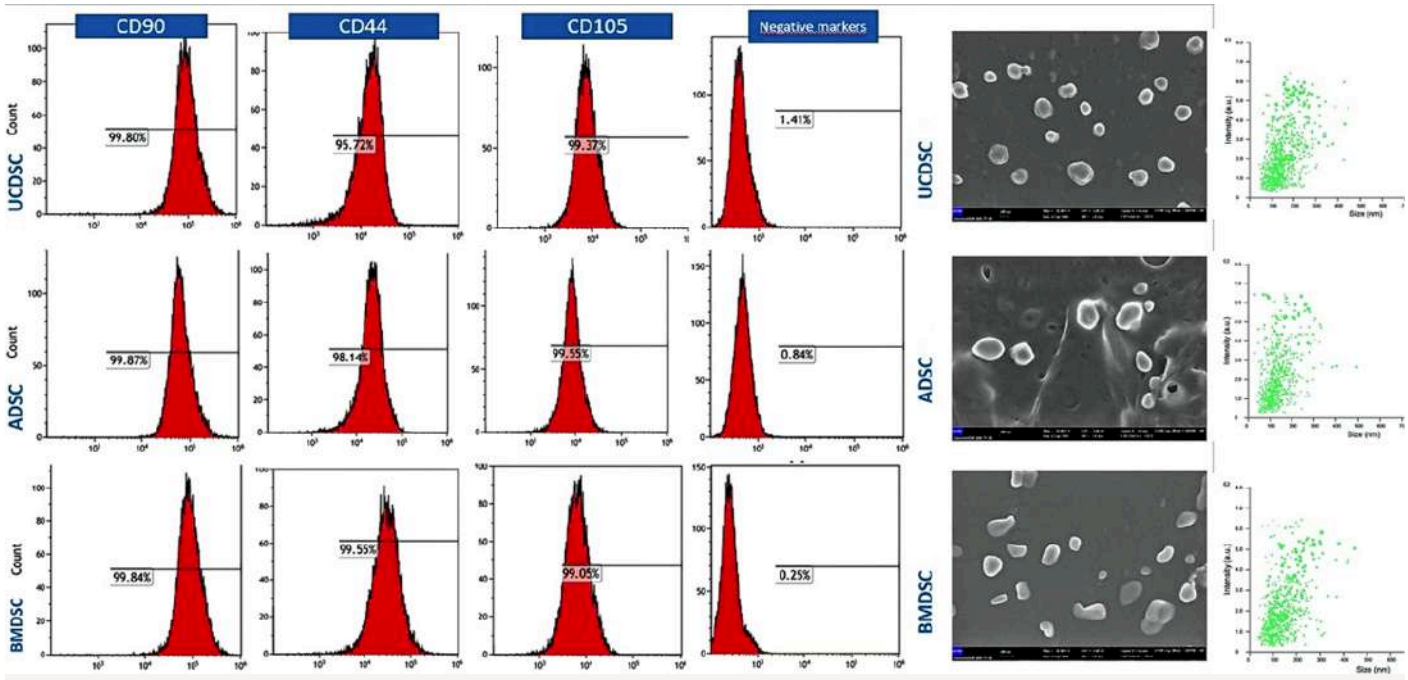

Figure 2. Flow cytometric analysis demonstrated that mesenchymal stem cells (MSCs) were positive for CD90, CD44, and CD105 and negative for CD11b, CD19, and CD34. Morphology of microvesicles was examined using scanning electron microscopy (SEM), and size distribution was analyzed by nanoparticle tracking analysis (NTA); scale bar=200 nm.

95.64)=278.98, p<0.001), a significant main effect of group (F(3, 33)=14.44, p<0.001), and a significant time × group interaction (F(8.70, 95.64)=6.72, p<0.001). Overall, microvesicle-treated wounds exhibited significantly improved wound closure compared with saline-treated controls throughout the observation period. Bonferroni-adjusted post hoc analyses demonstrated that the control group exhibited significantly poorer wound-healing outcomes compared with the ADSC-MVs, UCDSC-MVs, and BMDSC-MVs groups (all p<0.001) (Table 1), whereas no statistically significant differences were observed among the microvesicle-treated groups (p>0.05). By postoperative day 14, wound closure outcomes were comparable among the three microvesicle-treated groups (Fig. 3, 4).

Table 1. Results of repeated-measures analysis of variance (ANOVA) for wound closure rate

Effect	df	F	p
Time	2.90, 95.64	278.98	<0.001
Group	3, 33	14.44	<0.001
Time × group	8.70, 95.64	6.72	<0.001

Greenhouse–Geisser correction was applied when the assumption of sphericity was violated.

Histological Findings

Epithelialization

Epithelial tongue length was evaluated on H&E-stained sections. On day 3, epithelial tongue length differed significantly among groups (p<0.001). Post hoc analysis demonstrated that the UCDSC-MVs group exhibited significantly greater epithelial tongue length compared with the control group (p=0.008) and the BMDSC-MVs group (p=0.007). No statistically significant differences were observed among the other microvesicle-treated groups.

On day 14, epithelial tongue length also differed significantly among groups (p<0.001). The UCDSC-MVs group showed significantly greater epithelial tongue length compared with the control group (p<0.001), whereas no significant differences were detected among the microvesicle-treated groups (Table 2).

Angiogenesis

Angiogenic activity was assessed using Masson's trichrome staining. On day 3, angiogenesis scores differed significantly among groups (p<0.001). Post hoc analysis revealed significantly higher angiogenesis scores in the UCDSC-MVs group compared with the control group (p<0.001) and the BMDSC-MVs group (p=0.002). No statistically significant difference was observed between the control and ADSC-MVs groups.

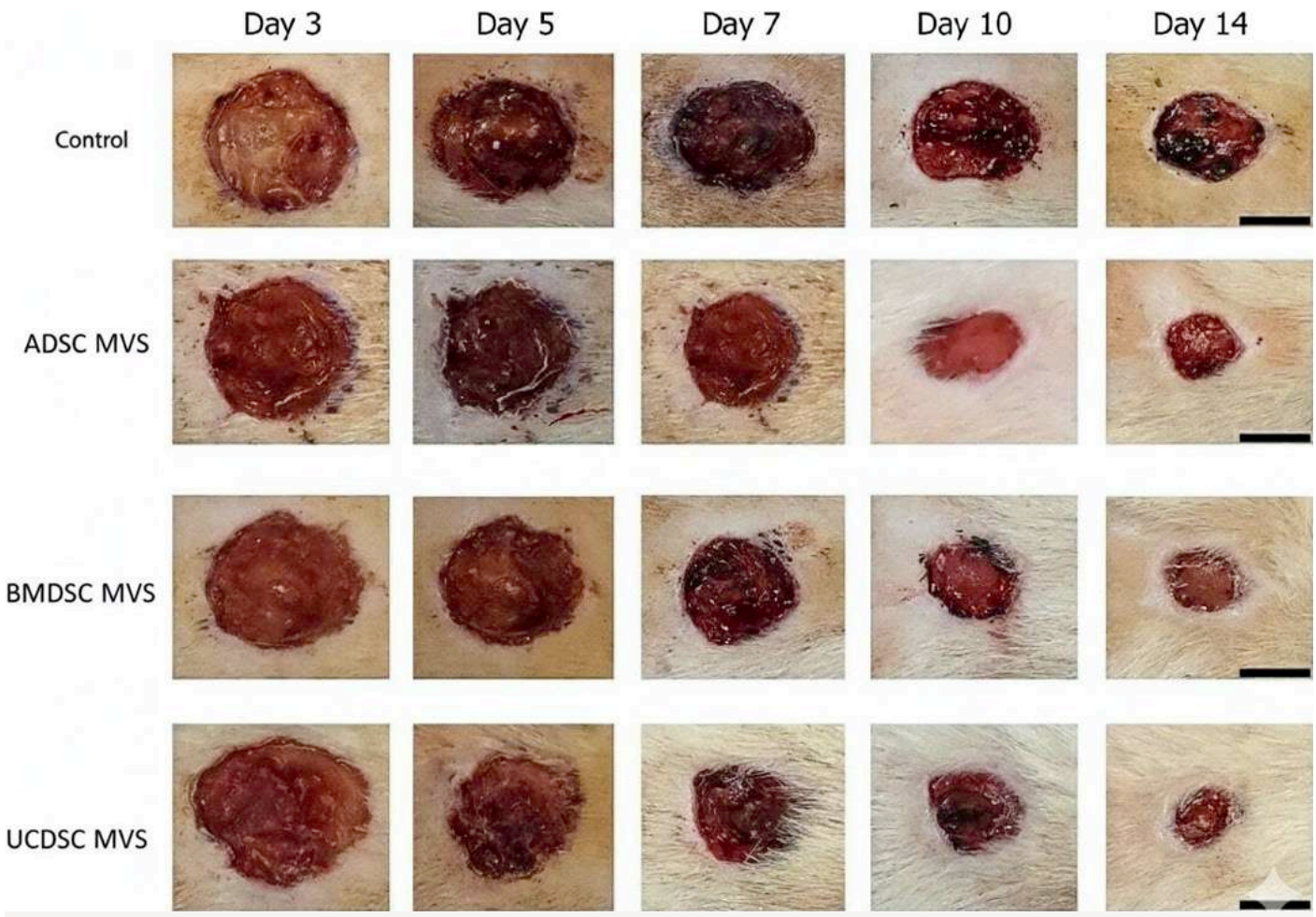

Figure 3. Images of the wound-healing process in all groups; scale bar=1 cm.

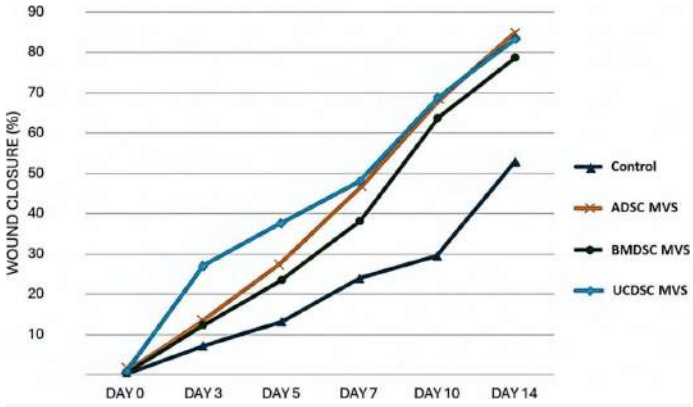

Figure 4. Wound closure rates among all groups.

On day 14, angiogenesis scores remained significantly different among groups (p=0.001). Angiogenesis scores in the UCDSC-MVs group were significantly higher than those in the control group (p=0.039), while no other intergroup differences reached statistical significance

Collagen Deposition (Fibrosis)

Fibrosis was evaluated using Masson's trichrome staining. On day 3, fibrosis scores differed significantly among groups (p<0.001). Post hoc analysis showed significantly lower fibrosis scores in the UCDSC-MVs and BMDSC-MVs groups compared with the control group (both p=0.001). No significant differences were observed among the microvesicle-treated groups (p>0.05).

On day 14, fibrosis scores also differed significantly among groups (p<0.001). Fibrosis scores were significantly lower in the UCDSC-MVs and BMDSC-MVs groups compared with the control group (p≤0.007), whereas no statistically significant differences were detected among the microvesicle-treated groups (Fig. 5).

Table 2. Histological outcomes on postoperative day 3 and day 14

Parameter	Group	Day 3		Day 14	
		n	Median (25^{th}–75^{th})	n	Median (25^{th}–75^{th})
Epithelial tongue length (μm)	Control	10	**462.378 (400.104–560.538)**	10	**782.594 (694.162–871.263)**
	BMDSC-MVs	10	**512.738 (476.316–535.692)**	9	**906.574 (825.473–996.450)**
	UCDSC-MVs	10	**605.465 (524.705–765.569)***	10	**1085.951 (1015.302–1346.942)***
	ADSC-MVs	10	**498.831 (445.909–552.816)**	8	**828.584 (746.562–932.561)**
Angiogenesis score	Control	10	**1.0 (1.0–2.0)**	10	**2.0 (2.0–3.0)**
	BMDSC-MVs	10	**2.0 (2.0–2.0)**	9	**3.0 (3.0–3.0)**
	UCDSC-MVs	10	**3.0 (3.0–3.0)***	10	**3.5 (3.0–4.0)***
	ADSC-MVs	10	**2.0 (2.0–2.0)**	8	**2.5 (2.0–3.25)**
Fibrosis score	Control	10	**0.0 (0.0–1.0)**	10	**2.0 (2.0–2.0)**
	BMDSC-MVs	10	**0.0 (0.0–1.0)***	9	**2.0 (2.0–2.0)***
	UCDSC-MVs	10	**1.0 (1.0–1.0)***	10	**2.0 (2.0–3.0)**
	ADSC-MVs	10	**0.0 (0.0–1.0)**	8	**2.0 (2.0–3.0)**
Epithelial regeneration score	Control	10	**1.0 (1.0–1.0)**	10	**1.0 (1.0–2.0)**
	BMDSC-MVs	10	**1.0 (1.0–2.0)**	9	**2.0 (1.0–2.0)**
	UCDSC-MVs	10	**2.0 (1.0–2.0)**	10	**2.0 (2.0–2.75)***
	ADSC-MVs	10	**1.0 (1.0–1.0)**	8	**1.0 (1.0–2.0)**

Data are presented as median (25^{th}–75^{th} percentiles). Group comparisons were performed using the Kruskal–Wallis test followed by Dunn's post hoc test. An asterisk (*) indicates a statistically significant post hoc difference ($p<0.05$). Specific between-group comparisons are detailed in the Results section.

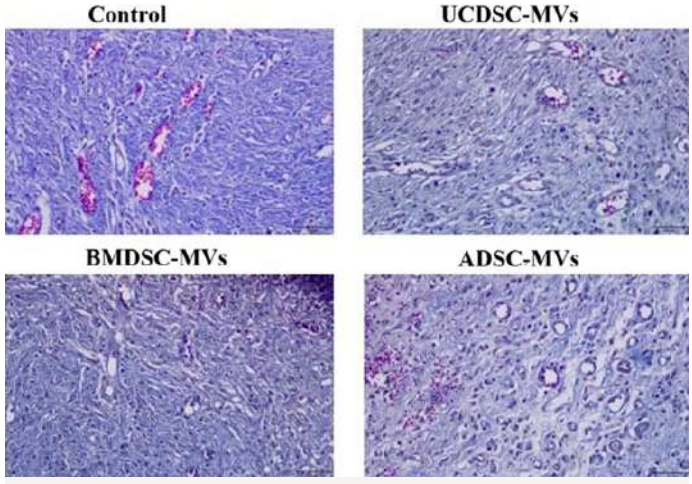

Figure 5. Masson's trichrome–stained sections obtained from the control, umbilical cord–derived stem cell microvesicles (UCDSC-MVs), bone marrow–derived stem cell microvesicles (BMDSC-MVs), and adipose-derived stem cell microvesicles (ADSC-MVs) groups on postoperative day 14 (40× magnification; scale bar=50 μm). Well-organized collagen bundles are observed in the microvesicle-treated groups, whereas the control group shows a more disorganized collagen structure.

DISCUSSION

Diabetes mellitus remains a major contributor to impaired wound healing, largely because of glucose-induced cellular toxicity. The primary mechanisms underlying this delay include microvascular dysfunction, peripheral neuropathy, and sustained inflammation.[19] Although surgical closure is often required for diabetic wounds, it cannot fully address the underlying vascular and neural injury that drives ulcer formation.

Emerging evidence suggests that transplantation of MSCs can enhance angiogenesis, modulate extracellular matrix (ECM) remodeling, and support keratinocyte function in DFUs.[20–22] MSCs contribute to tissue repair both by differentiating into endothelial or stromal cells and by secreting paracrine mediators that regulate immunity, suppress inflammation, and stimulate regeneration. Despite their therapeutic promise, MSC-based cell therapies face challenges such as storage instability, mutation-related tumorigenicity, immune rejection, and ethical limitations.[23] MSC-MVs offer a viable alternative, recapitulating many of the parent cells' regenerative effects while avoiding these drawbacks.

Mesenchymal stem cell–derived microvesicles act as natural nanocarriers for the paracrine signaling factors of stem cells, thus representing a novel, cell-free therapeutic strategy for

wound repair. Their bioactive cargo, including cytokines such as vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-β1), interleukin 6 (IL-6), and IL-10, as well as proteins, DNA, and noncoding RNAs, can be internalized by recipient cells, where it modulates the local microenvironment. Previous research has demonstrated that microvesicles obtained from various MSC sources can promote tissue regeneration, with ADSC-MVs, BMDSC-MVs, and UCDSC-MVs being the most extensively studied.

