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İç Kapı No: 105, Keçiören/Ankara, Turkiye
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EDITORIAL

Dear Readers,

As a part of Medihealth Academy, we are pleased to announce the publication of the first issue of the bi-monthly Journal of Medicine and Palliative Care (JOMPAC) in 2025. We are in our sixth year this year.

We would like to thank all of the authors, researchers, reviewers, and editorial board members who helped with this publication. We would also want to thank the publishing staff for their diligent efforts in preparing the journal for publication.

Ethics and scientificity are always prioritized in the blind evaluation procedures of articles submitted to our publication. Our primary goals are to uphold the journal's principles and establish an alternative path for researchers for the resarchers to contribute to the literature in the fields of General Medicine and Palliative Care. Being included in international indexes like SCI-Expanded, Scopus, ESCI, and Pubmed is another objective we have for JOMPAC. This 2025 first launch issue includes review and original researchs. Journals are created and made popular by their authors and readers. We would like to thank everyone who helps with the publishing in any form.

Best regards,

Assoc. Prof. Deniz ÇELİK, MD
Editor in Chief

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Original Article

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The digital dilemma of Haglund deformity: assessing online information's reliability and readability-a cross-sectional study

Mehmet Okan Atahan[1], Alper Özer[2], Aydoğan Aşkın[3], Furkan Soy[4], Göker Yurdakul[5], Mehmet Fatih Uzun[6]

[1]*Department of Orthopedics and Traumatology, Afyonkarahisar State Hospital, Afyonkarahisar, Turkiye*
[2]*Department of Orthopedics and Traumatology, Kayseri City Hospital, Kayseri, Turkiye*
[3]*Department of Orthopedics and Traumatology, Antalya Training and Research Hospital, Antalya, Turkiye*
[4]*Department of Orthopedics and Traumatology, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkiye*
[5]*Department of Orthopedics and Traumatology, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkiye*
[6]*Department of Orthopedics and Traumatology, Ceylanpınar State Hospital, Şanlıurfa, Turkiye*

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ABSTRACT

Aims: This study aimed to evaluate the quality, reliability, and readability of online information on Haglund deformity.

Methods: The three most popular browsers were selected, and two reviewers categorized the websites by type. The quality of each site was assessed based on its adherence to the HONcode and evaluated using scoring instruments like the DISCERN, JAMA benchmark, and GQS. The Flesch-Kincaid grade level (FKGL) score was utilized to evaluate the readability of the websites.

Results: Academic webpages exhibited markedly superior ratings in DISCERN, JAMA, GQS, and HCS compared to other subcategories ($p<0.05$). Websites with a HON code also demonstrated higher scores across most metrics, except for FKGL and FKRS. However, readability scores indicated that much of the content was above the recommended comprehension level for the general public. A strong positive correlation was observed between DISCERN and JAMA scores ($r=0.935$; $p<0.05$), while a negative correlation was noted between FKRS and HCS scores ($r=-0.723$; $p<0.05$).

Conclusion: The study highlights significant variability in the quality and accessibility of online information on Haglund deformity. While academic sources offer higher-quality information, their complexity may limit public understanding. These findings emphasize the need for accessible, high-quality online resources to enhance patient education and support informed decision-making.

Keywords: Haglund, internet, online information, search engine

INTRODUCTION

The internet serves as a prominent source of medical information.[1] The rising prevalence of self-educated individuals is establishing a novel dynamic for physicians, as patients are now more informed about their conditions than ever before. Self-educated patients may enhance patient management by improving their ability to critically evaluate treatment options and fostering realistic expectations regarding treatment outcomes.[2,3] However, the quality of data available on the Internet remains inconsistent and lacks regulation. The absence of regulation for search engines may result in websites via commercial or financial biases misleading patients.[4,5]

Haglund's deformity refers to an unusual bony enlargement located at the posterosuperior part of the calcaneus, first identified by Patrick Haglund in 1927.[6] Recurrent irritation of the retrocalcaneal bursa among the tendon of the Achilles and the calcaneal prominence may contribute to retrocalcaneal bursitis, a notable contributor to posterior heel pain.[7,8] Patients with this condition commonly report pain localised to the retrocalcaneal area.[9]

In addition to providing reliable information, websites should be easy to read for their intendedusers. The National Institutes of Health (NIH) recommends that patient education content be composed at a reading level of seventh grade or lower.[10] Previous studies have shown that a considerable percentage of health-related websites exceed the recommended readability level,suggesting that an important percentage of the patient population may struggle to understand the information presented on these sites.[11,12] The current study aimed to examine the reliability,readability, and accuracy of the data accessible on the Internet regarding Haglund's deformity. As far as we are aware, no research has been published that

Corresponding Author: Mehmet Okan Atahan, mehmetokanatahan@gmail.com

assesses the performance of internet-based resources for Haglund's deformity. This study sought to address the research gapby assessing the content, quality, and readability of online information regarding Haglund's deformity.

METHODS

As this article does not contain any studies with human participants or animals by any of the authors, no ethics committee report is required. The three most popular search engines, Google, Yahoo!, and Bing, were employed when browsing the Internet using the term 'Haglund's deformity.' Google is the leading search engine worldwide, followed by Bing and Yahoo![13] The scans were conducted on November 1, 2024, after deleting all cookies associated with the search engines before the scanning. After removing any websites that were either duplicates or had paywalls, a final count of 60 websites remained. Assigning the kinds of scanned websites was the initial step in the examination. Wesorted the websites into four categories: academic, medical, commercial, and professional physician.

Each website underwent a thorough evaluation using a comprehensive set of assessment tools designed to measure quality, readability, and credibility. The tools applied included the DISCERN instrument for evaluating reliability, the Flesch-Kincaid grade level (FKGL) to assess readability and comprehension level, and the global quality score (GQS) for overall content quality. Additionally, the JAMA benchmark was used to gauge adherence to established health information standards, and the Haglund-specific content (HSC) score provided a specialized measure tailored to Haglund-related content (Table 1). The evaluation also included verification of health on the net (HON) certification to confirm compliance withethical standards in health information dissemination.

The DISCERN tool is widely acknowledged as an effective and reliable method for assessingthe quality and trustworthiness of health information found online, helping users identify credible sources. This assessment framework comprises 16 distinct questions, each contributing one point to the final score. Websites can therefore earn up to a maximum score of 80, providing a clear measure of the quality and credibility of health content offered to the public.[14]

The JAMA benchmark criteria evaluate websites based on four primary factors: authorship, attribution, disclosure, and currency.[15] Each factor is scored with one point, for a total possiblescore of four points in this assessment. Additionally, each website's quality is rated using the GQS on a 5-point scale, which assesses the informational value and potential benefits providedto the patient.[16]

To evaluate the readability of each website, two widely recognized tools were employed: the FKGL, which estimates the educational grade level required to understand the text, and the Flesch reading ease score (FKRS), which provides a readability rating based on sentence structure and word complexity. The FKRS evaluates the understandability of a subject, with scores from 0 to 100. The FKRS score runs from 0 to 100,with a lower number signifying a more challenging reading passage. The text from each page was extracted without accompanying figures or table legends and input into an open-access readability calculator.[17,18]

Table 1. Haglund content score (HCS)

Haglund content score	Score
Retrocalcaneal bursitis	1
Achilles tendon	1
Calcaneal prominence	1
Heel pain	1
Physical therapy	1
Surgical treatment	1
Orthotic devices	1
Radiography	1
MRI	1
NSAIDs	1
Stretching exercises	1
Footwear modification	1
Ultrasound	1
Corticosteroid injection	1
Heel padding	1
Pain management	1
Bone spur removal	1
Endoscopic surgery	1
Haglund syndrome	1
Calcaneal osteotomy	1
Foot mechanics	1
Posterior heel pain	1
Inflammation	1
Conservative treatment	1
Shock wave therapy	1
Calcaneal spur	1
Custom orthotics	1
Cold therapy	1
Topical analgesics	1
Tendon repair surgery	1

We evaluated each website for adherence with the health on the net code (HON code), a benchmark created by the HON foundation to uphold the quality and integrity of online health information. Recognized as a leading standard, the HON code is instrumental in verifying thatdigital health resources meet essential criteria for credibility, transparency, and ethical presentation.[19]

In addition, we have created our own grading system that takes into account the accuracy of the material offered by the websites (Table 1). In the HCS scoring, 30 phrases or themes received one point if they were included on the webpage. The HCS evaluation was performed by the two authors of this study. Websites with varying rankings were reassessed until an agreement was achieved.

Statistical Analysis

The statistical analysis of the study was carried out using IBM SPSS Statistics 22 software (SPSS IBM, Turkey). The normality of variable distributions was checked through the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics, including mean, standard deviation, median, and frequency, were utilized. The Kruskal-Wallis test was applied

to compare different categories, and Dunn's test identified the group causing significant differences. The Mann-Whitney U test evaluated scores based on HON specifications while correlations between scores were analyzed with Spearman's rho. The intraclass correlation coefficient (ICC) provided lower and upper bounds to assess interobserver agreement, with a significance level set at p<0.05.