Adipose-derived stem cell microvesicles have attracted particular attention due to their ease of isolation and abundant availability. These vesicles can promote endothelial progenitor cell proliferation, stimulate angiogenesis, optimize fibroblast function, and reduce ulcer size.[24–26] They also mitigate oxidative stress by reducing reactive oxygen species (ROS) generation and improving mitochondrial activity under hyperglycemic conditions.[27] BMDSC-MVs, another promising cell-free approach, have demonstrated similar benefits. Yu et al.[28] showed that atorvastatin-pretreated BMDSC-MVs enhanced angiogenesis and wound closure in diabetic rats, while Hu et al.[29] reported that pioglitazone pretreatment improved collagen organization, ECM remodeling, and vascularization. In comparison, UCDSCs are advantageous because of their low immunogenicity, rapid expansion, and ethical acceptability. UCDSC-MVs have been shown to alleviate oxidative stress, promote fibroblast proliferation and migration, and augment the angiogenic activity of endothelial cells.[30–33] Despite these promising findings, no prior in vivo study has directly compared the wound-healing potential of microvesicles derived from different MSC sources within a diabetic model.

In the present study, all microvesicle-treated groups exhibited faster wound closure than controls. Although overall healing rates did not differ significantly among the microvesicle types, variations were observed at specific stages. Notably, epithelial tongue length was significantly greater in the UCDSC-MVs group on days 3 and 14. Hoang et al.[34] similarly reported that only UCDSC-MVs secreted high levels of TGF-β, a potent regulator of keratinocyte migration. Moreover, UCDSC-MVs are known to be enriched in miR-21-3p, which enhances re-epithelialization.[35]

Regarding angiogenesis, microvesicle treatment was associated with increased angiogenic activity compared with controls, with the most pronounced effects observed in the UCDSC-MVs group. Although microvessel density varied among treatment groups, significant differences were primarily detected between the UCDSC-MVs and control groups, particularly at earlier stages. Previous studies have shown that ADSC-, BMDSC-, and UCDSC-derived vesicles can promote angiogenesis through distinct signaling pathways.[36] For instance, UCDSC-MVs act through Wnt/β-catenin signaling,[31] BMDSC-MVs via the TGF-β/Smad axis,[37] and ADSC-MVs through modulation of the Delta-like 4 pathway.[24] These findings suggest that, while the molecular mediators may differ, microvesicle-based therapies can support angiogenic processes during wound healing.

Extracellular matrix remodeling, particularly collagen synthesis and degradation, is a pivotal determinant of scar quality. Although collagen scores did not differ significantly among the microvesicle-treated groups, treated wounds displayed more organized collagen bundles compared with controls. ADSC-MVs enhance fibroblast function by upregulating genes such as N-cadherin, cyclin-1, and proliferating cell nuclear antigen (PCNA),[37–39] whereas BMDSC-MVs exert antifibrotic effects by suppressing TGF-β/Smad signaling and increasing TGF-β3 expression.[37] UCDSC-MVs have also been shown to regulate fibroblast activity through inhibition of phosphatase and tensin homolog (PTEN), sprouty homolog 1 (SPRY1), and the TGF-β2/SMAD2 pathway.[35,40] Collectively, these findings support a beneficial role of microvesicle-based therapies in fibroblast-mediated tissue remodeling.

To the best of our knowledge, this work represents the first in vivo comparative evaluation of MSC-MVs from different sources for diabetic wound repair. While each microvesicle type offered unique advantages at particular stages of healing, several limitations merit consideration. First, although macroscopic and histological improvements were evident, the molecular mechanisms underlying these effects were not explored. Given that MSC-MVs carry a wide array of bioactive molecules that act synergistically on multiple cell types, single-pathway analyses may not fully explain their complex biological activity. Thus, more comprehensive molecular studies are required.

Second, despite providing a useful experimental model, rodent wounds differ from chronic human diabetic foot ulcers; therefore, our results may not directly translate to clinical outcomes. In addition, the ideal dose and frequency of MSC-MV administration remain undetermined. In this study, a single standardized local application was employed; however, varying doses or repeated treatments might yield different results. Further basic and translational research, followed by controlled clinical trials, is needed to establish optimal protocols and ensure the safe application of MSC-MVs in diabetic wound therapy.

Finally, although termed microvesicles, the vesicle population analyzed in this study may include other extracellular vesicle subtypes due to the precipitation-based isolation method; therefore, future studies incorporating molecular marker analysis are warranted for more precise subclassification.

CONCLUSION

In conclusion, this study represents the first in vivo comparison of MSC-MVs from different tissue origins in the treatment of diabetic wounds. Our findings demonstrate that microvesicles obtained from adipose tissue, bone marrow, and umbilical cord stem cells accelerated wound closure following subcutaneous administration to diabetic skin defects. Angiogenic activity was enhanced, with the most pronounced effects observed in the UCDSC-MVs group. Among the tested microvesicles, umbilical cord–derived microvesicles exhibited a notably stronger ability to promote re-epithelialization, highlighting their potential as a promising, cell-free therapeutic option for diabetic wound management.

Ethics Committee Approval: Ethics committee approval was obtained from Erciyes University Animal Experiments Ethics Committee (date: 07.12.2022, number: 22/265).

Informed Consent: Informed consent was not required for this study.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Relationships Between Swallowing Function, Sialorrhea, and Cervical Proprioception in Parkinson's Disease

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ABSTRACT

Objective: In patients with Parkinson's disease (PD), alterations in position sense, swallowing function, and sialorrhea may occur. In neurological disorders, impairment of sensory input from the cervical region has been shown to adversely affect swallowing function. The aim of this study was to investigate the relationship between swallowing function, sialorrhea, and cervical position sense in patients with PD.

Materials and Methods: In this cross-sectional study, 55 patients with PD completed assessments of swallowing function using the Turkish version of the Eating Assessment Tool-10, sialorrhea using the Sialorrhea Clinical Scale for Parkinson's Disease, and position sense using a digital inclinometer. The relationships among these variables were evaluated using the Spearman correlation test.

Results: A significant correlation was found between swallowing function and sialorrhea ($p<0.05$). However, no correlation was observed between cervical position sense and swallowing function or sialorrhea ($p>0.05$).

Conclusion: In conclusion, there was an association between swallowing function and sialorrhea. However, cervical position sense was not associated with these parameters. Future studies should consider these findings, and more comprehensive research including a control group and patients with advanced-stage PD is needed.

Keywords: Parkinson's disease, position sense, sialorrhea, swallowing disorders.

INTRODUCTION

Parkinson's disease (PD) is among the most prevalent neurodegenerative disorders globally and is characterized by a multitude of motor and non-motor symptoms.[1] Swallowing dysfunction develops in most patients during the course of the disease, with prevalence varying according to disease severity. The exact pathology of PD-related swallowing dysfunction remains unclear. However, current evidence suggests that it may be associated with substantia nigra degeneration, dysfunction of the non-dopaminergic system, and neuronal loss in the medullary swallowing pattern generator.[2] Swallowing dysfunction in PD leads to aspiration pneumonia, prolonged hospitalization, increased mortality, and decreased quality of life.[3]

Impaired saliva production and/or control, as well as salivary deficiency or excess, can result in a range of adverse effects, from mild discomfort to significant health and social complications. Control of sialorrhea is impaired in patients with PD and is associated with multiple factors such as dysphagia, orofacial stiffness/hypomimia, lingual bradykinesia, cognitive status, male sex, and disease stage.[4] Additional contributing factors include changes in sensory and postural processes. Despite the identification of numerous contributing factors to sialorrhea in PD, the precise etiology of this phenomenon remains unclear. The strongest evidence supports an established link between dysphagia and sialorrhea.[5] In the cohort study conducted by Perez-Lloret et al.,[5] oro-buccal symptoms such as dysarthria, sialorrhea, and swallowing function were examined. The study revealed that two-thirds of patients with moderate PD exhibited oro-buccal symptoms, and a significant relationship was observed between the occurrence of each symptom and the presence of the other two.

Swallowing is considered a sensorimotor function; it is a process that involves the harmonious interaction of sensory and motor components. Since many structures related to swallowing are located in the cervical region, problems in this region may negatively affect swallowing function. Therefore, proper alignment of the head and neck is extremely important for maintaining the dynamic processes occurring in the cervical region.[6] In addition, the cervical region has a high density of proprioceptors, which play a role in head, neck, and trunk alignment. Impairment of proprioception results in the inability to perform desired motor movements.[7]

Impairment of proprioception in the cervical region can negatively affect swallowing function as well as head and neck movements. Sevim et al.[8] reported a relationship between dysphagia severity and decreased head and neck proprioception in neurological disorders. Although it can be concluded that the sensory receptor system of the cervical region should be taken into consideration, the relationship between head and neck proprioception and swallowing in PD has not been sufficiently investigated in the literature. Therefore, the purpose of this study was to explore the relationship between swallowing function, sialorrhea, and cervical position sense in patients with PD. We hypothesized that there is a relationship between swallowing function, sialorrhea, and cervical position sense in patients with PD.

KEY MESSAGES

- There is a relationship between swallowing function and sialorrhea in patients with Parkinson's disease.
- There is no relationship between cervical position sense and swallowing function or sialorrhea in patients with Parkinson's disease.
- Future studies are recommended to include patients with advanced-stage PD, as well as a control group, to assess cervical motor function.

MATERIALS AND METHODS

Ethical Approval

This cross-sectional study was conducted at Neurology Clinic of Ankara City Hospital. Approval was granted by the Ankara Yıldırım Beyazıt University Health Sciences Ethics Committee (Approval Number: 2023-482, Date: 06.12.2023), and prospective participants were provided with information about the study before their consent was obtained.

Study Design and Participant Selection

Sample size estimation was performed using G*Power software (version 3.1). Assuming an expected correlation coefficient of 0.50, a significance level (α) of 0.05 and a statistical power of 95%, the required minimum sample size was determined to be 46 participants.[9]

The inclusion criteria were as follows: a diagnosis of PD according to the Movement Disorder Society–Parkinson's Disease (MDS-PD) criteria made by a specialist neurologist; age ≥18 years; and a Standardized Mini-Mental State Examination (SMMSE) score of 24 or higher. The exclusion criteria included the presence of a cervical pathology such as cervical disc herniation or radiculopathy; a history of cervical surgery; a history of neck pain within the previous month; the presence of any visual impairment; and receiving treatment for salivation.

Measurements

The severity of PD was assessed by a specialist neurologist using the Modified Hoehn and Yahr Scale, and cognitive status was evaluated using the SMMSE. Demographic information,

swallowing function, sialorrhea, and position sense of patients with PD who met the inclusion criteria were evaluated by a physiotherapist. All assessments in patients with PD were conducted during the medication "on" phase to minimize the effects of motor fluctuations. Evaluations were performed approximately 1–2 hours after the intake of dopaminergic medication, when patients were in their optimal functional state.

Swallowing Function

Symptoms of swallowing disorders were evaluated using the Turkish Eating Assessment Tool (T-EAT-10). The T-EAT-10 is a reliable and valid assessment tool that evaluates swallowing-related symptoms through specific self-reported questions. The test consists of 10 items. The T-EAT-10 is a screening tool that is simple to administer, easy to score, and applicable across a wide range of swallowing dysfunctions. A total score of 3 or higher on the T-EAT-10 indicates a risk of swallowing disorder.[1]

Sialorrhea

The Sialorrhea Clinical Scale for Parkinson's Disease consists of seven subscales that assess various aspects of salivation in patients with PD. These include diurnal sialorrhea (A), nocturnal sialorrhea (B), severity of drooling (C), speech disturbance (D), eating disturbance (E), frequency of drooling (F), and social disturbance (G). Patients are asked to respond subjectively regarding the extent to which increased salivation has bothered them during the past week. In all seven sections, increased salivation is rated on a scale from 0 to 3. A score of 0 indicates that the patient was not affected by increased salivation at all, whereas a score of 3 indicates that the patient was maximally affected. The lower the total score on the scale, the less the patient is affected by increased salivation.[11] The Turkish reliability and validity of the Sialorrhea Clinical Scale were established by Genç et al.,[12] and it has been reported to be a reliable and valid tool for patients with PD.

Position Sense

Cervical position sense was measured using the Cervical Joint Position Error Test (CJPET), which employed a Dualer IQ digital inclinometer (J-Tech Medical, Midvale, UT, USA). The digital inclinometer has been shown to demonstrate good test-retest reliability when used to measure spinal range of motion.[13] The test was conducted by the same experienced physiotherapist to ensure consistency. To administer the CJPET, the target head position to be reproduced by the patients was set by the assessor at 50% of the available range of motion (ROM). Patients were instructed to keep their eyes closed during the test. Measurements were performed in two positions:

(1) The sitting position for flexion, extension, and right and left lateral flexion,

(2) The supine position for right and left rotation.

Table 1. Sociodemographic characteristics of the patients

	Median	Minimum-Maximum
Age (years)	65	45-82
BMI (kg/m^2)	28.23	21.72-41.52
	n	**%**
Gender		
Male	36	65.5
Female	19	34.5
Education level		
Primary school	26	47.3
Secondary school	4	7.3
High school	9	16.4
University	16	29.1

n: Number of patients; %: Percentage; kg: Kilogram; m: Meter; BMI: Body mass index.

During the CJPET, the digital inclinometer was positioned at different points on the head: on the lateral aspect for flexion and extension, on the forehead for right and left lateral flexion, and on the vertex while the participant was in the supine position to assess right and left rotation. Each movement direction (flexion, extension, rotation, and lateral flexion) was repeated three times, and the mean of these trials was used for analysis. The absolute error value represented the CJPET outcome measure.[14]

Statistical Analysis

Data analyses were conducted using IBM SPSS Statistics version 26.0 (SPSS Inc., Chicago, IL, USA). The normality of the variables was examined using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Descriptive data were summarized as frequencies and percentages for categorical variables, and as median (minimum–maximum) and mean (standard deviation) for continuous variables. As the data were non-normally distributed, relationships among variables were analyzed using Spearman's correlation test. Statistical significance was set at a p-value <0.05.

RESULTS

A total of 55 patients with PD (median age=65 years, min/max= 45/82 years; 65.5% male) were included in the study. The sociodemographic characteristics of the patients are presented in Table 1.

The median disease duration was 6 years, and the median disease stage was 1.5. Intraoral anatomical structures were missing in 33 (60%) patients. In addition, 11 (20%) patients exhibited chewing difficulties, and 13 (23.6%) reported cough symptoms. The clinical features of the patients are detailed in Table 2.

A positive, moderate, and statistically significant correlation was found between swallowing function and sialorrhea (rho=0.618, p<0.001). There was no significant relationship between swallowing function and any parameter of cervical position sense (p>0.05). Similarly, no significant relationship was detected between sialorrhea and any cervical position sense parameters (p>0.05) (Table 3).

DISCUSSION

According to the findings of our study, greater sialorrhea severity was associated with increased swallowing dysfunction. However, cervical position sense was not related to either swallowing function or sialorrhea.

Swallowing dysfunction is frequently observed in patients with PD and can occur at all stages of the disease. It is estimated that 40-80% of patients with PD worldwide experience swallowing problems.[15] Hallmarks of swallowing dysfunction in patients with PD include festinated or repetitive tongue movements (also called tongue pumping),[16] decreased chewing speed and coordination, significantly prolonged oropharyngeal transit time, and pharyngeal spill.[17] In the study known as the Barcelona and Lisbon cohort, it was reported that the prevalence of swallowing dysfunction was 68% in patients with PD at Hoehn and Yahr stages 4 and 5.[18] These data indicate that swallowing problems increase in the later stages of the disease. This may result from a sustained reduction in dopaminergic activity in advanced PD. The results of our study showed that the EAT-10 scores of patients with early-stage PD averaged 3±6, indicating a risk of swallowing dysfunction.

Another clinical symptom observed in patients with PD is sialorrhea. Sialorrhea is essential for preserving oral health

Table 2. Clinical characteristics of the patients

	Median (Min-Max)	Mean±SD
Disease duration (years)	6 (1-20)	7.6±4.9
Hoehn and Yahr stage	1.5 (1-5)	1.6±0.9
Standardized mini-mental state examination (score)	27 (24-30)	27±2
Turkish eating assessment Tool-10 (score)	0 (0-30)	3±6
Sialorrhea clinical scale for Parkinson's Disease (score)	2 (0-14)	3±4
Position sense		
Cervical flexion JPE (°)	2.66 (0-6.33)	2.44±1.51
Cervical extension JPE (°)	2 (0.33-8)	2.56±1.58
Cervical right lateral flexion JPE (°)	2 (0.33-8.33)	2.45±1.61
Cervical left lateral flexion JPE (°)	2.33 (0-6.33)	2.58±1.49
Cervical right rotation JPE (°)	2.33 (0.33-5.33)	2.38±1.26
Cervical left rotation JPE (°)	2.17 (0.33-6.66)	2.38±1.47
	n (%)	**%**
Turkish eating assessment Tool-10 (score)		
≥3	18	32.7
<3	37	67.3
Cough after eating/drinking		
Yes	13	23.6
No	42	76.4

Min: Minimum; Max: Maximum; SD: Standard deviation; n: Number of patients; %: Percentage; JPE: Joint position error.

Table 3. Correlations between swallowing function, saliva, and cervical position sense

	T-EAT-10		SCS-PD	
	rho	p	rho	p
SCS-PD (score)	0.618	**<0.001***	–	–
Position sense				
Cervical flexion JPE (°)	-0.107	0.436	-0.126	0.359
Cervical extension JPE (°)	0.005	0.973	-0.083	0.549
Cervical right lateral flexion JPE (°)	-0.078	0.570	-0.007	0.962
Cervical left lateral flexion JPE (°)	-0.096	0.485	-0.128	0.351
Cervical right rotation JPE (°)	0.061	0.667	0.090	0.524
Cervical left rotation JPE (°)	-0.152	0.282	-0.037	0.794

T-EAT-10: Turkish Eating Assessment Tool-10; SCS-PD: Sialorrhea Clinical Scale for Parkinson's Disease; JPE: Joint position error; r: Spearman's correlation coefficient; *p<0.05.

and plays a crucial role in facilitating swallowing.[19] Xerostomia is observed in approximately 50% of patients with PD, and similarly, sialorrhea is reported in approximately 50% of cases.[20] Although sialorrhea appears to result from excessive saliva production, the primary underlying cause is a reduction in the frequency of spontaneous swallowing.[21] Patients with PD have been shown to swallow 39% less frequently than young healthy controls.[22] In addition, poor head control, a persistently open mouth, inadequate lip control, irregular tongue mobility, decreased tactile sensation, macroglossia, dental malocclusion, and nasal congestion have been identified as causes of sialorrhea.[23] However, many patients with PD may be unaware of their swallowing or sialorrhea problems, possibly due to deficits in proprioception.[8,24] Sensory dysfunction, such as impaired proprioception, may reduce an individual's ability to recognize sialorrhea. Similarly, anatomical or motor dysfunction related to swallowing may limit the ability to manage increased secretions. A cohort study examining the prevalence and clinical associations of sialorrhea and swallowing dysfunction in patients with PD reported that sialorrhea was more prevalent in patients with Unified Parkinson's Disease Rating Scale (UPDRS) II + III scores higher than 28 and in those with swallowing dysfunction.[5] In the present study, sialorrhea showed a positive correlation with swallowing dysfunction, and our findings are consistent with the literature.

Research has indicated that cervical proprioception plays a crucial role in various neurological disorders. Impaired cervical proprioception may disrupt body orientation and lead to insufficient stabilization of the head and neck muscles during swallowing.[25,26] Edwards et al.[26] demonstrated that cervical proprioceptive input has direct neural connections with brainstem swallowing centers. Using an experimental animal model, they showed that sensory afferents from the upper cervical region project to the intermedius nucleus of the medulla, which in turn connects with key nuclei responsible for tongue, pharyngeal, laryngeal, respiratory, and autonomic control. Functional findings indicated that stimulation of cervical afferents can modify brainstem motor output and respiratory patterns. These results suggest that cervical proprioception is neurally integrated into the swallowing network and may influence swallow initiation, coordination of oropharyngeal movements, and airway protection. There are numerous studies in the literature examining swallowing problems and sialorrhea in patients with PD;[21,27] however, no study has yet investigated the relationship between cervical position sense and swallowing function or sialorrhea in this population. A discrepancy of 4-5 degrees or more in position sense measurement is considered indicative of impaired cervical position sense. In the present study, the mean cervical position sense errors were as follows: flexion, 2.66; extension, 2.00; right and left lateral flexion, 2.00-2.33; right and left rotation, 2.33-2.17. Furthermore, Abakay et al.[28] investigated the relationship between cervical proprioception and dysphagia severity in patients with multiple sclerosis. They reported that significant proprioceptive deficits were associated with increased swallowing difficulties, suggesting that pronounced position sense impairment may contribute to dysphagia severity. Our findings indicate that the magnitude of position sense errors was insufficient to suggest the presence of a position sense disorder. Therefore, the lack of correlation between cervical position sense and both swallowing function

and sialorrhea may be attributable to this relatively preserved function. This finding may also suggest that the primary factors contributing to swallowing impairment and sialorrhea in patients with PD are motor deficits rather than sensory impairments, such as deficits in position sense.

This study has several limitations. First, dysphagia risk was assessed using self-report measures rather than objective imaging techniques, such as videofluoroscopic or fiberoptic endoscopic evaluation, which are considered gold-standard methods. Although self-reported questionnaires provide valuable information regarding patients' perceived swallowing difficulties, they may not fully capture subclinical or physiological aspects of dysphagia. Second, the mean disease severity of the participants with PD was 1.5, which may limit the generalizability of the findings. It is possible that the relationship between cervical position sense and swallowing function may differ in patients with more advanced disease severity.

CONCLUSION

In patients with Parkinson's disease, swallowing dysfunction is associated with sialorrhea, whereas cervical position sense does not appear to be related to either swallowing function or sialorrhea. These findings suggest that interventions targeting swallowing function may also contribute to the management of sialorrhea. Future studies should consider including patients with advanced-stage PD, as well as a control group, to further evaluate cervical motor function.

Ethics Committee Approval: Approval was granted by the Ankara Yıldırım Beyazıt University Health Sciences Ethics Committee (Approval Number: 2023-482, Date: 06.12.2023).

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Impact of Oxacillinase-48 (OXA-48) and New Delhi Metallo-β-Lactamase (NDM) Co-existence on Mortality in Critically Ill Patients with *Klebsiella pneumoniae* Bloodstream Infections: A Prospective Controlled Study

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ABSTRACT

Objective: Carbapenem-resistant *Klebsiella pneumoniae* (CRKp) is associated with high mortality rates due to carbapenemase production. Türkiye is endemic for oxacillinase-48–producing carbapenemase (OXA-48), and New Delhi metallo-β-lactamase-1 (NDM-1) has also recently emerged. This study aimed to investigate mortality risk factors and carbapenemase types in patients with CRKp bacteremia.

Materials and Methods: This prospective study included 83 adult patients with CRKp bacteremia at Kayseri City Training and Research Hospital between September 2023 and March 2024. The control group consisted of patients without infection from the same hospital units. Pathogens were identified using the Vitek 2 system. Susceptibility to ceftazidime-avibactam and colistin was determined using disk methods. Carbapenemase genes were detected by polymerase chain reaction (PCR).

Results: In the multivariable logistic regression analysis, central venous catheter use, rectal colonization, and prior piperacillin–tazobactam use were identified as significant risk factors for CRKp bacteremia. Among the isolates, 95% carried OXA-48, 48.2% carried NDM, and 3.6% carried *Klebsiella pneumoniae* carbapenemase (KPC); OXA-48 and NDM co-production was detected in 45.7% of isolates. Verona integron-encoded metallo-β-lactamase (VIM) and Imipenemase metallo-β-lactamase (IMP) were not detected. Fourteen-day mortality was 25.3%. Respiratory system-related bacteremia was identified as an independent predictor of mortality, whereas clinical response on day 5 was independently associated with lower mortality. No difference was observed between monotherapy and combination therapy.

Conclusion: This study demonstrates that OXA-48 remains the most prevalent carbapenemase type in CRKp bloodstream infections in Türkiye, while NDM is increasing rapidly and frequently co-occurs with OXA-48, worsening clinical outcomes. Strengthening infection control measures, promoting the prudent use of antibiotics, and ensuring access to new treatment agents are crucial for reducing the spread and impact of CRKp infections.

Keywords: Carbapenemase, New Delhi metallo-β-lactamase-1 (NDM), oxacillinase-48 (OXA-48).

INTRODUCTION

The global epidemic of antibiotic resistance is endangering public health. The incidence and prevalence of carbapenem-resistant Gram-negative bacteria have increased significantly worldwide over the past decade.[1] The 2014 antimicrobial resistance report from the World Health Organization (WHO) indicates that carbapenem-resistant *Klebsiella pneumoniae* (CRKp) is present in all regions of the world.[2] According to Türkiye's 2021 National Antimicrobial Resistance and Surveillance System report on healthcare-associated infections, the rate of carbapenem resistance among *K. pneumoniae* strains increased from 63.5% in 2021 to 73.43% in 2024.[3,4]

Carbapenemases are β-lactamases that hydrolyze at least one carbapenem and represent one of the primary mechanisms of carbapenem resistance. These enzymes can also hydrolyze other β-lactam antibiotics in addition to carbapenems.[5,6] Oxacillinase-48 (OXA-48) is the most commonly detected carbapenemase enzyme in CRKp strains in Türkiye.[7] Although data on carbapenemase-producing pathogens in Türkiye are limited, other carbapenemases, such as New Delhi metallo-β-lactamase (NDM-1), the NDM-1/OXA-48 co-production, Verona integron-encoded metallo-β-lactamase (VIM), and Imipenem metallo-β-lactamase (IMP), have also been identified in outbreak settings.[7–9]

The development of CRKp infection is influenced by multiple factors, and patients often present with one or more risk factors. Recognized risk factors include severe underlying diseases (such as solid tumors, diabetes mellitus, and hemiplegia), high disease severity, invasive procedures, prolonged stay in the intensive care unit (ICU), prior colonization, advanced age, and prior use of intravenous broad-spectrum antibiotics. Identifying risk factors associated with mortality is important for optimizing patient management and recognizing high-risk individuals.[10]

There are limited safe and effective alternative treatment options for healthcare-associated CRKp bacteremia, contributing to high mortality and morbidity rates that vary across years and regions.[11] Despite the use of various antibiotic combinations and the development of novel agents, treatment options may vary depending on the specific carbapenemase genes present. However, there is no consensus on the optimal treatment strategy for CRKp infections. When new drugs are unavailable or contraindicated, monotherapy or combination therapy with existing antibiotics may be the only viable option.[12]

The aim of this study was to evaluate episodes of bloodstream infections caused by CRKp in our hospital and to determine associated risk factors, the carbapenemase genotypes of the causative agents, and their relationship with patient prognosis. Additional objectives were to assess disease severity and mortality outcomes, to evaluate the effectiveness of monotherapy versus combination therapy, and to examine the relationship between carbapenemase resistance patterns and genotypes.

KEY MESSAGES

- This study evaluated the risk factors and clinical outcomes of bloodstream infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKp).
- OXA-48 carbapenemase remains the most prevalent type in CRKp bloodstream infections in Türkiye, whereas NDM is rapidly increasing, often co-occurring with OXA-48 and leading to poorer clinical outcomes.
- Given the limited treatment options and the high morbidity and mortality associated with CRKp, rational antibiotic use and strict infection-control practices are essential to prevent further resistance.

MATERIALS AND METHODS

Study Design and Participant Selection

This study was designed as a single-center, prospective observational cohort study. A total of 83 patients who were treated in the Kayseri City Training and Research Hospital Department of Infectious Diseases and Clinical Microbiology between September 7, 2023 and March 7, 2024, and who had CRKp isolated from blood cultures were evaluated. The control group consisted of patients admitted to the intensive care unit who had stayed for at least 48 hours, had similar demographic and clinical characteristics to the case group, and showed no evidence of infection. In total, 81 patients were enrolled in the study.

Based on previous literature, we evaluated mortality occurring within 14 days. The infection-related mortality period was defined as 14 days. Patients who developed CRKp-associated bloodstream infections were categorized into two groups according to their 14-day survival status: survivors and non-survivors. Risk factors for mortality were analyzed, and the genotypic characteristics of carbapenemase production in the causative strains were examined.

Ethics Committee Approval

Ethics approval was obtained from the Kayseri City Hospital Clinical Research Ethics Committee (Approval Number: 904, Date: 05.09.2023).

Patients and Data Collection

Clinical, laboratory, and demographic data of the patients were recorded. Risk factors for CRKp-associated bloodstream

infection were identified by comparing the clinical and demographic characteristics of the study groups. The study group consisted of patients aged 18 years and older who developed bloodstream infection due to CRKp while being followed at Kayseri City Training and Research Hospital. Pregnant women, patients under 18 years of age, and patients with polymicrobial infections (defined as isolation of *K. pneumoniae* together with other bacterial or fungal species) were excluded from the study. For each patient included in the case group, control patients were selected from individuals admitted to critical care units who had stayed in the intensive care unit for at least 48 hours, had similar demographic and clinical characteristics to the case group, and showed no evidence of infection. Microsoft Excel was used to record the following data for both study and control groups: demographic characteristics; comorbidities; rectal *K. pneumoniae* carriage within the three months prior to infection; risk scores calculated at hospital admission and on the day of bacteremia onset; interventional procedures and their durations; changes in key laboratory parameters and clinical response on the fifth day of targeted therapy after bacteremia diagnosis; empirical antibiotic therapy administered after diagnosis and its duration; appropriateness of empirical antibiotic therapy according to antimicrobial susceptibility results; microbiological response status on day 5 after treatment initiation; 14-day mortality following bacteremia diagnosis; antibiotic susceptibility results and minimum inhibitory concentration (MIC) values; and carbapenemase resistance genes (OXA-48, NDM, *Klebsiella pneumoniae* carbapenemase [KPC], VIM, and IMP).

Microbiological and Molecular Analyses

Bacteremia was diagnosed based on laboratory and clinical findings consistent with blood culture results. Blood culture samples sent to the microbiology laboratory from various departments during the study period were loaded into the BacT/Alert 3D automated blood culture system. Blood culture bottles were incubated in the device for seven days until a positive signal was detected. Bottles that yielded a positive signal during the incubation period were further processed.

Following Gram staining, samples were inoculated onto blood agar (Oxoid) and Eosin Methylene Blue (EMB) agar (Oxoid). After incubation for 18-24 hours, grown colonies were evaluated by Gram staining and then identified using an automated system (VITEK 2 Compact; bioMérieux, France). The isolates were identified as *K. pneumoniae*, and their sensitivities were evaluated. For isolates identified as carbapenem-resistant, sensitivity was re-evaluated using a meropenem disk test to confirm resistance.

A total of 83 isolates identified as *K. pneumoniae* were included in the study. Carbapenem-resistant isolates were additionally tested for ceftazidime-avibactam susceptibility using the disk diffusion method and for colistin susceptibility using the disk elution method. All antibiogram evaluations were performed and interpreted according to the clinical breakpoints established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), version 14.0 (2024).[13] Species identification of the obtained strains was further confirmed by 16S rRNA gene sequencing analysis at the Molecular Microbiology Laboratory of the Turkish Public Health Institution.

Statistical Analyses

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Prior to the initiation of this prospective observational cohort study, an a priori sample size calculation was conducted using PASS software (NCSS, LLC, USA). The calculation was based on the primary study hypothesis regarding the difference in 14-day mortality between treatment groups. Assuming an anticipated effect size of 0.60 for mortality outcomes, a two-sided alpha level of 0.05, and a statistical power of 95%, the minimum required sample size was estimated to be at least 35 patients per group.

Histogram analyses and the Shapiro-Wilks test were used to assess the normality of continuous variables. Parametric data are presented as mean ± standard deviation, and the Student's t-test was used to compare differences between groups. The Mann-Whitney U test was used to evaluate differences between groups, and non-parametric data are presented as median (range). The chi-square test was used to compare categorical variables, which are reported as numbers and percentages.

To identify factors associated with the outcome, logistic regression analyses were performed. First, univariate logistic regression analyses were conducted for all potential independent variables. Variables with a p value <0.20 in the univariate analysis were considered candidate variables and were subsequently included in the multivariable logistic regression model. A backward stepwise likelihood ratio method was used to derive the final model. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs).

The linearity of the logit assumption for continuous variables was assessed using the Box–Tidwell test. Multicollinearity among independent variables was evaluated using the variance inflation factor (VIF), with VIF values >5 considered indicative of significant multicollinearity. Model calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test. A p value <0.05 was considered statistically significant in all analyses.