RESULTS

The 60 websites were categorized according to the type of resources they offered. The distribution was as follows: 15 academic websites (25%), 12 physician websites (20%), 25 medical websites (41%), and 8 commercial websites (14%) (Figure 1). Table 2 presents the overall scores across all assessment tools, while Table 3 details each website's results for DISCERN, JAMA, GQS, FKGL, FKRS, and HCS.

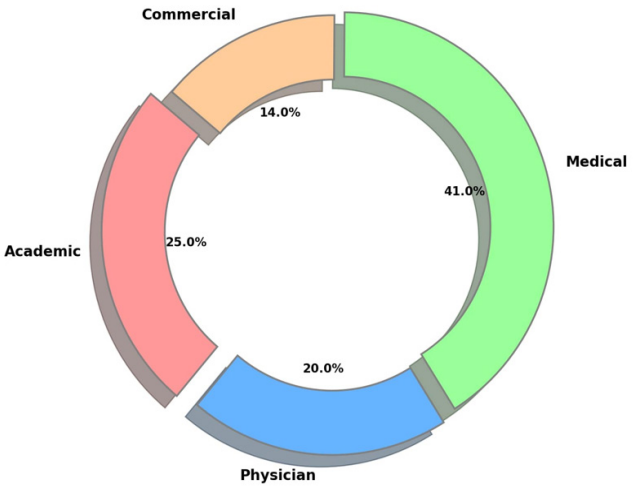

Figure 1. Website distribution based on sources

Table 2. Range, mean, and standard deviation values of the assessment tools

Tool	Range	Mean±SD
DISCERN reviewer A	18.2-63	35.15 ± 11.95
DISCERN reviewer B	18.2-63	37.05±13.05
DISCERN score	19- 63.5	36.10±12.75
JAMA reviewer A	1 - 4.5	2.05±1.01
JAMA reviewer B	1-4.5	2.30±1.12
JAMA score	1-4.5	2.15±1.07
GQS reviewer A	1-4.5	2.22±1.12
GQS reviewer B	1-5	2.40±1.20
GQS score	1-4.6	2.30±1.15
FKGL	4.0-12.5	9.35±2.15
FKRS	7.2-81.0	47.10±19.25
HCS	6-30	20.15±8.05

SD: Standart deviation, GQS: Global quality score, FKGL: Flesch-Kincaid grade level, FKRS: Flesch reading ease score, HCS: Haglund content score

The study's results revealed that academic websites achieved higher average scores across various assessment criteria compared to physician, medical, and commercial sites. Detailed analysis demonstrated that, within the academic category, scores on metrics such as DISCERN, JAMA, GQS, FKGL, and HCS were notably higher than those in physician, medical, and commercial categories (p<0.05). Conversely, academic websites had a significantly lower FKRS score compared to the other groups (p<0.05) (Table 3).

The scores on DISCERN and JAMA were found to have a noteworthy positive correlation of 0.939, which reflects the strong association that exists between the two metrics (p<0.05). There was a statistically significant positive correlation of 0.621 between DISCERN and FKGL scores and an even larger connection of 0.928 between DISCERN and HCS scores (Figure 2). Both of these correlations were statistically significant (p<0.05). However, the FKRS and HCS scoreswere found to have a statistically significant negative correlation of 0.752 (p=0.000; p<0.05) (Figure 3). The histogram (Figure 4) illustrates the variation in readability levels, with FKGL scores clustering around a mean of approximately 9, indicating a readability level above the recommended sixth-grade standard. The FKRS scores show a broader range, with values spread widely, highlighting the significant variation in content complexity across websites. This variation suggests that many sites may not be accessible for readers with lower health literacy, potentially impacting patient understanding and engagement.

Only 26.8% of the websites displayed a HON code. Notably, websites bearing the HON code had significantly different assessment scores in DISCERN, JAMA, GQS, and HCS compared to those without the code, with these differences being statistically significant (p<0.05). Nonetheless, Table 4 shows that neither group's FKGL nor FKRS scores changed significantly (p>0.05).

DISCUSSION

A quick, effective, and mostly unrecognised way to obtain medical information is the internet. Nevertheless, accessing in-depth information can be difficult. Patients frequently rely on commercial websites for guidance, often judging a site's reliability more on its visual design than on the credibility of its information source.[18]

This study's findings, derived from established assessment tools, indicate that websites commonly available to those researching Haglund deformity typically exhibit a low quality of information. The findings align with prior orthopaedic research regarding information quality.[20,21] Individuals seeking information on Haglund deformity can access a variety of sources, including online journals, anecdotal personal accounts, and commercial websites.

In the current study, the academic group outperformed the other groups on DISCERN, JAMA, GQS, FKGL, and HCS. Our results align with previous research, showing that information from academic sources was the most relevant and of the highest quality. The websites in this study yielded an average DISCERN score of 36.10±12.75, reinforcing these findings. These findings corroborate those of previous studies that showed the low quality of data accessible online.[24,25] On the contrary, some studies found no correlation between groups and quality ratings.[17] These findings show that academic research and other online materials may vary in quality and substance.

Table 3. Evaluation of scores by category and correlation analysis

Category	DISCERN score (Mean±SD)	JAMA Score (Mean±SD)	GQS Score (Mean±SD)	FKGL (Mean±SD)	FKRS (Mean±SD)	HCS (Mean±SD)
Academic	52.25±6.65	3.54±0.48	3.64±0.52	11.34±0.44	22.84±7.34	27.01±2.02
Physician	37.29±9.48	2.63±0.45	2.63±0.90	9.22±1.97	47.66±17.79	20.45±4.78
Medical	30.67±8.56	1.59±0.58	1.79±0.87	8.36±1.85	53.57±13.54	16.95±6.59
Commercial	21.76±7.43	1.09±0.26	1.35±0.35	6.45±1.04	60.70±12.47	10.56±4.82
p1	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***
Correlation pair		**Correlation (r)**	**p-value (p2)**			
DISCERN & JAMA		0.939	0.000*			
DISCERN & GQS		0.928	0.000*			
DISCERN & FKGL		0.621	0.000*			
DISCERN & FKRS		-0.674	0.000*			
DISCERN & HCS		0.928	0.000*			
JAMA & GQS		0.965	0.000*			
FKGL & FKRS		-0.958	0.000*			
HCS & FKRS		-0.752	0.000*			

*1Kruskal-Wallis Test (p1), 2Spearman Rho Correlation Analysis (p2), p<0.05
SD: Standart deviation, GQS: Global quality score, FKGL: Flesch-Kincaid grade level, FKRS: Flesch reading ease score, HCS: Haglund content score

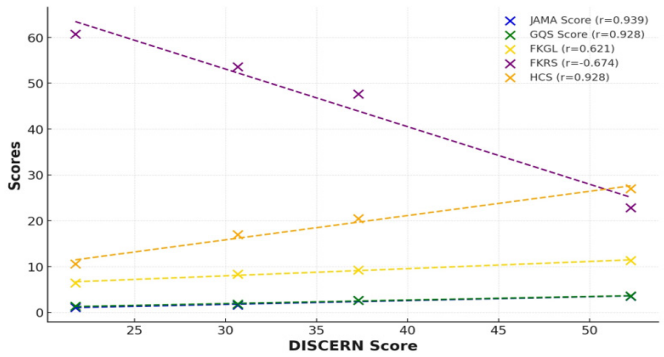

Figure 2. The correlation between DISCERN and other assessments

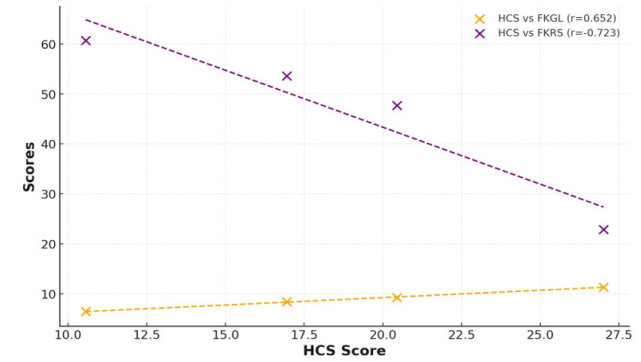

Figure 3. Correlation between TCS scores, FKGL scores, and FCRS scores
FKGL: Flesch-Kincaid grade level, FKRS: Flesch reading ease score, HCS: Haglund content score

The average JAMA benchmark result has been 2.15±1.07 on a scale of 4, comparable to findings in prior studies.[26] The low JAMA scores may be due to the absence of references or resources on most websites. The results of the current study revealed a significant positive association between the DISCERN scores and the JAMA benchmark values, with the statistical significance level reaching p<0.05. This could be because the JAMA benchmark parameter evaluation uses the DISCERN scale's two items—the publication date and the availability of references—to establish the ultimate score.