Table 1. Demographic and clinical characteristics of the study population

Characteristics	CRKp n=83 (%)	Control n=81 (%)	p
Age, mean (±SD)	69.4±16.5	70.1±15.8	0.781
Male sex	37 (44.6)	42 (51.9)	0.438
APACHE II score (on admission), mean (±SD)	17.7±6.6	16.1±6.7	0.125
SOFA Score (on admission), mean (±SD)	3.6±3.1	2.3±2.0	0.001
Charlson Comorbidity Index (on admission), mean (±SD)	5.5±3.0	5.0±2.7	0.232
Invasive procedures			
Central venous catheter	70 (84.3)	17 (21.0)	<0.001
Intubation	40 (48.2)	10 (12.3)	<0.001
Tracheostomy	11 (13.3)	–	–
Urinary catheter	82 (98.8)	77 (95.1)	0.207
Decubitus	21 (25.3)	–	
Drainage catheter	9 (10.8)	1 (1.2)	0.025
Percutaneous endoscopic gastrostomy	12 (14.5)	–	–
Nephrostomy	4 (4.8)	2 (2.5)	
Surgery	20 (24.1)	2 (2.5)	<0.001
Other (chest tube, etc.)	2 (2.4)	3 (3.7)	0.630
Rectal *K. pneumoniae* carriage	49 (59.8)	2 (2.5)	<0.001
Prior antibiotic use			
Beta-lactam/beta-lactamase inhibitor	44 (53.0)	14 (17.9)	<0.001
Carbapenem	57 (68.7)	13 (16.0)	<0.001
Quinolone	13 (15.7)	11 (13.6)	0.706
Aminoglycoside	13 (15.7)	1 (1.2)	–
Polymyxin	13 (15.7)	–	–
Tigecycline	8 (9.6)	–	–
Ceftazidime–avibactam	4 (4.8)	–	–

APACHE: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; SD: Standard deviation; CRKp: Carbapenem-resistant *Klebsiella pneumoniae*.

RESULTS

The case group consisted of 46 (55.4%) female and 37 (44.6%) male patients, with a mean age of 69.4±16.5 years. Age and sex distributions were similar between the case and control groups. The Sequential Organ Failure Assessment (SOFA) score at admission was higher in the case group than in the control group (p=0.007). In the case group, the most common comorbidities were hypertension (38 patients, 45.8%), diabetes mellitus (32 patients, 38.6%), congestive heart failure (19 patients, 22.9%), chronic kidney disease (14 patients, 16.9%), chronic obstructive pulmonary disease (10 patients, 12%), solid tumors (9 patients, 10.8%), and hematological malignancies (3 patients, 3.6%). There was no significant difference in comorbidities between the case and control groups.

Central venous catheterization, intubation, and surgical procedures were more frequent in the case group than in the control group (p<0.001). The rate of rectal *K. pneumoniae* carriage was also significantly higher in the case group. Regarding prior antibiotic use, beta-lactams or beta-lactam/beta-lactamase inhibitors (53%), carbapenems (68.7%), quinolones (15.7%), and aminoglycosides (15.7%) were used significantly more frequently in the case group (Table 1).

In the univariable logistic regression analysis for infection, central venous catheter use increased the risk of infection approximately eightfold (odds ratio [OR]: 7.80, 95% confidence interval [CI]: 2.30–26.52; p=0.001). Rectal *K. pneumoniae* carriage showed the strongest association, increasing the risk

Table 2. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infections

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
APACHE II score (on admission), mean (±SD)	1.06 (0.95–1.19)	0.297		
SOFA score (on admission), mean (±SD)	0.97 (0.74–1.28)	0.850		
Central venous catheter	7.80 (2.30–26.52)	0.001	8.42 (2.53–27.99)	0.001
Intubation	2.25 (0.55–9.26)	0.262	3.12 (0.86–11.29)	0.082
Rectal *K. pneumoniae* carriage	41.01 (6.03–278.74)	<0.001	50.65 (9.62–266.77)	<0.001
Piperacillin–tazobactam use	7.38 (2.24–24.28)	0.001	6.16 (2.02–18.78)	0.001
Carbapenem use	1.96 (0.55–7.02)	0.303		

APACHE: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; SD: Standard deviation; OR: Odd ratios; CI: Confidence interval.

more than fortyfold (OR: 41.01, 95% CI: 6.03–278.74; p<0.001). Piperacillin–tazobactam use was also significantly associated with infection (OR: 7.38, 95% CI: 2.24–24.28; p=0.001). Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, intubation, and carbapenem use were not significantly associated with infection. In the multivariable logistic regression analysis, central venous catheter use (OR=7.803; p=0.001), rectal colonization (OR=41.009; p<0.001), and piperacillin–tazobactam use (OR=7.377; p=0.001) were significantly associated with infection. Other variables, including APACHE II score, SOFA score, intubation, and carbapenem use, lost statistical significance after adjustment. The final model demonstrated good calibration according to the Hosmer–Lemeshow test (χ^2=1.677; p=0.976) (Table 2).

OXA-48 was detected in 95% of CRKp strains, NDM in 48.2%, and KPC in 3.6%, while co-production of OXA-48 and NDM was observed in 45.7% of strains. None of the isolates harbored VIM or IMP (Fig. 1).

Among patients with CRKp-associated bloodstream infection, the 14-day mortality rate was 25.3%. There was no significant difference in age between survivors and non-survivors. Similarly, no significant differences were observed between the two groups in terms of comorbidities. The APACHE II score on the day of bloodstream infection onset was higher in the survivor group (p=0.032).

When comparing the sources of bacteremia between survivors and non-survivors, respiratory tract–related bacteremia was more frequent in the non-survivor group (p=0.003). The most common source of bacteremia overall was central venous catheters, followed by the respiratory system. In 17 patients, *K. pneumoniae* was isolated from sputum cultures within 72 hours prior to blood culture growth. These cases were classified as respiratory-associated bacteremia, including pneumonia and ventilator-associated pneumonia.

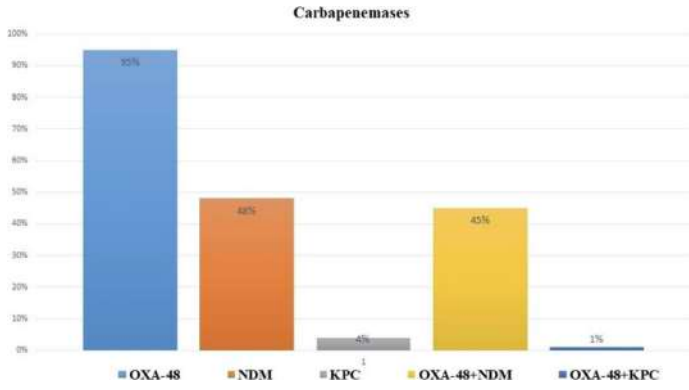

Figure 1. Distribution of carbapenemases.

OXA-48: Oxacillinase-48; NDM; New Delhi metallo-β-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase.

In the analysis of carbapenemase enzymes according to 14-day mortality, co-production of OXA-48 and NDM was more frequent in the non-survivor group (p=0.026). Empirical therapy was administered more frequently in non-survivors (47.6%), and the most commonly prescribed empirical antibiotic was a carbapenem (38.1%). There was no notable difference between survivors and non-survivors regarding the appropriateness of empirical therapy (p=0.38).

Targeted therapy was used more frequently in survivors (80.6%) (p=0.011). Ceftazidime-avibactam was the most frequently used antibiotic in monotherapy, whereas the most commonly administered combination therapy was carbapenem plus polymyxin. Carbapenem plus aminoglycoside was the second most frequently used combination regimen. No statistically significant difference was observed between survivors and non-survivors with respect to either monotherapy or combination

Table 3. Characteristics of survivors and non-survivors

	Non-survivors n=21	Survivors n=62	p
Age, mean (±SD)	68.6±19.0	69.7±15.8	0.790
Comorbidities			
Hypertension	9 (46.9)	29 (46.8)	0.756
Diabetes	10 (47.6)	22 (35.5)	0.323
Congestive heart failure	7 (33.3)	12 (19.4)	0.188
Chronic obstructive pulmonary disease	3 (14.3)	4 (6.5)	0.362
Chronic kidney disease	2 (9.5)	12 (19.4)	0.298
Solid tumor	1 (4.8)	8 (12.9)	0.300
Hematological malignancy	–	3 (4.8)	–
APACHE II score, mean (±SD) (day of bacteremia)	19.6±7.9	24.4±9.9	0.032
SOFA score, mean (±SD) (day of bacteremia)	5.2±4.2	7.6±5.4	0.056
Charlson Comorbidity Index, mean (±SD) (day of bacteremia)	5.3±2.7	5.6±3.0	0.792
Source of bacteremia			
Central catheter	7 (33.3)	25 (40.3)	0.570
Primary bacteremia	4 (19.0)	10 (16.1)	0.758
Respiratory tract	9 (42.9)	8 (12.9)	0.003
Urinary tract infection	1 (4.8)	13 (21.0)	0.087
Other (skin, soft tissue, and intra-abdominal)	1 (4.8)	6 (9.7)	0.484
Carbapenemases			
OXA-48	21 (100)	58 (93.5)	0.233
NDM	14 (66.7)	26 (41.9)	0.076
KPC	1 (4.8)	2 (3.2)	0.744
OXA-48 + NDM	14 (66.7)	24 (38.7)	0.026
OXA-48 + KPC	1 (4.8)	–	–
Treatment			
Empirical treatment	10 (47.6)	11 (17.7)	0.006
Carbapenem	8 (38.1)	9 (14.5)	0.030
Beta-lactam/beta-lactamase inhibitor	1 (4.8)	1 (1.6)	0.416
Cephalosporin	1 (4.8)	1 (1.6)	0.416
Appropriateness of empirical treatment	1 (4.8)	7 (11.3)	0.381
Targeted treatment	11 (52.4)	50 (80.6)	0.011
Monotherapy	1 (4.8)	14 (22.6)	0.067
Ceftazidime–avibactam	1 (4.8)	13 (21.0)	0.087
Trimethoprim-sulfamethoxazole	–	1 (1.6)	–
Combination therapy	9 (42.9)	37 (59.7)	0.180
Carbapenem + polymyxin	5 (23.8)	17 (27.4)	0.746
Carbapenem + aminoglycoside	3 (14.3)	8 (12.9)	0.872
Carbapenem + tigecycline	–	3 (4.8)	–
Carbapenem + trimethoprim-sulfamethoxazole	–	1 (1.6)	–
Ceftazidime–avibactam + polymyxin	1 (4.8)	2 (3.2)	0.744
Ceftazidime–avibactam + trimethoprim-sulfamethoxazole	–	1 (1.6)	–
Polymyxin + tigecycline	–	3 (4.8)	–
Beta-lactam/beta-lactamase inhibitor + aminoglycoside	1 (4.8)	–	–
Trimethoprim-sulfamethoxazole + fosfomycin	–	1 (1.6)	–
Carbapenem + polymyxin + tigecycline	–	1 (1.6)	–
Clinical response on day 5 of treatment	4 (19.0)	35 (56.5)	0.003

APACHE: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; SD: Standard deviation; OXA-48: Oxacillinase-48; NDM: New Delhi Metallo-β-Lactamase; KPC: *Klebsiella pneumoniae* carbapenemase.

Table 4. Risk factors for mortality in patients with carbapenem-resistant *Klebsiella pneumoniae* infections

	Univariate analysis	Multivariate analysis
Congestive heart failure	1.39 (0.42–4.63), p=0.59	
APACHE II score, mean (±SD) (day of bacteremia)	1.01 (0.94–1.09), p=0.77	
SOFA score, mean (±SD) (day of bacteremia)	0.95 (0.80–1.12), p=0.55	
Respiratory tract	4.98 (1.61–15.39), p=0.005	5.92 (1.41–24.77), p=0.015
Urinary tract infection	0.63 (0.22–1.79), p=0.38	
NDM	2.35 (0.87–6.35), p=0.091	1.88 (0.57–6.19), p=0.300
OXA-48 + NDM	3.28 (1.17–9.21), p=0.024	2.41 (0.72–8.07), p=0.150
Empirical treatment	1.31 (0.47–3.63), p=0.60	
Carbapenem	0.144 (0.006-3.365), p=0.228	
Targeted treatment	0.075 (0.003-1.641), p=0.100	
Monotherapy	1.62 (0.59–4.44), p=0.35	
Ceftazidime–avibactam	0.37 (0.14–1.02), p=0.054	0.46 (0.14–1.55), p=0.210
Combination therapy	0.72 (0.26–1.97), p=0.52	
Clinical response on day 5 of treatment	0.18 (0.06–0.49), p=0.001	0.16 (0.05–0.48), p=0.001

APACHE: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; OXA-48: Oxacillinase-48; NDM: New Delhi Metallo-β-Lactamase.

therapy. On the fifth day of treatment, the survivor group demonstrated a better clinical response (Table 3).

Fourteen-day mortality risk factors were evaluated using logistic regression analysis. In univariable analyses, respiratory source of infection, NDM production, OXA-48+NDM co-production, ceftazidime–avibactam therapy, and clinical response on day 5 were associated with mortality at p<0.20 and were therefore included in the multivariable model. In the final model, respiratory source of infection remained an independent predictor of mortality (OR: 5.92, 95% CI: 1.41–24.77; p=0.015), whereas clinical response on day 5 was independently associated with lower mortality (OR: 0.16, 95% CI: 0.05–0.48; p=0.001). The model demonstrated good calibration (Hosmer–Lemeshow p=0.48) and excellent discriminative ability (area under the curve [AUC]=0.86) (Table 4).

DISCUSSION

K. pneumoniae infections have increased in recent years and represent a serious public health issue due to limited treatment options and high mortality rates. According to the WHO, CRKp is classified among bacteria of critical priority, along with carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, for which the development of new antibiotics is urgently needed.[14]

Due to the limited range of available treatment options, the aim of this study was to investigate the clinical and molecular characteristics of patients who developed CRKp bacteremia in order to contribute to the development of strategies for reducing CRKp infections, identifying associated risk factors, and optimizing treatment approaches. The study included 83 patients with CRKp bacteremia and 81 patients in the control group.

Although risk factors vary across studies of CRKp infections and bacteremia, the most commonly reported factors include prior history ICU admission, previous antibiotic or carbapenem use, prolonged hospitalization, renal dysfunction, gastrointestinal colonization, mechanical ventilation or tracheostomy, history of surgery, and gastric catheterization.[15–23] In the present study, central venous catheter use and rectal *K. pneumoniae* carriage were also identified as risk factors for CRKp bacteremia.

Antibiotic selective pressure is believed to contribute to the emergence of antibiotic-resistant infections.[24] A meta-analysis by Liu et al.[25] demonstrated that prior use of quinolones, carbapenems, aminoglycosides, anti-pseudomonal agents, and glycopeptides was associated with the development of CRKp infections. Similarly, in our study, prior use of piperacillin–tazobactam was found to increase the likelihood of carbapenem resistance.

The 14-day mortality rate in our cohort was 25.3%. Notably, respiratory source-related bacteremia was significantly more frequent among non-survivors (p=0.003), suggesting that pulmonary infections may serve as a reservoir for more virulent or resistant strains. In Italy, a study conducted between 2015 and 2018 reported a 14-day mortality rate of 32.4% among 102 ICU patients with bloodstream infections caused by

KPC-producing CRKp.[26] In a study conducted in South Korea between 2016 and 2019, the 14-day and 30-day mortality rates for carbapenem-resistant *Enterobacteriaceae* bacteremia were 34.0% and 42.2%, respectively.[27] In several meta-analyses, mortality in cases of CRKp-associated infections has been estimated to range from 37.2% to 42.1%.[28,29]

Studies assessing CRKp bacteremia have identified various risk factors for mortality. Commonly reported predictors include ICU admission, mechanical ventilation, septic shock, high comorbidity-mortality or bacteremia scores (APACHE II, SOFA, and Pitt bacteremia score), absolute neutrophil count <500/mm^3, the presence of immunosuppressive comorbidities, inappropriate empirical antibiotic therapy, and colistin resistance.[7,26,27,30–33] In the present study, respiratory tract-related bacteremia was identified as an independent predictor of mortality, whereas clinical response on day 5 was independently associated with reduced mortality. Interestingly, the APACHE II score was higher in the survivor group (p=0.032), a finding that appears paradoxical. This discrepancy may reflect confounding factors such as small sample size, patient heterogeneity, or differences in the intensity of clinical management between groups.