The average FKGL assessment was 9.35±2.15 and the average FCRS evaluation was 47.10±19.25, according to the present study. After comparing the FKGL score to the sixth-grade level of reading recommended by the NIH[27], the data shows that it is around 3.5 points higher. The insufficient readability and quality of online information have been extensively discussed in medical literature, particularly within the field of orthopaedics. While 38 studies were reviewed by Cassidy and Baker[28] in 2016 for readability, only 2-5% of the sites included in these studies were rated as having a reading level below sixth grade. A comparable study investigating online materials regarding ankle arthrodesis identified merely 7 out of 98 results (7.1%) as being at an appropriate reading level.[29] The FCRS score derived from this study indicates thatthe data found on the internet was "difficult to read," suggesting that

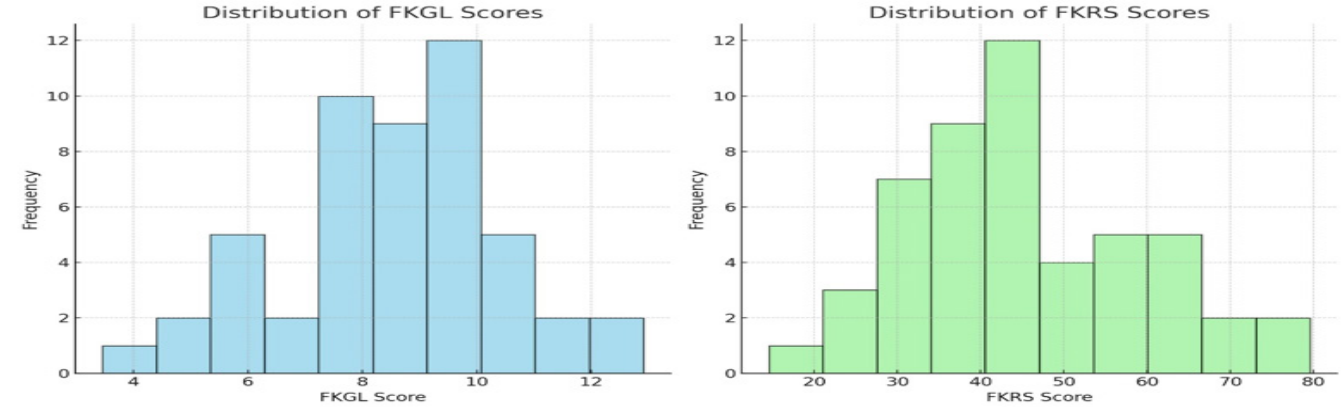

Figure 4. The distribution of readability scores (FKGL and FKRS) across the evaluated websites
FKGL: Flesch-Kincaid grade level, FKRS: Flesch reading ease score

Table 4. Evaluation of scores based on the presence of HON code

HON status	DISCERN score (Mean±SD)	JAMAscore (Mean±SD)	GQS score (Mean±SD)	FKGL (Mean±SD)	FKRS (Mean±SD)	HCS (Mean±SD)	p-value
Absent	31.50±11.50	1.80±0.90	1.95±1.05	9.15±2.20	46.80±18.50	17.50±7.50	
Present	43.50±11.00	2.65±0.85	2.90±0.95	9.05±2.05	47.90±17.50	23.00±6.20	
p-value	**0.003***	**0.011***	**0.005***	0.810	0.740	**0.019***	

HON: Health on the net, SD: Standart deviation, GQS: Global quality score, FKGL: Flesch-Kincaid grade level, FKRS: Flesch reading ease score, HCS: Haglund content score

patients require almost a high school-level English proficiency to adequately understand the knowledge available on the internet.

Consistent with the literature, the level of quality of online articles having a HON code was superior, reinforcing the notion that the content of HON code-compliant websites can be trusted to deliver higher quality information.[30-32] The content assessed concerning websites with a HON code exhibited markedly superior DISCERN, JAMA, GQS, and TCS scores compared to those lacking a HON code. On the other hand, websites with HON codes performed similarly to those without in terms of FKGL and FCRS ratings.

Limitations

This study's content score may be lacking in thoroughness because it was developed with the help of two orthopaedic doctors. The evaluation involved only two orthopedic specialists, which may limit the generalizability of the findings. Although patients may seek information through audiovisual mediums, this research did not evaluate it as it solely focused on web-based text content. Because the Internet is always evolving, search results and ranking positions are not always consistent. The study included only three search engines (Google, Yahoo!, Bing); incorporating more platforms could enhance the comprehensiveness of the analysis. Lastly, the lack of a survey assessing orthopedic specialists' knowledge about Haglund deformity leaves a gap that future research should address.

CONCLUSION

Consistent with earlier research, this study discovered that most of the informative websites did not have high-quality information, even if the number of such websites has increased. There were websites that provided better quality information, particularly academic ones, but their content was sometimes difficult to interpret. As far as we know, this is the first study specifically examining online information about Haglund deformity. This study can thus provide a valuable perspective on evaluating online resources, which may play a crucial role in supporting balanced interactions between patients and healthcare providers. Future studies should involve more orthopedic specialists and include additional search engines to broaden the scope and reliability of findings. Conducting surveys on orthopedic specialists' knowledge of Haglund deformity would also provide valuable insights to bridge gaps between clinical expertise and online information.

ETHICAL DECLARATIONS

Ethics Committee Approval

As this article does not contain any studies with human participants or animals by any of the authors, no ethics committee report is required.

Informed Consent

As this article does not contain any work with human participants or animals by any of the authors, informed consent is not required.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Measurement of mean platelet volume in the diagnosis of acute ischemic stroke and transient ischemic attack

Erkan Boğa

Department of Emergency Medicine, Esenyurt Necmi Kadıoğlu State Hospital, İstanbul, Turkiye

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ABSTRACT

Aims: The primary aim of this study is to investigate the potential role of mean platelet volume (MPV) in the diagnostic and prognostic evaluation of acute ischemic stroke (AIS) and transient ischemic attack (TIA), focusing on its clinical significance and role in diagnostic processes. This study aims to evaluate the clinical significance of MPV in patients with AIS and TIA and its role in diagnostic processes.

Methods: This retrospective study was conducted between June 15 and December 15, 2014, at the Emergency Medicine Department of Haydarpaşa Numune Training and Research Hospital. The study included 300 patients diagnosed with AIS or TIA and 100 healthy individuals matched for demographic characteristics as the control group. Participants were evaluated based on vascular risk factors such as hypertension, diabetes, and hyperlipidemia, and AIS subgroups were analyzed according to the TOAST classification. MPV and platelet counts, along with biochemical parameters, were measured, and statistical analyses were performed utilizing SPSS software.

Results: MPV values in the case group were significantly higher compared to the control group ($p<0.001$). No significant differences were observed in platelet counts between the two groups ($p>0.05$). MPV demonstrated no significant associations with hypertension, hyperlipidemia, or other vascular risk factors; however, patients with a history of TIA exhibited higher MPV levels compared to those without, although the difference was not statistically significant. A weak but statistically significant negative correlation was identified between MPV and platelet counts ($r=-0.20$, $p<0.05$).

Conclusion: MPV demonstrates potential as a significant biomarker in the diagnostic and prognostic processes of AIS and TIA. Nevertheless, the inconsistencies observed in the literature regarding the relationship between MPV and risk factors necessitate more extensive, prospective, and standardized studies to elucidate its clinical utility. The evidence suggests that MPV may contribute to enhancing diagnostic accuracy in clinical practice.

Keywords: Acute ischemic stroke, transient ischemic attack, mean platelet volume, biomarker, cerebrovascular disease

INTRODUCTION

Acute ischemic stroke (AIS) and transient ischemic attack (TIA), caused by cerebrovascular disorders, rank among the leading global causes of mortality and morbidity, representing a significant public health challenge.[1,2] AIS is characterized by ischemic damage to local brain tissue caused by the abrupt cessation of blood flow to the cerebral arteries. In contrast, TIA typically manifests as transient neurological dysfunction without permanent brain injury, often serving as a warning sign for more severe vascular events.[3] This underscores the critical importance of early diagnosis and timely intervention to mitigate long-term adverse outcomes.[4]

In recent years, platelet parameters, particularly mean platelet volume (MPV), have gained attention for their potential role in understanding the pathophysiology and diagnosis of vascular diseases. MPV, an indirect marker of platelet activation and functional status, has been recognized as a significant biomarker in evaluating thrombotic processes.[5,6] Elevated MPV levels are thought to reflect increased platelet activation, which contributes to the development of thrombotic and ischemic events.[7]

Despite its potential clinical relevance, the current literature reveals a paucity of studies investigating the specific relationship between MPV and the pathogenesis of AIS and TIA. A detailed exploration of this association is essential to address this gap and establish MPV's role as both a diagnostic and prognostic biomarker.[8] Moreover, limited perspectives exist regarding the clinical significance of MPV in distinguishing between AIS and TIA cases. This study aims to evaluate MPV levels in patients with AIS and TIA to determine its practical applicability as a diagnostic and prognostic tool in clinical settings.[9]

Corresponding Author: Erkan Boğa, drerkanboga@gmail.com

Such findings could improve diagnostic accuracy and facilitate the development of effective early intervention strategies. Additionally, this analysis seeks to provide evidence supporting MPV's dual role in diagnosis and prognosis, laying the foundation for its better integration into clinical practice.[10]

METHODS

Ethics

The study was conducted with the permission of the Clinical Researches Ethics Committee of Haydarpaşa Numune Training and Research Hospital (Date: 11.08.2014, Decision No: HNEAH-KAEK2014/43). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Population

This study was performed between the dates of June 15 and December 15, 2014 in the Department of Emergency Medicine at Haydarpaşa Numune Training and Research Hospital. The study population consisted of 300 patients diagnosed with AIS or TIA. A control group of 100 individuals without cerebrovascular or active vascular disease, matched for demographic characteristics, was included. These individuals were free from malignancy, infection, or medications affecting platelet function. Exclusion criteria included pyrexia at admission or a diagnosis of infection during first five days.