Aslan et al.[34] evaluated 124 patients with bacteremia and detected OXA-48 in 85.5%, NDM in 3.2%, and OXA-48 + NDM in 8.9% of cases, while no KPC-producing strains were identified. A multicenter study conducted between 2018 and 2019, including 187 patients with CRKp bacteremia, reported OXA-48-like genes in 79.1%, OXA-48-like genes + NDM in 15.5%, NDM alone in 5.9%, and KPC in 3.2% of cases.[7] In another study conducted in Ankara, Bursa, and Trabzon Zarakolu et al.[8] detected OXA-48 in 62.6%, NDM in 9.2%, OXA-48 + NDM in 6.9%, and KPC in 14.5% of 131 bacteremia cases. In our study, the distribution of resistance genes was as follows: OXA-48 in 95%, NDM in 48%, KPC in 4%, OXA-48 + NDM in 45.7%, and OXA-48 + KPC in 1%. This is the first study to report such a high rate of OXA-48 + NDM co-occurrence. We believe this finding is particularly important for Türkiye and will influence empirical treatment strategies.

Another study investigated the clonal and genetic relationships of carbapenem- and colistin-resistant isolates in a large hospital in Sofia, Bulgaria. Co-production of NDM-5 and OXA-232 was detected in 72% of 14 isolates, which were susceptible only to cefiderocin. In our study, NDM was detected in 48% of cases, and OXA-48 + NDM co-production was observed in 45.7%. The lack of effective therapeutic options complicates the management of infections caused by these strains, and their rapid spread emphasizes the urgent need for effective control measures.[35]

A retrospective two-year study conducted in a tertiary hospital in Romania evaluated CRKp-resistant infections and reported that the predominant carbapenemases were OXA-48 + NDM (49.4%), highlighting an increasing frequency of this combination. In our study, the OXA-48 + NDM co-production rate was 45.7%. The Romanian study emphasized the need for caution in the management of infected patients and in preventing the spread of these infections, as well as the need for strengthening public health policies promoting rational antibiotic use and a One Health approach.[36]

Studies investigating the efficacy of antibiotic therapy in CRKp bacteremia have produced conflicting results. While some studies suggest that certain antibiotic combination therapies may influence mortality, others have found no difference.[7,19,26,33,34,37–39] In the INCREMENT study (International Network for Optimal Resistance Monitoring and Epidemiology of Carbapenemase-Producing *Enterobacteriaceae*), which included 437 patients with carbapenem-resistant *Enterobacteriaceae* bacteremia from 10 countries, monotherapy was compared with combination therapy. The 30-day mortality rate did not differ significantly between the groups; however, lower 30-day mortality was observed in patients who received at least one effective antibiotic within five days of bacteremia onset.[37]

Another study conducted at Hacettepe University between 2014 and 2018 compared 30-day mortality rates among 124 patients with CRKp bacteremia who received appropriate treatment (defined as initiation of at least one active antibiotic within five days of bacteremia onset) and those who did not. Mortality was lower in the group that received appropriate treatment. Furthermore, within this group, patients receiving monotherapy (primarily carbapenem and, less frequently, colistin) were compared with those receiving combination therapy (carbapenem- and colistin-based regimens), and no significant difference in 30-day mortality was observed between the two groups.[34]

In our study, empirical carbapenem use was more common among non-survivors, underscoring the limited efficacy of carbapenems in the treatment of CRKp infections. In contrast, targeted therapy based on susceptibility results was significantly more frequent among survivors (p=0.011), highlighting the importance of early microbiological diagnosis and individualized treatment. Pathogen-directed therapy was associated with improved 14-day survival. No statistically significant difference in 14-day survival was observed between survivors and non-survivors with respect to ceftazidime-avibactam monotherapy or combination regimens (including carbapenem-based, polymyxin-based, ceftazidime-avibactam-based, and other combinations). These findings are consistent with previous studies questioning the

superiority of combination therapy. The absence of an optimal standardized treatment regimen continues to be a major challenge in the management of CRKp infections.

CONCLUSION

Central venous catheter use, rectal *K. pneumoniae* carriage, and prior piperacillin–tazobactam use were identified as risk factors for CRKp-associated bloodstream infections. Respiratory system-related bacteremia emerged as an independent predictor of mortality, whereas clinical response on day 5 was independently associated with reduced mortality. No significant difference in mortality was observed between patients receiving monotherapy and those receiving combination therapy. CRKp bacteremia has limited treatment options, is difficult to manage, and is associated with high morbidity and mortality. Therefore, rational antibiotic use, strict adherence to hand hygiene, appropriate use of personal protective equipment, and rigorous implementation of hospital infection-control protocols are essential to prevent the emergence and spread of resistance.

Ethics Committee Approval: Ethics committee approval was obtained from Kayseri City Hospital Clinical Research Ethics Committee (Approval Number: 904, Date: 05.09.2023).

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An Overlooked Risk Group in Adult Immunization: Vaccination Awareness and Knowledge in Patients with Cushing's Syndrome: A Cross-sectional Study

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ABSTRACT

Objective: The aim was to quantify registry-documented adult vaccine uptake among patients with Cushing's syndrome and to assess vaccine awareness in a voluntary survey subgroup.

Materials and Methods: We conducted a record-based, retrospective, cross-sectional analysis of adults with confirmed Cushing's syndrome who were followed at a tertiary endocrinology clinic in Türkiye between January 1, 2017, and May 31, 2023. Vaccine histories were obtained from the national vaccination registry. In addition, a structured telephone questionnaire assessed awareness of vaccines for hepatitis A–D, pneumococcal disease, herpes zoster, influenza, and COVID-19 in a convenience sample of reachable, consenting patients. Analyses were descriptive; subgroup comparisons were exploratory.

Results: The registry cohort included 119 patients (83.2% female; median age 51 years). Beyond SARS-CoV-2 vaccination, uptake of routinely indicated adult vaccines was low: tetanus–diphtheria vaccine (38.7%), pneumococcal conjugate vaccine (23.5%), hepatitis B vaccine (18.5%), influenza vaccine (2.5%), and meningococcal vaccine (1.7%). In the survey subgroup (n=35), awareness of hepatitis B infection was high (80.0%), but knowledge of hepatitis A (28.6%) and hepatitis B (51.4%)vaccines was limited. Although 80.0% reported awareness of pneumococcal vaccination, only 20.0% reported having received it; the most common reason cited was that it was perceived as unnecessary.

Conclusion: Adults with Cushing's syndrome had high SARS-CoV-2 vaccine uptake but substantial gaps in uptake and awareness of other recommended vaccines. Routine immunization assessment and proactive counseling in endocrinology clinics may help close preventable vaccination gaps.

Keywords: Adult immunization, Cushing's syndrome, influenza vaccine, pneumococcal vaccine, vaccine awareness, vaccine uptake.

INTRODUCTION

Cushing's syndrome is an endocrine disorder resulting from chronic exposure to elevated glucocorticoids, either due to endogenous overproduction or exogenous corticosteroid therapy.[1–4] Cortisol excess exerts systemic effects across many organs, most critically impairing the immune system.[5,6]

Glucocorticoids disrupt both innate and adaptive immunity by suppressing cytokine signaling altering T-cell differentiation, inhibiting lymphocyte proliferation, and promoting lymphocyte apoptosis.[7,8] These effects lead to lymphopenia, reductions in CD4+ T-helper cells, alterations in CD4/CD8 ratios, and diminished natural killer (NK) cell cytotoxicity.[9] B-cell function may also be compromised, attenuating humoral immune responses even after natural infections or vaccination.[10] Neutrophil numbers paradoxically rise via demargination, while their functional activity (chemotaxis, phagocytosis, reactive oxygen species production) declines.[11] Macrophage cytokine secretion and dendritic cell maturation are suppressed, reducing antigen presentation and downstream T-cell activation.[12,13] The combined consequence is impaired pathogen clearance and weakened antiviral defense.[14]

Cushing's syndrome patients exhibit increased susceptibility to bacterial, viral, fungal, and opportunistic infections including *Nocardia* spp., *Pseudomonas* spp., *Candida* spp., *Aspergillus* spp., *Pneumocystis jirovecii*, *Herpesviridae*.[15,16] Circadian disruption of cortisol secretion further exacerbates immune dysfunction and infection risk.[1,17] Observational studies confirm infections remain a leading cause of morbidity and mortality in Cushing's syndrome.[18,19] Proactive immunization provides a critical preventive strategy by priming adaptive immunity even in the context of impaired immune function.[16] Although vaccine responses may be attenuated, they remain clinically meaningful.[8] Current guidelines recommend vaccinations for influenza, pneumococcal disease, COVID-19, and other pathogens during periods of stable disease control.[16]

Overall, patients with Cushing's syndrome have increased susceptibility to infection; adult vaccination is recommended as part of routine preventive care for immunocompromised populations. Despite these recommendations, real-world implementation of adult immunization in endocrine outpatient settings remains poorly characterized.

The present study aimed to evaluate documented vaccination coverage in a tertiary-care Cushing's syndrome cohort using national registry data and to explore vaccination awareness through a pilot telephone survey conducted among a voluntary subgroup.

KEY MESSAGES

- Registry-documented adult vaccination coverage was low in patients with Cushing's syndrome, except for SARS-CoV-2 vaccination.
- While COVID-19 vaccination rates were high, likely reflecting national and global pandemic-driven initiatives, the uptake of other routinely recommended vaccines, including pneumococcal, tetanus-diphtheria, hepatitis, and influenza, was substantially lower.
- Routine immunization assessment and proactive counseling in endocrinology clinics may help close preventable vaccination gaps. Collaboration with infectious disease specialists and primary care providers is essential to ensure adherence to adult vaccination guidelines and to close the existing care gaps.

MATERIALS AND METHODS

Study Place and Design

This record-based, retrospective, cross-sectional analysis included adults with a confirmed diagnosis of Cushing's syndrome who were followed at the Endocrinology and Metabolism Outpatient Clinic of Ege University Faculty of Medicine between January 1, 2017, and May 31, 2023. The study comprised two complementary components: (i) a registry-based retrospective audit evaluating documented vaccination coverage in all eligible patients, and (ii) a voluntary telephone survey conducted in a reachable subgroup to explore vaccination awareness and self-reported vaccination history. These components were analyzed separately because the survey subgroup was a convenience sample and was not intended to represent the full cohort.

Ethical Approval

The study protocol was approved by the Ege University Medical Research Ethics Committee (Approval Number: 23-8T/63, Date: 24.08.2023). For the telephone survey, verbal informed consent was obtained at the beginning of each call.

Patients and Data Collection

Demographic and clinical data were extracted from electronic medical records. Vaccination histories were obtained from the Turkish Ministry of Health Vaccination Tracking System and reflected all vaccinations recorded up to each patient's last clinic visit within the study period. For the survey component, a structured telephone questionnaire assessed awareness of infections (hepatitis A, B, C, and D; pneumococcal disease; herpes zoster; influenza; and COVID-19) and related vaccines. Participation was voluntary, and responses were anonymized and kept confidential.

Diagnostic Criteria

Cushing's syndrome was defined as endogenous hypercortisolism confirmed by an endocrinologist based on standard biochemical testing, with at least two abnormal screening tests (overnight 1-mg dexamethasone suppression test, late-night salivary cortisol, and/or 24-hour urinary free cortisol) followed by etiologic work-up, in line with Endocrine Society guidance.[3]

Definitions

"Registry cohort" refers to all eligible patients with Cushing's syndrome whose vaccine uptake was assessed through the national vaccination registry. "Survey subgroup" refers to the reachable, consenting patients who completed the telephone questionnaire that assessed vaccine awareness and self-reported vaccination history.

Inclusion Criteria

For the registry cohort, inclusion required age ≥18 years and a confirmed diagnosis of Cushing's syndrome with follow-up during the study period. For the survey subgroup, additional inclusion criteria required successful telephone contact and consent to participate.

Exclusion Criteria

Patients without a confirmed diagnosis of Cushing's syndrome and those not followed during the study period were not included in the registry cohort. For the survey component, patients who could not be reached by telephone or who declined participation were excluded.

Clinical, Surgical and Laboratory Investigations

Clinical data were obtained from electronic medical records. Diagnostic biochemical testing and etiologic work-up for Cushing's syndrome were performed as part of routine endocrine care, as described above. Vaccination status was assessed using the national vaccination tracking system. Vaccine awareness and self-reported vaccination history were assessed using a structured telephone questionnaire.

Statistical Analysis

All statistical analyses were performed using Jamovi version 2.5.7 (The Jamovi project; AGPL-3.0 license). Categorical variables are presented as numbers and percentages. Normality of continuous variables was assessed using visual inspection (histograms, Q–Q plots) and the Shapiro–Wilk test. As continuous variables were not normally distributed, they were reported as medians (interquartile range [IQR], 25th–75th percentiles). The chi-square or Fisher's exact test was used to compare categorical variables, as appropriate. Statistical significance was set at $p<0.05$. No correction for multiple comparisons was applied; therefore, subgroup comparisons were interpreted as exploratory. No a priori sample-size calculation was performed because this was a retrospective study that included all eligible patients during the study period.

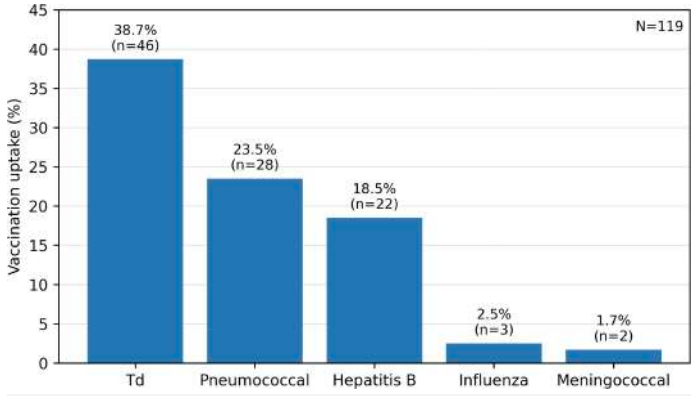

Figure 1. Registry-documented uptake of adult vaccines in the cohort with Cushing's syndrome (n=119). Bars represent the proportion of patients with documentation of each vaccine in the national registry during or prior to the study period.

Td: tetanus–diphtheria; PCV13: 13-valent pneumococcal conjugate vaccine.

RESULTS

A total of 119 patients were included in the study: 99 females (83.2%, n=99) and 20 males (16.8%, n=20). The median age was 51 years (IQR: 39–59.5). The most commonly administered vaccines were tetanus-diphtheria (Td) (38.7%, n=46), pneumococcal (PCV13) (23.5%, n=28), and hepatitis B (18.5%, n=22). In contrast, the uptake of influenza (2.5%, n=3) and meningococcal (1.7%, n=2) vaccines was markedly low (Fig. 1). Of the patients, 43.7% (n=52) had no documented vaccination history other than SARS-CoV-2 vaccination.

When vaccination status was examined by sex, no statistically significant differences were observed in receipt of PCV13, Tdap/Td, influenza, hepatitis B, and meningococcal vaccines (p=0.999, 0.053, 0.999, 0.198, and 0.999, respectively). However, women were more likely than men to have received a Td vaccine (17/41, 41.5% vs 2/78, 2.6%; p=0.004).

According to the national vaccination registry, all patients in the record-based cohort had received at least one dose of a SARS-CoV-2 vaccine. Most participants had received at least one dose of the mRNA vaccine BNT162b2 (n=104, 87.4%), at least one dose of the CoronaVac vaccine (n=99, 83.2%), or both. In total, 84 patients (70.6%) had received both vaccines.

In addition to vaccination records, we conducted a telephone questionnaire to evaluate vaccination knowledge and awareness among a subgroup of 35 patients who voluntarily

Table 1. Hepatitis and vaccine awareness in survey participants, overall and by age group

Variables	All participants (n=35) n (%)	<60 years (n=25) n (%)	≥60 years (n=10) n (%)	p
Hepatitis A infection awareness	23 (65.7)	16 (64.0)	7 (70.0)	0.999
Hepatitis A vaccine awareness	10 (28.6)	8 (32.0)	2 (20.0)	0.686
Hepatitis B infection awareness	28 (80.0)	21 (84.0)	7 (70.0)	0.381
Hepatitis B vaccine awareness	18 (51.4)	13 (52.0)	5 (50.0)	0.999
Hepatitis C awareness	21 (60.0)	15 (71.0)	6 (60.0)	0.999
Pneumococcal vaccine awareness	28 (80.0)	21 (84.0)	7 (70.0)	0.381
Pneumococcal vaccine awareness	28 (80.0)	21 (84.0)	7 (70.0)	0.381

P values are from Fisher's exact test.

agreed to participate. The surveyed subgroup consisted of 30 females (85.7%) and 5 males (14.3%), with a median age of 51 years (IQR: 42–61). The subgroup was predominantly female, reflecting the composition of the overall cohort. The median duration of Cushing's syndrome among surveyed patients was 4 years (IQR: 2–7). 25 participants (71.4%) were under 60 years of age, and 10 (28.6%) were 60 years of age or older.