I) AIS patients were further classified according to the TOAST criteria into two subgroup: large vessel disease and small vessel disease; II) TIA patients were stratified in two subgroups, TIA with focal deficient symptom and TIA without focal deficient symptom. Stroke severity on admission was assessed with the Modified Rankin Scale (mRS) which classifies patients as mild (0–2) or severe (3–6).

Availability of Data and Material

All study subjects completed comprehensive medical histories. CNP and CTNP level of evidence: diagnoses were based on findings from comprehensive physical and neurological examinations that were recorded in detail. Laboratory tests were carried out with a battery of tests including full blood count, urinalysis, routine biochemistry (blood glucose, urea, creatinine, electrolytes, bilirubin and total protein among few others), C-reactive protein (CRP), prothrombin time (PT), activated partial thromboplastin time (aPTT) and international normalized ratio (INR). SD Biochemical markers, including LDL C, HDL C, triglycerids (TG), fibrinogen and ESR were extracted from patients records as well as follow up outcomes.

All the participants underwent ECG. Computed tomography (CT) scans of the brain were obtained upon admission. Additional imaging modalities, including echocardiography, carotid vertebral Doppler ultrasound, transesophageal echocardiography, cranial magnetic resonance imaging (MRI), and cranial angiography were reviewed from hospital records for inpatients.

Blood samples were obtained in the hospital during a period of at least 8 hours fasting (for patients hospitalized in the neurology ward) or at admission to the emergency department right after physical examinations. A hemogram analysis was conducted on venous blood samples which were placed into K3 EDTA tubes and run using a Coulter Gen.S System 2 analyzer. The samples for MPV measurements were drawn in tubes with citrate, processed within one hour of blood sampling using an ABX Roche analyzer and then quantified using the following formula:

MPV (fL)=Pct (%)×1000/Plt (×103/µL).

Risk Factor Evaluation

The following risk factors were assessed:

Hypertension: History of hypertension preceding stroke.

Diabetes: Elevated blood glucose or diagnosed diabetes noted on initial presentation of the stroke.

Hypercholesterolemia: Serum cholesterol levels greater than 200 mg/dl.

Embologenic sources: documented past of cardiac risk factors or embrological bases.

Current smoking: active or recent tobacco use within the last year.

Drinking: Continued or excessive consumption of ethanol.

Family history stroke in immediate relatives.

Obesity: Body-mass index (BMI) greater than 30 kg/m^2.

Stroke Categorization

Ischemic strokes were categorized aetiologically into one of four different types:

Lacunar infarcts: Classic lacunar syndromes and deep infarcts <15 mm on neuroimaging.

Type of emboli box: Cardioembolic infarcts; non-lacunar lesions with known embolic sources, lack of important arterial atherosclerotic disease or inconsistency between infarcts and findings on atherosclerosis.

Atherothrombotic infarcts: Non-lacunar lesions with significant atherosclerosis in large extracranial or intracranial vessels, or border-zone infarcts due to hemodynamic mechanisms.

Unclassified: Infarcts that do not fit within the aforementioned classifications.

Stroke localization was determined using the Bamford classification [9]. MPV reference range: 7.4–10.4 fL, PLT count from 130000 to 400000/µL (measured upon admission and three weeks after admission). Data on any laboratory results performed later were obtained from hospital records.

Statistical Analysis

SPSS for Windows version 10.0 was used for data analysis. Data were presented in descriptive statistics including mean and standard deviation. T-tests, Mann-Whitney U tests, Kruskal-Wallis tests and Wilcoxon signed-rank tests were performed to compare quantitative variables. Categorical variables were tested using chi-square tests. Spearman's rho

test was used to evaluate correlations. p<0.05 was considered statistically significant.

Modified Rankin Scale (mRS)

The mRS was used to stratify stroke severity:

0: Asymptomatic.

1: No disability; able to carry on normal activities.

2: Slightly disabled; unable to do all but self-care activities.

Disability rating scale (DRS) 3: None to moderate disability; ambulatory but requires assistance with activities of daily living.

4: Charcot marie tooth and its effects of needed assistance to walk or even need help with bodily functions.

Level 5: Very severe disability; confine to bed.

6: Deceased.

Methods: Cohorts of study and outcome measures:

A total of 300 patients were included for outcome determination on the first day and at two months post-admission, and they were classified into independent (mRS 0–2) and dependent (mRS 3–6). We assessed MPV values along with other laboratory parameters to evaluate their diagnostic and prognostic significance. ificance.

RESULTS

The study included a total of 300 patients (cases diagnosed with cerebrovascular disease), comprising 177 males (59.1%) and 123 females (40.9%), aged between 18 and 99 years. The mean age of the case group was 66.38±13.70 years. The control group consisted of 100 individuals, including 36 males (36%) and 64 females (64%), also aged between 18 and 99 years, with a mean age of 57.20±14.01 years.

When comparing the MPV and platelet counts between the case (cerebrovascular disease group) and control groups, the MPV values in the case group were significantly higher than those in the control group (p<0.001). No significant differences were observed in platelet counts between the two groups (p>0.05). MPV was not significantly associated with hypertension, hyperlipidemia, or other vascular risk factors. However, patients with a history of TIA showed higher MPV levels compared to those without, although this difference was not statistically significant (Table 1, Table 2).

Table 1. Comparison of MPV and platelet count between case (patients diagnosed with cerebrovascular disease) and control groups

	Case group		Control group		
	Mean	SD	Mean	SD	p
MPV (fl)	9.25	1.38	8.56	1.27	0.002**
Platelet 10^3/µL	270.14	83.95	261.02	72.72	0.485

MPV: Mean platelet volume, SD: Standart deviation

When examining the case group in terms of risk factors such as hypertension, hyperlipidemia, rheumatic heart disease, myocardial ischemia, smoking, alcohol consumption, and obesity, no statistically significant difference in MPV values at admission was found between patients with and without these risk factors (p>0.05) (Table 3).

Table 2. Correlations of MPV in the case group (patients diagnosed with cerebrovascular disease)

	MPV	
	r	p
Leukocyte (10^3/µL)	0.056	0.570
Cholesterol (mg/dl)	0.029	0.763
Triglyceride (mg/dl)	-0.039	0.687
LDL (mg/dl)	0.051	0.598
Glucose (mg/dl)	0.041	0.668
ESR (mm/s)	0.015	0.874
aPTT (sn)	0.135	0.161
Platelet (10^3/µL)	-0.204	0.033*
CRP (mg/dl)	0.000	0.997

MPV: Mean platelet volume, LDL: Low-density lipoprotein, aPTT: Activated partial thromboplastin time, CRP: C-reactive protein

Although there was a trend towards higher MPV values in patients with atrial fibrillation compared to those without, the difference was not statistically significant (p>0.05). Notably, patients with atrial fibrillation had higher MPV values than those without (Table 3).

Table 3. Evaluation of MPV at admission based on risk factors in the case group

	n	Mean	SD	p
Hypertension				
Absent	24	9.32	1.66	
Present	86	9.23	1.31	0.772
Hyperlipidemia				
Absent	64	9.31	1.41	
Present	46	9.17	1.36	0.615
Atrial fibrillation				
Absent	83	9.11	1.40	
Present	27	9.68	1.25	0.065
Rheumatic heart disease				
Absent	103	9.21	1.38	
Present	7	9.82	1.43	0.265
Myocardial ischemia				
Absent	93	9.26	1.43	
Present	17	9.18	1.17	0.827

SD: Standart deviation

There were no significant associations between MPV and hypertension, hyperlipidemia, or other vascular risk factors. However, patients with a history of TIA exhibited higher MPV levels compared to those without, although this difference was not statistically significant. (p>0.05) (Table 3).

Similarly, when comparing MPV values based on the presence of diabetes, no statistically significant difference was observed, although a trend towards higher MPV values was noted in diabetic patients compared to non-diabetic patients (p>0.05).

No statistically significant difference in MPV values was found in the case group based on a family history of stroke (p>0.05).

When comparing MPV values at admission between patients with and without a history of prior stroke or TIA, no statistically significant differences were observed (p>0.05).

Although there was no statistically significant difference in MPV values based on the presence of TIA (p>0.05), a trend was noted where patients with TIA had higher MPV values than those without.

No statistically significant differences in MPV values were found based on infarct localization (p>0.05) (**Figure**).

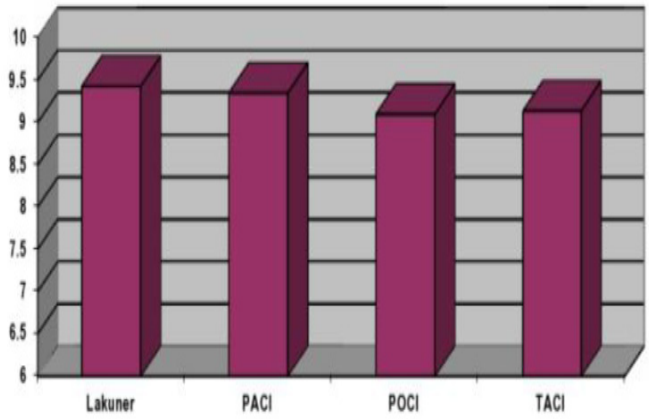

Figure. MPV values based on infarct localization
MPV: Mean platelet volume, PACI: Partial anterior circulation infarcts, TACI: Total anterior circulation infarcts, POCI: Posterior circulation infarcts

No significant differences in MPV values were observed between patients using anticoagulant or antiplatelet medications and those not using these medications (p>0.05).

Finally, no statistically significant differences in MPV values were detected among ischemic stroke subgroups classified according to TOAST and Bamford criteria (p>0.05).

DISCUSSION

Key demographic factors such as age and sex are intrinsic risk factors for ischemic stroke. Age is the most significant determinant of stroke risk, with males generally experiencing a higher incidence than females. However, among older age groups, the incidence in females surpasses that of males, consistent with previous findings.

However, in older age groups, the incidence in females surpasses that of males, consistent with prior findings.[10] Our study supported this male predominance among ischemic stroke patients, as reported in the literature.

Sharpe and Trinick compared MPV between diabetic patients and healthy controls, showing a significant increase in MPV among the diabetic group.[12] Similarly, Hekimsoy et al.[13] reported higher MPV values in diabetic patients compared to controls. In contrast, McCabe et al.[14] investigated MPV during the acute phase of cerebrovascular disease and at six months post-event, finding no significant differences between early and late MPV levels or any influence of risk factors on MPV.

In our study, no statistically significant differences were found between MPV values in diabetic patients and healthy controls. This divergence in findings aligns with the variability reported in the literature regarding the relationship between diabetes and MPV. Our results suggest that platelet size is determined during thrombopoiesis, potentially influenced by insulin or other factors promoting larger platelet production.[15]

Increased MPV has been described in patients with various vascular risk factors, including diabetes, hypercholesterolemia, smoking, preeclampsia, and renal artery stenosis.[16,18] Brown et al.[17] examined MPV and platelet counts in diabetic patients, reporting significantly elevated MPV in those with vascular disease compared to non-diabetic populations. However, in our study, no significant differences in MPV were observed between hypertensive or hyperlipidemic patients and those without these conditions. This lack of significance may be attributed to well-controlled blood pressure levels in our cohort, consistent with findings suggesting that blood pressure regulation reduces MPV over time.[19,20]

Patients with a history of TIA or prior stroke did not show statistically significant differences in MPV compared to those without such a history. However, patients with a history of TIA exhibited higher MPV values, although the difference was not statistically significant. This finding may be explained by thromboembolism originating from large extracranial arteries, which is common in TIA pathophysiology, and prior associations of increased MPV with atherosclerotic conditions.[21]

In our study, MPV was not significantly different between patients with and without myocardial infarction. This lack of association could be due to the small number of myocardial infarction cases in our cohort, limiting the statistical power required to detect true differences.[22]

No significant differences were found in platelet counts between cases and controls (p>0.05). In the case group, a weak but statistically significant negative correlation was observed between MPV and platelet counts (r=-0.20, p<0.05), while no significant correlations were found between MPV and other parameters such as cholesterol, triglycerides, LDL cholesterol, glucose, ESR, or CRP (p>0.05).[23]

Patients using antiplatelet or anticoagulant medications showed no significant differences in MPV compared to those not using these drugs, consistent with prior findings.[24]

When comparing stroke subtypes by etiology or localization, no significant differences in MPV were observed. This supports the hypothesis that localized thrombus-associated platelet consumption does not affect peripheral venous platelet parameters. Additionally, the lack of MPV differences between large cortical infarcts and smaller lacunar infarcts reinforces this hypothesis.[25,26] Notably, elevated MPV and decreased platelet counts persisted in post-stroke survivors, suggesting a role for larger platelets in stroke development rather than being a mere consequence of the acute event.[27,28]

Discrepancies in MPV findings across studies may stem from measurement errors and differences in methodology. Variability in instruments and anticoagulants used can influence MPV results. Standardized protocols addressing these issues, including anticoagulant type, sample processing timing, and measurement techniques, are essential for future research.[29,30]

Recent studies highlight the role of MPV in various thrombotic conditions and its potential as a biomarker for cardiovascular diseases. A systematic review by Giannini et al.[31] demonstrated

that elevated MPV is consistently associated with an increased risk of ischemic events and poorer outcomes in stroke patients. Furthermore, advancements in MPV measurement techniques, as discussed by Lippi et al.[32] emphasize the need for standardized methodologies to minimize variability in clinical studies. The potential integration of MPV into stroke risk assessment models has been explored in recent meta-analyses, suggesting improved predictive power when combined with other biomarkers.

CONCLUSION

MPV appears to have potential as both a diagnostic and prognostic biomarker. Our study suggests that MPV can enhance diagnostic accuracy in complex clinical conditions such as stroke and support early intervention strategies. However, no significant relationship was observed between MPV values and certain risk factors, nor did it provide a clear distinction between different types of strokes. This finding indicates that the use of MPV as a standalone clinical decision-support tool may be limited, but it could offer greater value when combined with other clinical and laboratory parameters.

Recent literature supports the idea that MPV is not only a standalone biomarker but also an essential component of multimodal diagnostic and prognostic approaches. In an era where multidisciplinary and personalized treatment strategies are gaining importance, integrating MPV with other hematological markers could lead to more precise diagnoses and improved long-term outcomes in stroke management. In particular, MPV may play a role in the diagnosis of ischemic stroke, predicting recurrent thrombotic events, and evaluating mortality rates.

Moreover, to improve the clinical applicability of MPV, further large-scale, multicenter studies with standardized protocols are essential. Future research should focus on clarifying MPV's individual diagnostic value and its contribution to multimarker models. Incorporating emerging data and modern technological approaches could significantly enhance the role of MPV in clinical decision-support systems.

In conclusion, our study highlights the potential role of MPV in clinical practice, while also emphasizing the need for further advanced research to optimize its use and establish a more robust evidence base. The effective application of MPV as a clinical tool will depend on comprehensive studies supported by accurate protocols.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was conducted with the permission of the Clinical Researches Ethics Committee of Haydarpaşa Numune Training and Research Hospital (Date: 11.08.2014, Decision No: HNEAH-KAEK2014/43).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Pneumothorax in patients with COVID-19 pneumonia in the intensive care unit: an indicator of poor prognosis

Onur Küçük[1], Fatih Seğmen[2], Semih Aydemir[3]

[1]*Department of Anesthesiology and Reanimation, Ankara Atatürk Sanatorium Training and Research Hospital, University of Health Sciences, Ankara, Turkiye*

[2]*Department of Intensive Care, Ankara Bilkent City Hospital, Ankara, Turkiye*

[3]*Department of Anesthesiology and Reanimation, Yıldırım Beyazıt University, Yenimahalle Training and Research Hospital, Ankara, Turkiye*

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ABSTRACT

Aims: The objective of this study was to investigate whether the incidence and development of pneumothorax in patients hospitalized in the intensive care unit (ICU) with coronavirus disease 2019 (COVID-19) pneumonia is associated with patient prognosis.

Methods: This retrospective, cohort, descriptive study was initiated following approval from the ethics committee. The study was conducted on patients with confirmed COVID-19 pneumonia admitted to the tertiary ICU between March 2020 and March 2022. Data were collected from the patient registry system and ICU files. The patients were divided into two groups: those who developed pneumothorax and those who did not. The factors associated with mortality in the ICU were evaluated by univariate analysis and multiple logistic regression analysis.

Results: The study included a total of 397 patients with confirmed cases of COVID-19 infection and pneumonia who were admitted to the ICU between March 2020 and March 2022. The mean age of the patients was 62±15 years. Of the patients, 56.1% were male. Pneumothorax was identified in 6.8% of patients. In addition to pneumothorax, six patients (1.5%) exhibited pneumomediastinum. The mortality rate was observed to be 40.5% among the total patient population. The mortality rate was 81.5% in the group with pneumothorax and 37.6% in the group without pneumothorax. The median time to mortality was 6 days (range 1–29 days) following the diagnosis of pneumothorax. Pneumothorax alone increased the likelihood of mortality in the ICU sevenfold (OR 7.3, 95% CI=2.70–19.75) and twofold when other variables were taken into account (OR 2, 95% CI=0.57-6.99).

Conclusion: Pneumothorax is a common and fatal complication affecting mortality in patients with COVID-19 pneumonia in the ICU, despite the use of protective ventilation strategies. Particular caution should be exercised in patients receiving respiratory support in the ICU and in patients with a severe inflammatory response.