When asked about their awareness of viral hepatitis infections, 28 participants (80.0%) reported having heard of hepatitis B; 22 (62.9%) reported hepatitis A; 21 (60.0%) reported hepatitis C; and only 6 (17.1%) reported hepatitis D. Awareness of hepatitis D was markedly lower than that of other hepatitis types. In terms of vaccine knowledge, 18 participants (51.4%) reported knowing that a hepatitis B vaccine exists, whereas only 10 (28.6%) were aware of the hepatitis A vaccine. Only 5 participants (14.3%) believed that there was a vaccine for hepatitis C, while 26 (74.3%) stated that they did not know.

Regarding prior serological testing for hepatitis B, 14 participants (40.0%) did not recall whether they had been tested; 9 (25.7%) reported they had never been tested; 8 (22.9%) reported being vaccinated after testing; and 4 participants (11.4%) did not provide an answer. For hepatitis A, 10 participants (28.6%) reported never being tested and 20 (57.1%) participants could not remember whether testing had been performed; 5 participants (14.3%) did not provide an answer. When asked about past childhood infections, 14 participants (40.0%) reported that a family member had told them they had measles; 11 (31.4%) reported rubella, and 17 (48.6%) reported mumps. Varicella (chickenpox) was the most frequently reported infection, with 23 participants (65.7%) stating they had it during childhood. Only 5 participants (14.3%) reported having received the measles, mumps, and rubella (MMR) vaccine; 26 (74.3%) reported not having received it; and 4 (11.4%) could not recall or did not respond.

While 28 participants (80.0%) reported awareness of the pneumococcal vaccine, only 7 (20.0%) stated they had ever received it. The most common reasons for not receiving the pneumococcal vaccine were that participants deemed it unnecessary (21 participants, 60.0%) or were unaware of its existence (6 participants, 17.1%). Influenza vaccination was infrequent: 22 participants (62.9%) reported never having received an influenza vaccine, 4 (11.4%) reported receiving it annually, 4 (11.4%) reported having received a single prior dose, and 5 (14.3%) reported receiving it occasionally. COVID-19 vaccination uptake was high: 32 participants (91.4%)reported more than one dose; one participant (2.9%) reported a single dose; and two participants (5.7%) reported no vaccination. In contrast, the national vaccination registry documented at least one SARS-CoV-2 vaccine dose for all patients in the record-based cohort, indicating disagreement between self-report and registry documentation. For tetanus-diphtheria (Td) vaccination, 14 participants (40.0%) had received one dose, 6 (17.1%) reported receiving regular vaccination, 11 (31.4%) could not remember, and 4 (11.4%) had never received it or were unaware of it.

Awareness was broadly similar across age groups (<60 years, n=25; ≥60 years, n=10). Awareness of hepatitis A was 16/25 (64.0%) in those aged <60 versus 7/10 (70.0%) in those aged ≥60 (p=0.999). The differences in awareness of hepatitis B (21/25, 84.0% vs 7/10, 70.0%; p=0.381) and of the pneumococcal vaccine (21/25, 84.0% vs 7/10, 70.0%; p=0.381) were not statistically significant. Awareness of the hepatitis A vaccine (8/25, 32.0% vs 2/10, 20.0%; p=0.686) and of the hepatitis B vaccine (13/25, 52.0% vs 5/10, 50.0%; p=0.999) was also similar (Table 1).

There were no statistically significant differences between male and female participants in awareness of hepatitis A, hepatitis B, and pneumococcal vaccination (all p>0.05). Participants receiving corticosteroid treatment (n=13) were more likely than those not receiving corticosteroid treatment (n=22) to be aware of hepatitis B but not of hepatitis A or of the hepatitis A

vaccine (p=0.004, 0.999, and 0.999, respectively). Participants diagnosed with Cushing's syndrome for less than 5 years (n=18) showed higher awareness of the hepatitis A vaccine (7/18, 38.9% vs. 3/17, 17.6%) and hepatitis B vaccine (11/18, 61.1% vs. 7/17, 41.2%) than those with a diagnosis ≥5 years. Awareness of hepatitis A infection was also slightly higher in the <5-year group (12/18, 66.7% vs. 10/17, 58.8%) (p>0.05). However, these observed differences were exploratory; therefore, these should be interpreted cautiously given the small sample size.

DISCUSSION

This study combined a registry-based vaccination audit with a pilot awareness survey. The primary finding is that documented adult vaccination coverage was low in a tertiary-care Cushing's syndrome cohort, while awareness remained limited among a voluntary subgroup of engaged patients. The two datasets address complementary but distinct aspects of preventive care implementation. Consistent with previous studies, our results indicate that patients with Cushing's syndrome are susceptible to infectious diseases because excess glucocorticoids exert immunosuppressive effects. Cortisol impairs both innate and adaptive immune responses through mechanisms such as lymphopenia, T-cell suppression, inhibition of pro-inflammatory cytokines, and impaired neutrophil and natural killer cell function.[3–6,17] These alterations predispose patients to a range of infections, including opportunistic pathogens, as shown in earlier studies.[5,7,8,11] Because of the immunological vulnerabilities resulting from this syndrome, ensuring adequate immunization is crucial to prevent infection in this high-risk group.

Our results are in line with those of Te Linde et al.,[2] who analyzed vaccination practices in a large cohort of immunocompromised adults and found substantial gaps in coverage despite high medical need. In their study, only 19% had received the pneumococcal vaccine and only 1% had received the herpes zoster vaccine, despite clear indications. By comparison, our study found pneumococcal vaccine uptake to be 23.5%, while herpes zoster vaccine awareness among Cushing's patients was remarkably low, less than 6% in the survey subgroup, even among those aged 60 or older. These parallel results indicate systemic gaps in adult immunization, even among clinically vulnerable populations. Interestingly, COVID-19 vaccine uptake exceeded 90% in both studies, likely reflecting the effects of strong public health messaging and pandemic-era interventions rather than standard preventive care delivery. This disparity between COVID-19 and uptake of other vaccines suggests that system-wide incentives, accessibility, and policy play critical roles in improving adult vaccination.

The coverage of other routinely recommended adult vaccines was notably low. Although 80.0% of participants were aware of the pneumococcal vaccine, only 23.5% had received it; only 38.7% had received at least one dose of the tetanus-diphtheria vaccine. Influenza vaccination was also underutilized: only 11.4% reported receiving it annually. Notably, 74.3% had never received the MMR vaccine, and awareness of hepatitis A and B was limited. Awareness of herpes zoster vaccines was remarkably low, with over 90% of patients unaware of vaccine availability. Despite recommendations for herpes zoster vaccination in older adults,[12] age-stratified analysis revealed no significant increase in awareness or uptake among participants aged 60 and above.

In our survey subgroup, awareness of hepatitis A, hepatitis B, and pneumococcal vaccination was broadly similar in participants aged <60 and ≥60 years, with no statistically significant differences (all p>0.05) (Table 1). For instance, patients aged <60 years had slightly higher awareness of hepatitis A (68.0% vs. 50.0%) and B (84.0% vs. 70.0%) than those ≥60 years, respectively, although the differences were not statistically significant. Patients on corticosteroids had higher awareness of the pneumococcal vaccine (92.3% vs. 72.7%) and hepatitis B vaccine (61.5% vs. 45.5%) compared with those not on treatment, although the differences were not statistically significant.

Greater awareness of the hepatitis A and B vaccines and the pneumococcal vaccine was observed among younger patients, corticosteroid users, and patients with a shorter duration of Cushing's syndrome. These findings may reflect increased healthcare engagement or education in these subgroups. Having said this, it should be kept in mind that adult vaccine awareness is shaped by multiple factors, including health literacy, clinician recommendation, and perceived susceptibility, and may vary across settings and populations.[12,14] While subgroup comparisons were exploratory and not statistically significant, certain descriptive differences were observed. These findings do not support conclusions regarding determinants of awareness, but may guide future hypothesis-driven research.

Interestingly, longer disease duration (≥5 years) was not associated with improved vaccine knowledge. One might expect that patients with chronic diseases would have more frequent contact with healthcare providers, and thus greater exposure to preventive care counseling. However, our findings align with previous reports that preventive care discussions, including immunization, are often underemphasized in specialty settings such as endocrinology clinics.[8–10]

These results suggest that vaccination is underutilized among patients with Cushing's syndrome. While the high rate of COVID-19 vaccination is encouraging, it appears to be an exception rather than the rule, influenced more by global public

health campaigns than by individualized preventive care.[11] The consistently low uptake of other vaccines highlights the need for more integrated care strategies involving endocrinologists, primary care providers, and infectious disease specialists.

There are several limitations to our study. First, it was conducted at a single tertiary center, and the findings may not be generalizable. Second, only 35 participants completed the telephone survey, and participation was voluntary, raising the possibility of selection bias. Third, we performed several subgroup comparisons without adjustment for multiple testing; therefore, subgroup findings should be regarded as exploratory. Finally, vaccination awareness and self-reported histories are subject to recall bias, as illustrated by discordance between self-reported and registry-derived SARS-CoV-2 vaccination data in a small number of participants. Future multicenter prospective studies with standardized vaccination counseling interventions may help to better define immunization needs and improve vaccination rates in patients with Cushing's syndrome.

CONCLUSION

Patients with Cushing's syndrome are uniquely vulnerable to infections because of cortisol-mediated immunosuppression. Documented adult vaccination coverage in patients with Cushing's syndrome was low in our routine clinical practice. A voluntary subgroup survey demonstrated limited awareness, even among engaged patients. Together, our findings suggest gaps in preventive care implementation, rather than patient-level behavioral resistance.

While COVID-19 vaccination rates were high, likely reflecting national and global pandemic-driven initiatives, the uptake of other routinely recommended vaccines, including pneumococcal, tetanus-diphtheria, hepatitis, and influenza, was substantially lower. Understanding the determinants of high COVID-19 and influenza vaccine uptake could provide valuable insights for enhancing the uptake of other guideline-recommended vaccines in immunocompromised populations.

Endocrinologists should routinely assess immunization status and initiate vaccination discussions during outpatient visits. Collaboration with infectious disease specialists and primary care providers is essential to ensure adherence to adult vaccination guidelines and to close the existing care gaps. Integrating vaccination assessments into electronic health records and specialist workflow may also enhance preventive care delivery. The need for proactive vaccination protocols, comprehensive patient education, and integration of preventive strategies into routine care for patients with Cushing's syndrome is evident. Vaccination should be a cornerstone of infection prevention, reducing morbidity and hospitalizations and improving survival outcomes in this high-risk population.

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From Anti-Neutrophil Cytoplasmic Antibody–Negative to Positive: Eosinophilic Granulomatosis with Polyangiitis Under Benralizumab After Steroid Cessation

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ABSTRACT

Background: Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare systemic vasculitis characterized by asthma, eosinophilia, and small- to medium-vessels inflammation.

Case Report: We present the case of a 28-year-old woman with severe eosinophilic asthma, chronic rhinosinusitis with nasal polyposis, and recurrent pruritic skin lesions. She was diagnosed with EGPA based on the presence of asthma, peripheral eosinophilia, histopathologically confirmed vasculitis, and sinus-related involvement. Long-term corticosteroid therapy resulted in adverse effects, necessitating a transition to mepolizumab and subsequently to benralizumab. Although systemic corticosteroids were successfully discontinued under benralizumab therapy, the patient developed pruritic papular-plaque skin lesions in the eighth month of treatment. Laboratory evaluation revealed new-onset perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) positivity despite normal eosinophil counts.

Conclusion: This case highlights the importance of close ANCA monitoring and increased awareness of skin manifestations in patients treated with benralizumab in whom systemic corticosteroids can be discontinued.

Keywords: Benralizumab, corticosteroid therapy, eosinophilic granulomatosis with polyangiitis, skin involvement.

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INTRODUCTION

Eosinophilic granulomatosis with polyangiitis (EGPA) is a systemic immunological disorder characterized by tissue damage resulting from hypereosinophilia and necrotizing vasculitis affecting small- to medium-sized blood vessels.[1] The hallmark features distinguishing EGPA from other vasculitides are eosinophilia and asthma. Perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) are detected in approximately 30–35% of patients.

Benralizumab, an anti-interleukin-5 receptor (IL-5R) monoclonal antibody, targets type 2 (T2)-driven eosinophilic inflammation. It was initially approved for the treatment of severe eosinophilic asthma and has since gained approval for use in EGPA.[2] In EGPA cases that are not life- or organ-threatening, mepolizumab may be utilized as a maintenance therapy option.

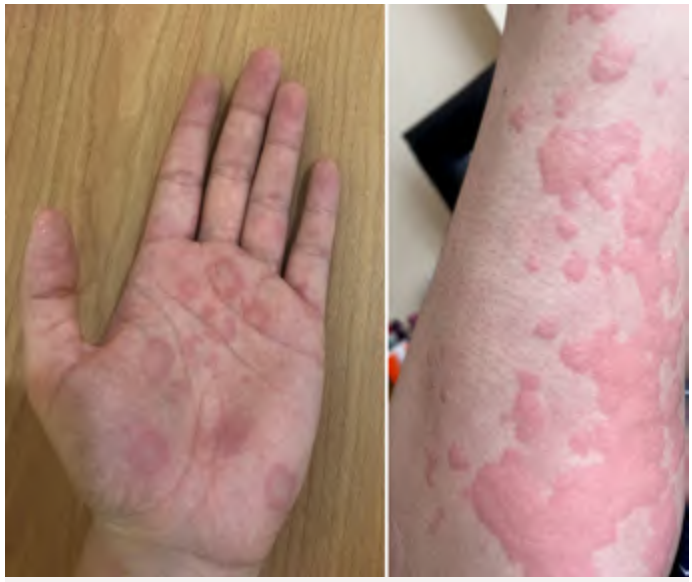

Figure 1. Skin lesions at the time of diagnosis of EGPA.

In this case, due to the development of adverse effects associated with long-term oral corticosteroid use, benralizumab was initiated as maintenance therapy. The patient, who was ANCA-negative at baseline, developed ANCA positivity following corticosteroid discontinuation and initiation of anti-IL-5R therapy. This case highlights the potential importance of monitoring ANCA status in patients with EGPA when transitioning from systemic corticosteroids to anti-IL-5R therapy.

CASE REPORT

A 28-year-old woman was referred to our clinic with a diagnosis of non-atopic severe eosinophilic asthma and chronic rhinosinusitis with nasal polyposis. She had been diagnosed with asthma approximately four years earlier. Despite treatment with high-dose inhaled corticosteroids, long-acting beta-2 agonists (LABAs), and montelukast, her symptoms persisted with minimal clinical improvement. Over the preceding year, she had received systemic corticosteroid therapy on six separate occasions. Although symptom control was achieved during corticosteroid treatment, symptoms recurred following tapering or discontinuation.

The patient also reported recurrent pruritic, erythematous papular lesions on the abdomen; pruritic papules with a pale erythematous center on the palmar surfaces; and erythematous, itchy plaques of varying morphology on the lower extremities. All skin lesions were unresponsive to high-dose antihistamine therapy. A skin biopsy of the lesions was reported as consistent with vasculitis (Fig. 1). Cutaneous involvement in EGPA exhibits a broad clinical spectrum. In this case, the skin lesions were atypical, lacking the classic presentations of palpable purpura or urticaria. Therefore, histopathological confirmation of vasculitis played a crucial role in establishing the diagnosis.