Keywords: Pneumothorax, pneumomediastinum, intensive care unit, prognosis, COVID-19

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus was initially identified in Wuhan, China, in December 2019.[1] On March 11, 2020, the World Health Organization (WHO) declared a global pandemic, a designation that remained in effect in our country until July 1, 2021. During this period, there was an increase in intensive care unit (ICU) hospitalization and mortality rates. A review of the extant literature reveals that the mortality rates reported for patients who were hospitalized in ICUs within our country exhibit variability, with rates ranging from 47% to 66% being documented.[2-4] The 2019 novel coronavirus disease (COVID-19), which continues to manifest in different variants, remains a significant public health concern.[5] The most commonly utilized diagnostic techniques for the identification of SARS-CoV-2 infection are reverse transcriptase-polymerase chain reaction (RT-PCR) testing and thorax computed tomography (CT) scanning.[6] In more than 70% of cases of patients with a positive RT-PCR test result for SARS-CoV-2, there are specific characteristics observed on thorax CT scans. These include ground-glass opacities, vascular enlargement, bilateral abnormalities, lower lobe and posterior involvement.[7] However, less common radiological findings have also been observed in patients with confirmed SARS-CoV-2 infection, including pneumothorax, bullae, pleural effusion, lymphadenopathy, central lesion distribution, and pericardial effusion.[8,9] COVID-19 differs from other

Corresponding Author: Semih Aydemir, drsemihaydemir@gmail.com

causes of acute respiratory distress syndrome (ARDS) due to its potential to induce vascular and parenchymal damage through inflammatory cascades.[10,11] Consequently, it is more probable that the development of pneumothorax and other associated complications will be observed.

In the lung parenchyma, COVID-19 pneumonia can produce cystic lesions that may disappear or develop into larger blisters.[12,13] As a result, patients may be at risk of rupture, which can subsequently lead to spontaneous pneumothorax or mediastinal and subcutaneous emphysema.[14] Primary spontaneous pneumothorax has no known etiology, but secondary spontaneous pneumothorax results from an underlying lung pathology.[15] It can occur in 1% of hospitalized patients,[16] 3% of patients hospitalized for pneumonia,[17] 6% of mechanically ventilated patients,[18] and 1% of deceased patients.[19] Due to the poor understanding of lung histology in these patients, it is unknown how effectively damaged lung tissue will heal and re-expand on its own in COVID-19 individuals.[20] Patients with neutrophilia and a prolonged clinical course are more likely to experience diffuse lung injury and pneumothorax.[21] Similarly, pneumothorax is considered a poor prognostic feature of Middle East Respiratory syndrome (MERS) coronavirus associated infection.[22] It has been suggested that rapid diagnosis and management of pneumothorax may reduce morbidity and mortality in COVID-19 patients.[21,23] However, clinical data are lacking in this regard. In addition, its incidence in ICU patients is not known exactly.

The objective of this study was to investigate the incidence of pneumothorax in patients hospitalized with COVID-19 pneumonia in the ICU and to determine whether its development is associated with a poor prognosis. The present study was designed in accordance with the hypothesis that the development of pneumothorax in patients with SARS-CoV-2 infection and pneumonia requiring ICU admission is associated with an adverse prognosis. The objective was to ascertain whether the mortality rate among patients with SARS-CoV-2 infection and pneumonia requiring ICU admission, with or without the development of pneumothorax, differs from that observed among patients without pneumothorax.

METHODS

Study Design and Patients

This retrospective cohort descriptive study was initiated following approval from the Clinical Researches Ethics Committee of Yıldırım Beyazıt University, Yenimahalle Training and Research Hospital (Date: 13/09/2023, Decision No: E-2023-43). All procedures were conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Declaration of Helsinki, as revised in 2013. The present study was conducted through a review of patient registries and ICU follow-up data on patients with confirmed COVID-19 pneumonia who were admitted to tertiary ICU at two medical centers between March 2020 and March 2022. Lung-protective ventilation strategies were a standard procedure in all ICUs.

The inclusion criteria were as follows: (1) admission to the ICU between March 2020 and 2022, (2) confirmation of SARS-CoV-2 infection via positive PCR test results on nasopharyngeal swabs or respiratory tract secretions, or via the presence of SARS-CoV-2 infection on thorax CT scans, and (3) presence of diagnosed lung involvement and pneumonia. Patients with incomplete or missing data in the registry system and ICU files, as well as those with duplicate hospitalizations (in which the data from the first hospitalization were evaluated), were excluded from the study.

Patients with a confirmed diagnosis of SARS-CoV-2 infection were identified through the presence of SARS-CoV-2 ribonucleic acid (RNA) in a nasopharyngeal swab or respiratory secretion, or through an official diagnosis in the hospital registration system with a documented involvement of the virus in the patient's thorax CT report. Pneumothorax detection was defined as patients with a diagnosis of pneumothorax in the hospital information registry system whose diagnosis was confirmed by chest radiography. The following data were recorded from the hospital information system: demographic data, Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II scores (at the 24th hour of admission), laboratory parameters (white blood cell count (WBC), neutrophil count, C-reactive protein (CRP), hemogram, D-dimer), and body-mass index (BMI). Furthermore, data regarding the administration of respiratory support devices, the duration of such support, the presence of intubation, the development of pneumothorax, the side of the lung affected by pneumothorax, ICU mortality, how many days after the pneumothorax the mortality occurred and ICU length of stay were obtained from ICU records. The highest laboratory parameter values during the ICU stay were included in the study. The data of patients diagnosed with COVID-19 pneumonia in the ICU with and without pneumothorax were compared. The effect of pneumothorax on estimated mortality was evaluated.

Outcome

The primary outcome measure of the study was the ICU mortality rate in patients admitted to the ICU with COVID-19 pneumonia with and without pneumothorax. Additional outcome measures included the factors associated with mortality in patients with COVID-19 pneumonia, the contribution of the pneumothorax factor to mortality, and descriptive characteristics of patients with pneumothorax.

Statistical Analysis

The data were subjected to statistical analysis using the statistical package for the social sciences (SPSS) 24.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as the number (n) of cases, percentage (%), mean±standard deviation ($\bar{x}$±SD), or median (Q-Q3), minimum (min) value, and maximum (max) value. Categorical and demographic data were tabulated as number of cases and percentage. A Chi-square test was employed to ascertain whether two rates exhibited a statistically significant discrepancy. The distribution of the obtained data was evaluated using the Shapiro-Wilk test. In accordance with the distribution outcomes of the numerical data, a comparison of

paired groups was conducted using Student's t-test and Mann-Whitney U test. The comparison of categorical data between groups was conducted using either the Pearson Chi-Square test or the Fisher exact test. The relative odds ratio (OR) was calculated using the ICU mortality values observed in patients with and without pneumothorax. A p-value of less than 0.05 was considered statistically significant.

A logistic regression analysis was conducted using the variables that were statistically significant in terms of ICU mortality in univariate analysis, as well as variables that were not statistically significant but had a p-value less than 0.2. Eight variables were identified as potentially suitable for inclusion in the multivariate logistic regression model. To ascertain the likelihood of multicollinearity, all variables were subjected to correlation analysis, variance inflation factor assessment, and tolerance value analysis. The WBC value was excluded from the model due to its high correlation with the neutrophil value. The residue and Cook distance values were subjected to rigorous control. No variables were excluded from the data set. The APACHE II score was excluded from the model due to its minimal contribution (likelihood ratio (LR) test X^2 values 0.58). Ultimately, six variables were incorporated into the model: age, neutrophil count, CRP value, male gender, D-dimer value, and the presence of pneumothorax. The omnibus test yielded a p-value of less than 0.001, confirming the overall fit of the model. The model demonstrated an accuracy power of 49% (Nagelkerke R^2 = 0.4880).

RESULTS

The data of 421 patients who met the study's inclusion criteria during the specified period were evaluated. However, 24 patients were excluded from the study due to incomplete data and duplicate hospitalizations, resulting in a final sample of 397 patients who were hospitalized in ICU between March 2020 and March 2022 and diagnosed with COVID-19 pneumonia. The mean age of the patients was 62±15 years. Of the total number of patients, 223 (56.1%) were male. Pneumothorax was identified in 6.8% (n=27) of the patients. Table 1 presents the demographic and clinical characteristics of patients with and without pneumothorax.

The APACHE II score, WBC count, neutrophil count, CRP value, and D-dimer value were found to be statistically higher in patients with pneumothorax compared to those without (p<.001). At the time of pneumothorax diagnosis, 5 patients (18.5%) were on non-invasive mechanical ventilation, 9 patients (33.3%) were on invasive mechanical ventilation, and 13 patients (48.2%) were on respiratory support with nasal high-flow oxygen therapy. Pneumothorax was identified on the right side in 13 patients (48%), on the left side in 7 patients (26%), and in both lungs in 7 patients (26%). Chest tubes were placed in all patients. In addition, 6 patients (22.2%) exhibited concomitant pneumomediastinum. Mortality occurred on median day 6 (min=1, max=29) after the diagnosis of pneumothorax.

The mortality rate was 40.5% (n=161) among the total patient population. The mortality rate was 81.5% (n=22) in the group with pneumothorax and 37.6% (n=139) in the group without pneumothorax. This difference between the groups was statistically significant and higher in the pneumothorax group (p<.001). Table 2 presents the demographic and clinical characteristics of patients with and without mortality.

The results of the logistic regression analysis for ICU mortality are presented in Table 3. Univariate logistic regression showed a sevenfold increase in ICU mortality with the occurrence of pneumothorax in COVID-19 pneumonia in the ICU (OR 7.3, 95% CI=2.70-19.75). This association was twofold in multivariable adjustment (OR 2, 95% CI=0.57-6.99). In the multivariate analysis, increasing age, increasing neutrophil count, high CRP value, and male gender were identified as independent variables significantly associated with increased mortality from COVID -19 pneumonia in the ICU. The logistic regression analysis demonstrated that age was the most influential factor in the model, with a LR test X2 value of 48.9 and a p-value of <.001. The model's performance in terms of mortality was determined to be 68.3% sensitivity and 84.7% specificity, with an accuracy of 78% and an area under the receiver operating characteristic curve (AUC) of 0.86 (Figure).

Table 1. Demographic and clinical characteristics of patients with and without pneumothorax

		Pneumothorax, No, (n=370)	Pneumothorax, Yes, (n=27)	p-value
Age, year, median (Q1-Q3)		63 (53-74)	65 (57.5-74)	0.459
Gender, n (%)	Female	165 (94.8%)	9 (5.2%)	0.255
	Male	205 (91.9%)	18 (8.1%)	
BMI, kg/m², mean±SD		27.04±3.86	26.45±3.26	0.367
APACHE II score, median (Q1-Q3)		20 (15-24)	25 (20.5- 29)	**<.001**
WBC /µL, median (Q1-Q3)		9460 (6198-13300)	20750 (15040-27995)	**<.001**
Neutrophil /µL, median (Q1-Q3)		8195 (4253-11928)	18230 (13515-24680)	**<.001**
CRP mg/L, median (Q1-Q3)		81.36 (19.70-164.3)	189.6 (149.1-277.5)	**<.001**
D-dimer mg/L, median (Q1-Q3)		1.12 (0.54-2.45)	6.48 (3.73-12.45)	**<.001**
ICU length of stay, median (Q1-Q3)		9 (5-19)	10 (4.5-23)	0.938
ICU mortality	No, n (%)	231 (97.9%)	5 (2.1%)	**<.001**
	Yes, n (%)	139 (86.3%)	22 (13.7%)	

Continuous variables are expressed as either the mean±standard deviation (SD) or median (Q1-Q3) and categorical variables are expressed as either frequency (percentage). Continuous variables were compared with Student-t test or Mann-Whitney U test, and categorical variables were compared using Pearson'schi-square test or fisher exact test.
APACHE II: Acute physiologic assessment and chronic health evaluation, BMI: Body-mass index, CRP: C-reactive protein, ICU: Intensive care unit, SD: Standart deviation, WBC: White blood cell

Table 2. Demographic and clinical characteristics of patients with and without mortality

		Mortality, No, (n=236)	Mortality, Yes, (n=161)	p-value
Age, year, median (Q1-Q3)		58 (47-67)	70 (63-78)	<.001
Gender, n (%)	Female	116 (66.7%)	58 (33.3%)	0.010
	Male	120 (53.8%)	103 (46.2%)	
BMI, kg/m², mean±SD		23.9±5.05	28.06±6.56	0.198
APACHE II skor, median (Q1-Q3)		19.5 (15-24)	20 (17-25)	**0.002**
WBC /µL, median (Q1-Q3)		7870 (5520-11493)	13470 (9230-18010)	**<.001**
Neutrophil /µL, median (Q1-Q3)		6150 (3365-9513)	12300 (8570-16920)	**<.001**
CRP mg/L, median (Q1-Q3)		63.29 (15.23-131.7)	149 (68.69-223.4)	**<.001**
D-dimer mg/L, median (Q1-Q3)		0.7 (0.41-1.7)	2.2 (1.14-6.46)	**<.001**
Pneumothorax	No, n (%)	231 (62.4%)	139 (37.6%)	**<.001**
	Yes, n (%)	5 (18.5%)	22 (81.5%)	

Continuous variables are expressed as either the mean±standard deviation (SD) or median (interquartile range) and categorical variables are expressed as either frequency (percentage). Continuous variables were compared with Student-t test or Mann-Whitney U test, and categorical variables were compared using Pearson'schi-square test or fisher exact test.
APACHE II: Acute physiologic assessment and chronic health evaluation, BMI: Body-mass index, CRP: C-reactive protein, SD: Standart deviation, WBC: White blood cell

Table 3. Univariate and multivariate logistic regression modeling for intensive care mortality in COVID-19 patients

Prediction variable	Unadjusted			Adjusted		
	OR	95% CI	p-value	OR	95% CI	p-value
Age, year	1.072	1.052-1.091	<.001	1.072	1.048-1.095	<.001
Neutrophil, /µL	1.0002	1.0001-1.0003	<.001	1	1.0001-1.00019	<.001
CRP, mg/L	1.008	1.006-1.0109	0,001	1.004	1.0013-1.0072	0.005
Gender, male	1.7167	1.1385-2.5885	0.010	2.133	1.2382-3.6758	0.006
D-dimer, mg/L	1.1091	1.0574-1.1633	<.001	1.029	0.9912-1.0684	0.134
Pneumothorax, Yes	7.3	2.7074-19.7492	<.001	2.008	0.5769-6.9925	0.273

CRP: C-reactive protein, OR: Odds ratio, CI: 95% confidence interval

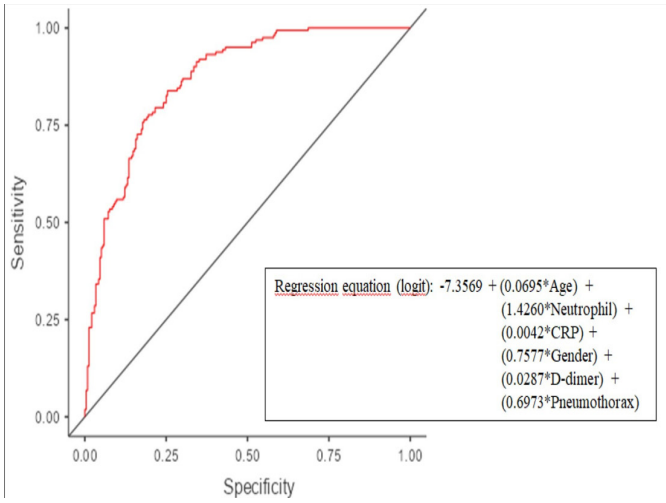

Figure. Area under the receiver operating characteristic curve for intensive care mortality in COVID-19 pneumonia

DISCUSSION

The objective of this study was to examine the influence of pneumothorax on mortality rates among patients admitted to the ICU with COVID-19 pneumonia. Pneumothorax was identified in 6.8% of patients hospitalized in the ICU with COVID-19 pneumonia. Pneumothorax was identified in the right lung in 48% of cases, in the left lung in 26% of cases, and in both lungs in 26% of cases. At the time of diagnosis of pneumothorax, all patients were receiving respiratory support. The mortality rate among patients with pneumothorax in the ICU was 81.5%. The median time to mortality was six days after diagnosis. Our findings indicate that the development of pneumothorax in the ICU in patients with COVID-19 pneumonia was associated with a seven-fold increase in mortality when considered as a single factor and a two-fold increase when other factors were taken into account. Furthermore, age, neutrophil count, CRP value, and male gender were identified as independent variables associated with mortality in patients with COVID-19 pneumonia. The most significant independent variable affecting mortality was the patient's age.

Pneumothorax is defined as the accumulation of air between the visceral and parietal pleurae, which surround the lungs. Secondary spontaneous pneumothorax is a consequence of underlying lung disease, whereas primary spontaneous pneumothorax can occur without any triggering event. The precise etiology of the injury remains uncertain. However, it is plausible that infection-related alveolar damage and alveolar wall rupture resulting from elevated pressure caused by intense coughing in response to the virus may be the primary causes.[24] Furthermore, an inflammatory response during lung infections has the potential to contribute to the development of secondary spontaneous pneumothorax. Some studies have indicated that inflammatory exudates may be involved in the pathogenesis of pneumothorax, even in the absence of mechanical ventilation.[11,25] Pneumothorax has been identified as a potential, albeit uncommon, consequence of SARS-CoV-2 infection since the initial reports of cases emerged. The autopsy studies of patients with COVID-19 have revealed that the most prevalent pulmonary pathological findings were diffuse alveolar damage, comparable to that observed in patients with severe ARDS, and the formation of pneumothorax. These findings lend support to the hypothesis that pulmonary parenchymal damage may be a consequence of the disease.[26,27] The incidence of pneumothorax exhibits

considerable variability in the literature, particularly in the context of ARDS patients, with reported rates ranging from 1.7% to 10%.[28,29] In their study of patients with COVID-19, Wang et al.[23] observed an incidence of pneumothorax of 10% in patients without mechanical ventilation and 24% in those who required it. Yang X et al.[30] observed a rate of 2.7% in patients with COVID-19 who were receiving mechanical ventilation, whereas Sihoe et al.[28] reported a rate of 1.7% in patients with severe acute respiratory syndrome (SARS) who had ARDS. In our study, we observed a pneumothorax rate of 6.8% in patients hospitalized in the ICU with COVID-19 pneumonia. Although this rate is consistent with the literature and relatively high, it is an expected finding when considering that the majority of studies in the literature were conducted with ward patients and that our patient group consisted of high-risk patients on respiratory support in the ICU. It is of the utmost importance to implement effective prevention, immediate recognition, and treatment strategies for pneumothorax in order to minimize mortality rates among patients with lung infections and predominant inflammatory processes, such as those observed in patients with SARS-CoV-2 infection who are hospitalized in the ICU and receive respiratory support.