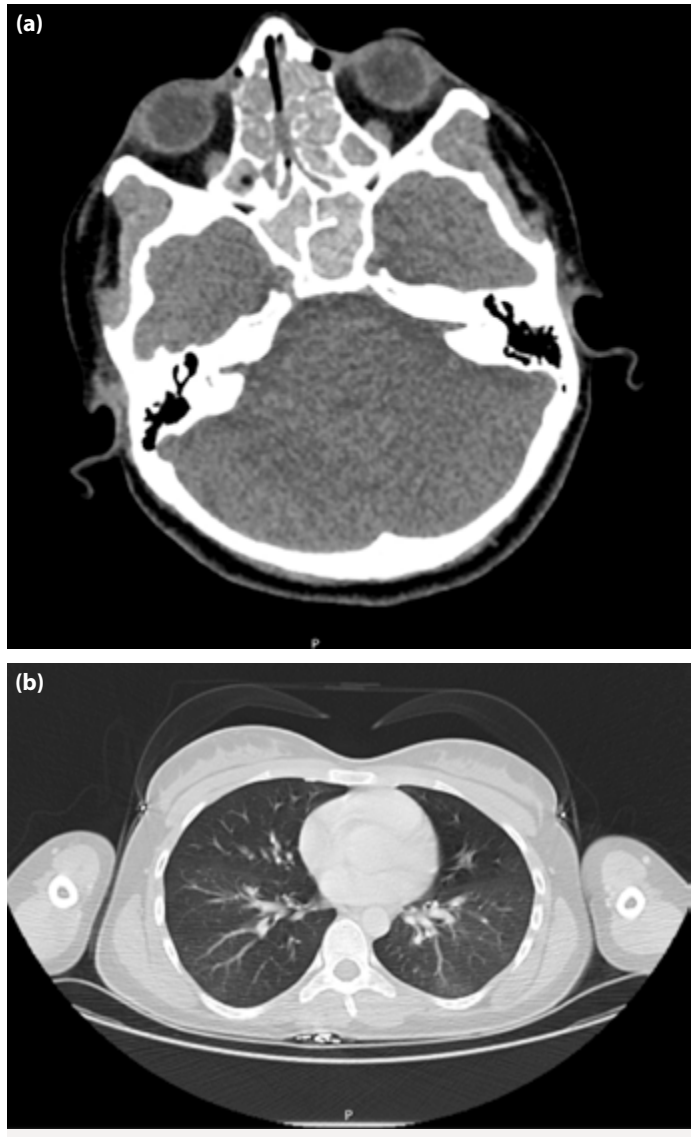

Figure 2. (a) Paranasal sinus CT image of the patient. **(b)** Thoracic CT image of the patient. Şeklinde düzeltilmeli.

On admission, the patient's vital signs were stable. Respiratory examination revealed widespread expiratory wheezing. Nasal endoscopy demonstrated visible bilateral nasal polyps, previously confirmed by otolaryngologic examination and paranasal sinus computed tomography (CT) (Fig. 2a). The patient reported no cardiac symptoms, and physical examination revealed no clinical signs suggestive of cardiac involvement. Electrocardiography and troponin levels were

Table 1. Laboratory findings at the time of diagnosis of eosinophilic granulomatosis with polyangiitis (EGPA)

Test	Result
Erythrocyte sedimentation rate (ESR), mm/s, [normal range: 0-20]	56
C-reactive protein (CRP), mg/dL, [normal range: 0-5]	3.15
Procalcitonin, ng/mL, [normal range: 0-0.5]	<0.02
Hemoglobin, g/dL, [normal range: 12-16]	14.6
Hematocrit, %, [normal range: 37-47]	41
Leukocyte count, 10^9/L, [normal range: 4-10]	10.32
Platelet count, 10^9/L, [normal range: 130-400]	224
Eosinophil count (absolute), /µL, [normal range: 0-200]	1,400
Total serum IgE, IU/mL, [normal range: 0-100]	223
LDH, U/L, [normal range: 135-250]	228
Creatinine, mg/dL, [normal range: 0.50-0.90]	0.66
BUN, mg/dL, [normal range: 6-20]	6.8
Urinalysis	Normal
Autoimmune panel [includes c-ANCA, p-ANCA, anti-dsDNA, ANA panel]	Negative
Parasitological examination (stool, ×3)	Negative

LDH: Lactate dehydrogenase; BUN: Blood urea nitrogen; ANCA: Antineutrophil cytoplasmic antibodies; ANA: Antinuclear antibodies; anti-dsDNA: Anti-double stranded DNA.

normal. As echocardiography was also normal, no further advanced investigations, such as cardiac magnetic resonance imaging (MRI) was performed. No abnormalities were detected on abdominal or other systemic examinations.

Thoracic CT imaging demonstrated peripheral ground-glass opacities (Fig. 2b). Pulmonary function testing revealed a forced vital capacity (FVC) of 5.25 L (104% predicted), a forced expiratory volume in one second (FEV1) of 2.91 L (69% predicted), and an FEV1/FVC ratio of 55%, consistent with an obstructive pattern. Skin prick testing, including testing for Aspergillus species, revealed no sensitizations. Aspergillus-specific immunoglobulin E (IgE) was also negative. Hematology consultation excluded lymphoproliferative malignancy. ANCA testing was performed using indirect immunofluorescence for screening, followed by antigen-specific immunoassays for myeloperoxidase (MPO) and proteinase 3 (PR3); all results were negative. Laboratory values at the time of diagnosis are summarized in Table 1.

The diagnosis of EGPA was established based on the presence of long-standing asthma, peripheral eosinophilia, chronic rhinosinusitis with nasal polyposis, and histopathological evidence of small-vessel vasculitis on skin biopsy. In addition, in patients with histopathologically confirmed small-vessel vasculitis, the disease was shown to meet the 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria for EGPA. Immunosuppressive therapy was initiated with methylprednisolone at a dose of 40 mg/day. The corticosteroid dose was gradually tapered to 6 mg/day; however, attempts to reduce the dose below this level resulted in asthma exacerbations. Maintenance therapy was therefore continued at 6 mg/day. During follow-up, further reduction of the dose to 4 mg/day led to recurrence of both asthma symptoms and cutaneous lesions, necessitating a return to the 6 mg/day maintenance dose.

The patient remained on low-dose corticosteroids for a total of two years. Due to corticosteroid-related adverse effects, including striae and hirsutism, mepolizumab was initiated. During mepolizumab therapy, methylprednisolone was successfully reduced to 4 mg/day without asthma exacerbations or recurrence of skin lesions. However, asthma control deteriorated when the dose was reduced below 4 mg/day. Consequently, maintenance therapy was continued for two years with methylprednisolone 4 mg/day in combination with subcutaneous mepolizumab 150 mg every four weeks. Because of systemic steroid-related adverse effects and the inability to further taper the steroid dose, treatment was switched from mepolizumab to benralizumab. Under benralizumab therapy, systemic steroids were gradually discontinued over four months without loss of asthma control or recurrence of skin lesions. However, in the eighth month of benralizumab treatment, pruritic, erythematous papular¬–plaque skin lesions developed and were unresponsive to

high-dose antihistamines, despite preserved asthma control. Laboratory evaluation demonstrated a normal eosinophil count; however, previously negative myeloperoxidase-ANCA (p-ANCA) results had converted to positive. Repeat testing confirmed persistent p-ANCA positivity.

Given the new onset of ANCA positivity and recurrent skin lesions during benralizumab therapy, methylprednisolone was reintroduced into the treatment regimen. Following the reinitiation of corticosteroids, the skin lesions resolved rapidly.

This case underscores the potential for ANCA seroconversion and disease flare following corticosteroid withdrawal in patients with EGPA undergoing benralizumab therapy. It highlights the importance of close monitoring of ANCA status and cutaneous manifestations in cases in which systemic steroids can be completely discontinued under benralizumab treatment.

DISCUSSION

Anti-neutrophil cytoplasmic antibodies are detected in approximately one-third of patients with EGPA and are associated with a distinct clinical phenotype. ANCA-positive patients more commonly present with manifestations of small-vessel vasculitis, including palpable purpura, glomerulonephritis, and mononeuritis multiplex. In contrast, ANCA-negative patients more frequently exhibit clinical features related to eosinophilic infiltration, particularly involving the respiratory, cardiac, and gastrointestinal systems.[3,4] Although these phenotypic differences between ANCA-positive and ANCA-negative EGPA are well recognized, it remains uncertain whether they influence therapeutic responses to maintenance treatments. The primary goal in the management of EGPA is to induce and maintain remission while minimizing cumulative exposure to systemic glucocorticoids and other immunosuppressive agents. In cases of life- or organ-threatening disease, induction therapy typically consists of systemic glucocorticoids in combination with cyclophosphamide or rituximab. For maintenance therapy, methotrexate, azathioprine, rituximab, or anti-IL-5/IL-5R agents may be used.[2]

In our case, the patient did not exhibit life- or organ-threatening disease; however, corticosteroid-related adverse effects developed during follow-up. Therefore, maintenance therapy was transitioned to mepolizumab and subsequently to benralizumab. While systemic steroids could be completely discontinued under benralizumab treatment, the patient subsequently developed cutaneous lesions, and p-ANCA seroconversion was observed during follow-up. Speculatively, this may suggest that systemic glucocorticoids play a broader role in suppressing the vasculitic component of EGPA beyond their eosinophil-lowering effects, whereas anti-IL-5R therapy alone may be insufficient to control vasculitic manifestations.

In our patient, the emergence of p-ANCA positivity was temporally associated with the appearance of skin lesions on the lower extremities, despite the absence of peripheral eosinophilia. Although firm conclusions cannot be drawn from a single case, we hypothesize that while benralizumab effectively controls eosinophil-mediated disease manifestations, it may have limited efficacy in suppressing ANCA-associated vasculitic processes.

Based on a single observation, it is not possible to establish a causal relationship between benralizumab therapy and ANCA seroconversion, particularly in the context of concurrent systemic glucocorticoid treatment. In this patient, MPO/p-ANCA positivity became evident following glucocorticoid discontinuation, suggesting that steroid withdrawal may have contributed to the unmasking of underlying vasculitic activity. Anti–IL-5 and anti–IL-5 receptor agents markedly reduce peripheral eosinophil counts, which may obscure clinical disease activity. Recent reports have described the development or relapse of EGPA during benralizumab therapy in the absence of eosinophilia. These observations indicate that vasculitic manifestations and ANCA positivity may occur despite apparently controlled eosinophil levels during biologic maintenance therapy, highlighting the importance of careful clinical monitoring.[2,5,6]

Consistent with our observations, a previously published case report also described a vasculitic relapse in an EGPA patient treated with mepolizumab despite persistently normal peripheral eosinophil counts. In that case, clinical improvement was achieved only after the reintroduction of high-dose systemic corticosteroids.[6] However, ANCA seroconversion was not reported.

CONCLUSION

Although similar cases are scarce in the literature, this report describes an EGPA patient with negative baseline ANCA who developed p-ANCA positivity and cutaneous lesions while receiving maintenance therapy with benralizumab. These findings raise important considerations regarding the need for continued ANCA monitoring during anti-IL-5R therapy and highlight potential limitations of such treatments in controlling the vasculitic component of EGPA.

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Written informed consent was obtained from patient who participated in this study.

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Rhabdomyolysis, Acute Kidney Injury, and Exudative Retinal Detachment Associated with Nonsteroidal Anti-Inflammatory Drug and Herbal Product Use: A Case Report

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ABSTRACT

Background: Rhabdomyolysis is a potentially life-threatening condition that may lead to acute kidney injury (AKI). Although nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used, diclofenac-associated rhabdomyolysis is exceedingly rare. Herbal products, often perceived as harmless, may also contribute to nephrotoxicity through contamination or pharmacokinetic interactions.

Case Report: A 20-year-old woman presented with muscle pain, weakness, and dark-colored urine after using diclofenac potassium and a homemade herbal mixture. Laboratory findings revealed severe rhabdomyolysis and AKI. Kidney biopsy confirmed myoglobin-induced acute tubular injury. During treatment, the patient developed hypertension and blurred vision; ophthalmologic evaluation revealed exudative retinal detachment. Hemodialysis, ultrafiltration, and blood pressure control resulted in complete renal and visual recovery.

Conclusion: This case highlights the unpredictable toxicity that may arise from the combined use of NSAIDs and herbal products. Clinicians should routinely inquire about herbal supplement use, particularly in patients presenting with unexplained rhabdomyolysis or AKI.

Keywords: Acute kidney injury, herbal product, nonsteroidal anti-inflammatory drug, retinal detachment, rhabdomyolysis.

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INTRODUCTION

Rhabdomyolysis is a clinical syndrome resulting from the breakdown of skeletal muscle cells and may lead to severe systemic complications. Acute kidney injury (AKI) is one of the most serious complications of rhabdomyolysis, with a reported incidence ranging from 10% to 55%. Patients typically present with myalgia, dark-colored urine, muscle weakness, and markedly elevated creatine kinase (CK) levels. In adults, rhabdomyolysis is most commonly associated with trauma, infections, and toxic exposures. The pathophysiology of kidney injury involves renal vasoconstriction, hypovolemia, myoglobin-mediated toxicity, and tubular obstruction. Although nonsteroidal anti-inflammatory drugs (NSAIDs) have a broad adverse-effect profile,

rhabdomyolysis is an exceptionally rare complication, with only a limited number of cases reported. While the precise role of NSAIDs in the development of rhabdomyolysis remains unclear, mitochondrial dysfunction, increased oxidative stress, and impaired stabilization of the myocyte membrane have been proposed as plausible mechanisms. The primary therapeutic goals include elimination of the precipitating trigger, prompt and adequate volume replacement, correction of electrolyte and acid–base disturbances, and initiation of renal replacement therapy when indicated.[1] Herbal products are widely used worldwide and are often consumed without physician awareness because they are perceived as "natural." However, nephrotoxicity may occur due to uncertain composition, contamination, improper processing, and pharmacodynamic or pharmacokinetic interactions.[2] In addition, herbal products may alter drug metabolism and renal elimination via cytochrome P450 enzymes, P-glycoprotein, and organic anion transporters (OATs), potentially increasing the risk of unexpected toxicities. Therefore, herbal product use should be routinely queried in cases of unexplained rhabdomyolysis or AKI. Rhabdomyolysis has been reported following the use of various herbal supplements.[3] Hypertension and hypervolemia may develop during the course of AKI and can affect the choroidal circulation, disrupting the retinal pigment epithelium barrier and leading to exudative retinal detachment. This ocular manifestation often resolves rapidly with appropriate blood pressure and volume control.[4] Exudative retinal detachment may also occur secondary to multiple etiologies beyond malignant hypertension, including inflammatory, infectious, autoimmune, neoplastic, and drug-related causes.[5] Nevertheless, in cases related to hypertension and volume overload, prompt clinical improvement following blood pressure and volume optimization is characteristic.

Herein, we report a distinctive case that contributes to the literature by describing the concurrent occurrence of rhabdomyolysis, AKI, and hypertension-associated exudative retinal detachment following the concomitant use of NSAIDs and unregulated herbal products.

CASE REPORT

A 20-year-old woman presented with fatigue, diffuse myalgia, restricted mobility, and dark-colored urine. Following an upper respiratory tract infection, she had taken a total of 1,000 mg of diclofenac potassium over seven days and had concurrently consumed a homemade herbal mixture ("Atom Tea") containing ginger, hibiscus, turmeric, allspice, clove, cinnamon, and galangal. Physical examination revealed tachycardia, oliguria, +2 pretibial edema, and marked muscle weakness. Laboratory testing demonstrated

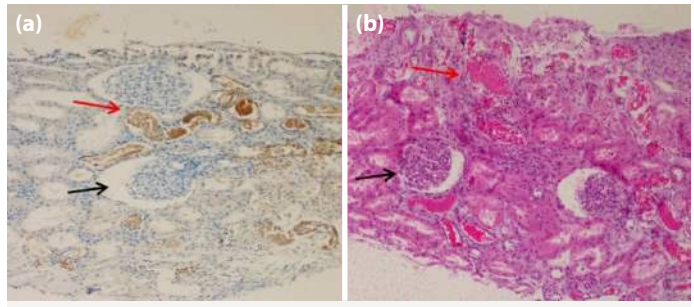

Figure 1. (a) Kidney core needle biopsy showing myoglobin-positive cast material within the tubular lumens (red arrows). Black arrows indicate glomeruli with preserved histological architecture (×200, immunoperoxidase). **(b)** Kidney core needle biopsy demonstrating tubular cast material (red arrows) with glomeruli within normal histological limits (black arrows) (×200, hematoxylin and eosin staining).

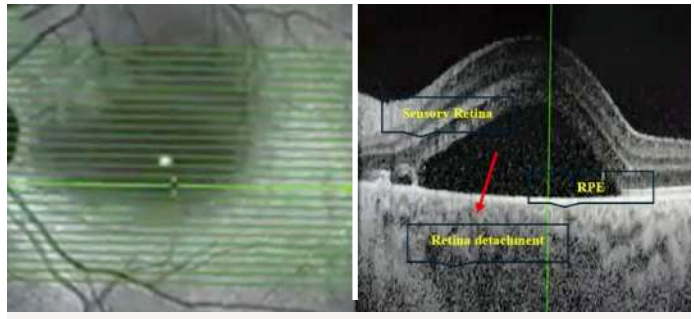

Figure 2. Optical coherence tomography (OCT) scan showing a cross-sectional view of the retina. The central dome-shaped elevation indicates retinal detachment. Retinal layers are clearly visualized; hyperreflective bands represent structural layers, while hyporeflective areas correspond to fluid accumulation. The green line marks the scanning axis used to obtain this cross-sectional image.

elevated renal function parameters and muscle enzyme levels (Table 1). Urine microscopy showed granular casts. Renal ultrasonography revealed bilateral grade 2 increased echogenicity. Kidney biopsy demonstrated pigmented tubular casts, interstitial edema, and mild mononuclear infiltration; myoglobin positivity on immunohistochemistry confirmed acute tubular injury (Fig. 1).

Isotonic fluid therapy was initiated at 200 mL/kg/day. Although early fluid resuscitation is recommended in rhabdomyolysis, the patient was closely monitored clinically and hemodynamically, as aggressive hydration in the setting of oliguria may increase the risk of hypervolemia and hypertension. Hemodialysis was initiated following the development of hyperkalemia,

Table 1. Laboratory testing

Laboratory parameter	Day 1	Day 7	Day 14	Day 21	Post-discharge follow-up
BUN, mg/dL	61.1	44.1	30.8	9.0	7.0
Creatinine, mg/dL	3.98	5.74	4.9	1.5	1.14
eGFR, mL/min	15.3	9.83	11.9	47	69.3
Sodium, mEq/L	133	136	140	145	141
Potassium, mEq/L	6.33	3.54	3.65	3.95	3.71
Calcium, mEq/L	5.62	10.2	7.98	7.79	7.9
Magnesium, mEq/L	1.33	0.86	0.69	0.41	0.7
Creatinine kinase, IU/L	38899	7600	856	56	25
Lactate dehydrogenase, u/L	5457	1641	955	692	559
Aspartate transaminase, u/L	6760	353	67	28	27
Alanine transaminase, u/L	1878	650	177	62	38
Proteinuria, g	6.3	1.1	0.3	0.02	0.01
Complete urinalysis	Granular casts				
Urine, blood, and stool cultures	Negative				
C3/C4 levels	Normal				
Anti-PLA2R	Normal				
Albumin, g/dL	3.6	3.01	4.4	3.72	3.9
White blood cell count	21.2	7.8	9.8	8.7	7.6
Hemoglobin	13.2	9.8	10.2	12.6	12.5
Platelet count	187	137	142	157	167
Free kappa/lambda ratio	2.1				
Hemoglobin A1c	5.4				
ANA, anti-dsDNA, ANCA Anti-PLA2R	Normal				
Hepatitis A, B, C, and HIV	Negative				
IgG, IgA, IgM, and IgG4 levels	Normal				
Blood gas (pH/HCO_3^-)	7.30/17	7.40/21	7.38/21	7.40/23	7.39/23
FENa	3%				
C-reactive protein	62	17	21	4	2
Procalcitonin	3.59	0.4	0.1	0.05	0.01

BUN: Blood urea nitrogen; eGFR: Estimated glomerular filtration rate; C3/C4: Complement components 3 and 4; Anti-PLA2R: Anti–phospholipase A2 receptor antibody; FENa: Fractional excretion of sodium; ANA: Antinuclear antibody; anti-dsDNA: Anti–double-stranded DNA antibody; ANCA: Antineutrophil cytoplasmic antibody.

metabolic acidosis, and volume overload. The primary and urgent indication for hemodialysis was life-threatening hyperkalemia, and ultrafiltration was additionally employed to achieve volume control and blood pressure regulation.

During follow-up, the patient developed hypertension and blurred vision. Ophthalmologic evaluation revealed macular edema and exudative retinal detachment (Fig. 2). These retinal findings were interpreted as objective manifestations of end-organ damage secondary to volume overload and uncontrolled hypertension. Hypervolemia and hypertension were managed with antihypertensive therapy and ultrafiltration. On day 16, AKI entered a polyuric phase, and renal function improved rapidly (Fig. 3). By day 19, the retinal findings had completely resolved, and visual acuity returned to baseline. Laboratory parameters also normalized.

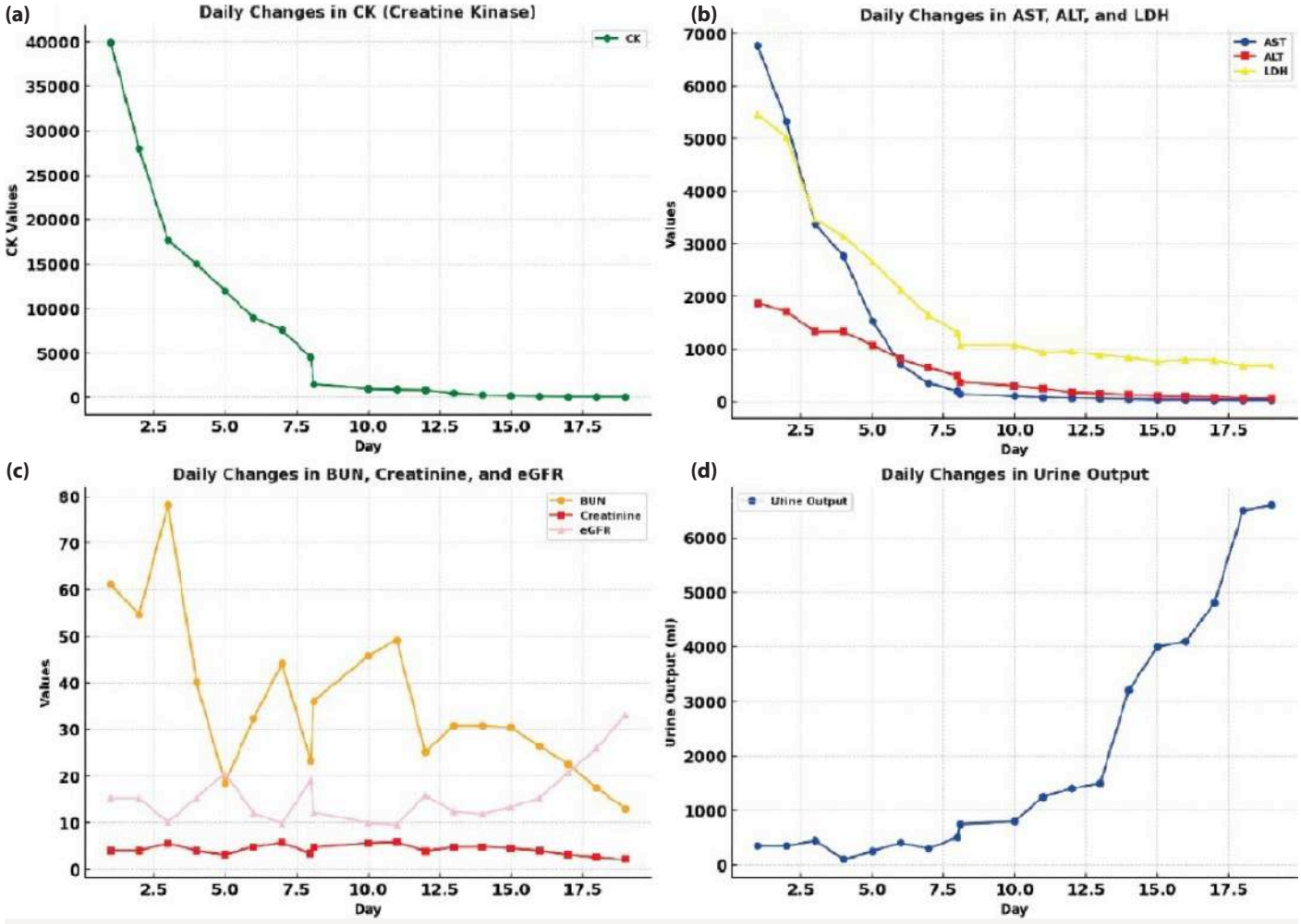

Figure 3. (a) The green arrow shows the day-to-day trend of creatine kinase (CK). **(b)** The blue arrow indicates the trend in aspartate aminotransferase (AST), the red arrow alanine aminotransferase (ALT), and the yellow arrow lactate dehydrogenase (LDH) over time. **(c)** The orange arrow represents daily changes in blood urea nitrogen (BUN), the red arrow creatinine, and the light yellow arrow estimated glomerular filtration rate (eGFR). **(d)** The blue arrow shows changes in urine output over the course of hospitalization.

DISCUSSION

This case represents one of the rare examples in the literature in which diclofenac use and unregulated herbal product consumption were temporally associated with rhabdomyolysis and AKI, accompanied by hypertension-related exudative retinal detachment. AKI develops in approximately 10%–55% of patients with rhabdomyolysis, and 5%–15% may require renal replacement therapy. Although diclofenac-associated rhabdomyolysis is rare, only a limited number of cases have been reported, including a pediatric case. Moreover, analyses of the World Health Organization (WHO) pharmacovigilance database have identified reports of diclofenac-associated rhabdomyolysis, with symptom improvement noted after drug discontinuation in some cases.[6–8] In our patient, although causality cannot be definitively established, the relatively high cumulative dose administered over a short period is noteworthy.

Although several components of the herbal mixture have been suggested to possess nephroprotective properties in experimental settings, herbal products may pose significant clinical risks due to contamination, pesticide residues, heavy metals, or unknown toxic substances. In addition, herbal preparations are known to modulate CYP450 enzymes, P-glycoprotein, and organic anion transporters, potentially affecting diclofenac metabolism and increasing systemic exposure. Such interactions may have amplified muscle and renal toxicity in this case.[9]

The exudative retinal detachment observed in conjunction with rhabdomyolysis and AKI was consistent with a hypertensive crisis and volume overload. Hypertensive retinopathy and serous retinal detachment associated with malignant hypertension have been previously described.[10] In our patient, the bilateral involvement, temporal association with hypertension, and rapid resolution following ultrafiltration strongly support a pathophysiological mechanism driven by volume overload and uncontrolled blood pressure.

Upper respiratory tract infections may precipitate rhabdomyolysis through viral myositis or systemic inflammatory responses. In this case, viral serologies and extended myositis panels were not evaluated, which should be acknowledged as a limitation.

CONCLUSION

This case highlights that herbal products are not inherently safe and that their concomitant use with nonsteroidal anti-inflammatory drugs may precipitate severe and unpredictable toxicities, including rhabdomyolysis and acute kidney injury. A structured assessment of herbal product exposure should therefore be routinely incorporated into the evaluation of unexplained rhabdomyolysis and acute kidney injury. Despite the absence of toxicological, pharmacogenetic, and pharmacokinetic analyses, this report underscores a critical and often overlooked source of preventable drug-related harm.

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Informed Consent: Written informed consent was obtained from the patient.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Author Contributions: Concept – SD, AD; Design – SD, AD; Supervision – HA, BT; Resource – HA, BT; Materials – SD; Data Collection and/or Processing – SD, AD; Analysis and/or Interpretation – SD, AD; Literature Review – AD; Writing – SD, AD; Critical Review – HA, BT.

Peer-review: Externally peer-reviewed.

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Comment on "Prognostic Value of Modified Glasgow Prognostic Score in Acute Decompensated Heart Failure with Reduced Ejection Fraction"

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Dear Editor,

We read with interest the article by Tunca et al.,[1] titled "Prognostic Value of Modified Glasgow Prognostic Score (mGPS) in Acute Decompensated Heart Failure with Reduced Ejection Fraction." The study offers valuable insights into the prognostic utility of the mGPS in a selected population of patients with heart failure with reduced ejection fraction. We would like to respectfully raise two points regarding the statistical methodology that could help readers better interpret the results.

The first point concerns the potential for interval-censored events in the survival data. The authors state that post-discharge outcomes were assessed via "follow-up telephone interviews." If these interviews did not ascertain the exact date of death, the event time would be known only to fall within the interval between the last known point of contact (e.g., hospital discharge) and the date of the interview. The standard Cox proportional hazards (Cox PH) model has theoretical limitations when applied to interval-censored data, as it is primarily designed for right-censored data in which event times are known precisely. Analyzing such data with a Cox PH model, for instance, by imputing a single event time, can introduce bias into the risk estimates. In contrast, statistical models specifically designed to accommodate interval censoring, such as certain formulations of accelerated failure time (AFT) models, adjust their likelihood function to handle this type of data appropriately.[2,3] A discussion of how post-discharge event times were handled, and potentially a sensitivity analysis using an AFT model, could provide valuable evidence to support the robustness of the reported hazard ratios.

Furthermore, for a precise understanding of temporal risk dynamics, the proportional hazards assumption underlying the chosen Cox model requires careful scrutiny. It is unclear whether this condition of time-invariant hazard ratios was formally tested, for example, through analysis of Schoenfeld residuals. If this assumption is violated, the reported risk estimates may represent a potentially misleading average over the follow-up period rather than capturing the changing nature of risk. Should non-proportionality be detected, other models—including Cox models with time-dependent effects, stratified Cox models, or accelerated failure time models—would offer a more nuanced characterization of mortality risk.[4,5]

We believe that clarification of these two methodological aspects would further strengthen the study and aid in the accurate interpretation of its important findings.

Conflict of Interest: The authors have no conflicts of interest to declare.

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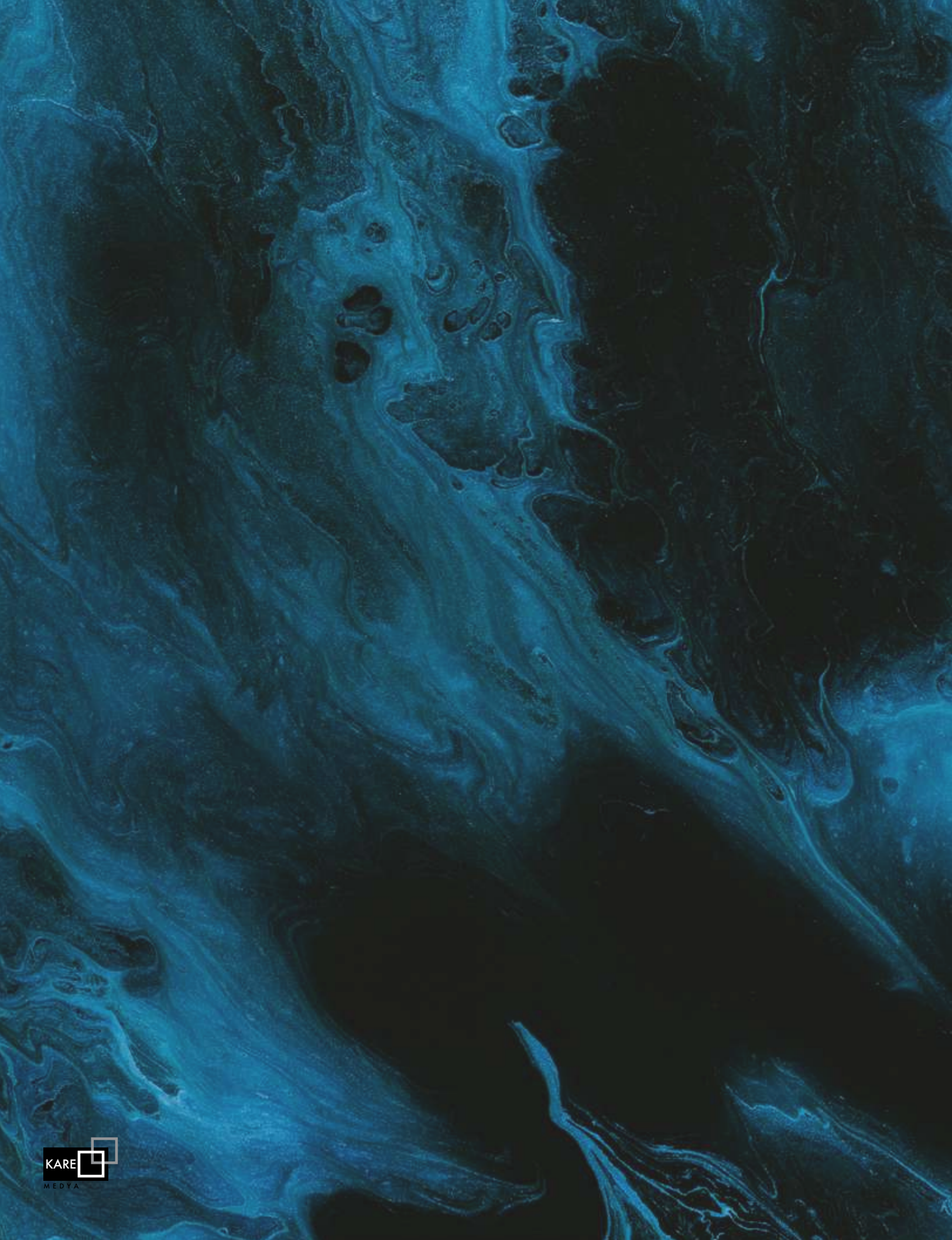
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