In the existing literature, cases of pneumothorax and pneumomediastinum induced by SARS-CoV-2 are frequently reported together. However, it should be noted that these studies are all case series.[24,31] The occurrence of spontaneous pneumothorax may be classified as primary or secondary, contingent upon the presence or absence of an underlying lung disease.[32] In contrast, the presence of pneumomediastinum may be classified as primary if the underlying cause is idiopathic or secondary if it responds to a spontaneous, traumatic, or iatrogenic etiology.[33] The coexistence and incidence of pneumothorax and pneumomediastinum remain unclear. However, it is evident that their association has a detrimental impact on prognosis.[31] Ulutas et al.[21] identified the presence of pneumothorax in eleven patients diagnosed with SARS-CoV-2 infection, yet no instances of pneumomediastinum observed. In our study, the occurrence of pneumothorax was observed in conjunction with pneumomediastinum in six of the 27 patients (22.2%). In comparison to the overall study population, this rate was 1.5%. The prognosis for these patients was poor, and mortality was observed in the ICU. Our findings indicate that, although the frequency of this association is low, it should be considered as a potential diagnosis in critically ill patients hospitalized in the ICU.

Previously, it was postulated that the presence of pneumothorax was a significant prognostic factor in patients infected with the novel coronavirus.[22,34] However, the literature on the subject of COVID-19 is limited to case series. Concurrently, the treatment of pneumothorax in conjunction with SARS-CoV-2 infection, which can result in severe manifestations, may potentially lead to an increased incidence of comorbidities and complications. In particular, the insertion of a chest drain to treat pneumothorax can be considered an aerosol-generating technique. Furthermore, recent postmortem findings have revealed the presence of RNA from the SARS-CoV-2 in pleural fluid.[35] In this regard, the management of pneumothorax represents a significant challenge. In a retrospective study by Ershadi et al.[36] of patients with COVID-19 and pneumothorax admitted to the ward, the 50-day mortality rate was 52.2%. An analysis of the data regarding the COVID-19 in our nation reveals that the mortality rate among patients admitted to the ICU with the disease ranges from 47% to 66%.[2-4] Satici et al.[37] reported a 30-day mortality rate of 60% in patients hospitalized in the ICU in their study on patients diagnosed with pneumonia caused by the COVID-19. This mortality rate was observed to be higher in the geriatric age group and in patients with comorbidities.[2] In our study, which was conducted on patients diagnosed with COVID-19 pneumonia in the ICU, the mortality rate was 81.5% in patients who developed pneumothorax. In the univariate analysis, it was determined that the presence of pneumothorax was associated with a sevenfold increase in mortality, which was reduced to twofold when other variables were taken into account. In light of these findings, the presence of pneumothorax in conjunction with the inflammatory process is associated with a poor prognosis in the ICU.

The use of non-invasive and invasive mechanical ventilation in the treatment of patients with COVID-19 has been associated with an increased risk of developing pneumothorax.[38] In a series of eleven cases, Ulutas et al.[21] reported the development of spontaneous pneumothorax in patients with COVID-19. While the provision of respiratory support increases the risk of pneumothorax in patients diagnosed with COVID-19, the development of pneumothorax represents a significant prognostic factor in patients who do not receive mechanical ventilation support.[36] In the our study, all patients who developed pneumothorax were receiving respiratory support. Additionally, Ulutas et al.[21] observed elevated levels of CRP, lactate dehydrogenase, ferritin, D-dimer, and interleukin-6 in the majority of patients who developed spontaneous pneumothorax. This finding is consistent with recently published studies examining potential mechanisms of lung injury induced by SARS-CoV-2.[39,40] Cytokine storms are postulated to be involved in the pathophysiology of the disease. However, it remains unclear whether there is a distinction between these inflammatory markers in patients with and without pneumothorax within the context of the broader patient group diagnosed with COVID-19. In our study, the levels of inflammatory markers (WBC, neutrophils, CRP, and D-dimer) were statistically significantly higher in the patient group with pneumothorax compared to the group without pneumothorax. Although protective ventilation strategies are employed in our ICUs, it is premature to draw any definitive conclusions. However, given that our patient cohort comprises critically ill individuals in the ICU, we advise caution with regard to the potential for pneumothorax in patients with a severe inflammatory process who are receiving respiratory support.

Limitations

The present study has some limitations. Firstly, it should be noted that this cohort study population is a specifically selected cohort of ICU patients, and that the number of patients is relatively small. Consequently, the sample is not

representative of all patients with COVID-19. Secondly, this was a retrospective cohort study, which precluded the possibility of conducting a therapeutic analysis within the cohort and of including all variables that influence mortality, given the limitations of the data. Moreover, the retrospective design of the study may have constrained its capacity to discern clinical predictors. Third, no histopathologic evidence of disease was obtained in patients who died. Fourth, the data available for analysis were limited to those collected during the ICU follow-up period, and the post-ICU ward follow-up data were not included in the study.

CONCLUSION

In conclusion, pneumothorax represents a common and potentially fatal complication affecting mortality in patients with COVID-19 pneumonia in the ICU, despite the implementation of protective ventilation strategies. It is of particular importance to ensure that patients who are receiving respiratory support and who are exhibiting a severe inflammatory response are diagnosed and treated promptly. It is imperative that the necessary precautions be taken to prevent contamination during the course of treatment. It should be noted that pneumomediastinum may also be observed in patients with pneumothorax. Although our study is limited by its retrospective nature, the reported findings are important for reducing the incidence of pneumothorax and mortality, as well as improving the prognosis in the ICU, given the high prevalence of infectious diseases in the current era. Due to the limited study population, prospective studies examining the association of viral infections with pneumothorax in a larger population of patients in ICUs are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the Clinical Researches Ethics Committee of Yıldırım Beyazıt University Yenimahalle Training and Research Hospital (Date: 13/09/2023, Decision No: E-2023-43).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Prolonged atrial electromechanical delay and P-wave parameters in asymptomatic carotid artery stenosis: novel insights from a non-invasive evaluation

Faruk Serhatlıoğlu[1], Şaban Keleşoğlu[2]

[1]*Department of Cardiovascular Surgery, Faculty of Medicine, Niğde Ömer Halisdemir University, Niğde, Turkiye*
[2]*Department of Cardiology, Faculty of Medicine, Erciyes University, Kayseri, Turkiye*

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ABSTRACT

Aims: Stroke is one of the top three causes of death in developed societies. Ischemic strokes are linked to carotid artery stenosis (CAS). Atrial fibrillation (AF) is a commonly encountered clinical arrhythmia. It has been shown that the prolongation of intra- and interatrial conduction times, known as atrial electromechanical delay (EMD), is associated with a higher risk of AF. We aimed to determine the correlation of atrial conduction abnormalities between surface electrocardiographic and TDI measurements in the CAS patient group.

Methods: The study included 76 patients diagnosed with extracranial internal carotid artery (ICA) stenosis. Asymptomatic severe CAS was defined as patients with 70-99% stenosis detected by carotid digital subtraction angiography (DSA). The longest P-wave and the longest atrial conduction time ACT were considered as the maximal P-wave duration. The difference between the longest P-wave (Pmax) and the shortest P-wave (Pmin) was accepted as PD. (PD=Pmax-Pmin). Atrial EMD was defined as the time interval from the onset of atrial electrical activity to the beginning of mechanical atrial contraction.

Results: The CAS group had significantly longer Pmax and PD values compared to the control group (Pmax 104.72±6.03 and 93.06±7.26 ms, p<0.001; PD 48.55±6.72 and 38.50±8.12 ms, p<0.001). In the TDI examination, the atrial EMD parameters (PA lateral, PA septum) were significantly longer in the CAS group compared to the control group. (77.88±5.13 vs 65.53±9.11 ms; p<0.0001; 63.77±3.95 vs 54.56±7.13 ms; p<0.001 respectively) Both interatrial and intra-atrial EMD times were found to be longer in the CAS group compared to the control group (31.72±7.39 vs 22.13±8.67 ms; p<0.001; 17.61±7.76 vs 11.16±7.76 vs 11.16±7.04 ms; p<0.001, respectively). In the correlation analysis, a positive relationship was found between interatrial and interatrial EMD and Pmax and PD (p<0.001, both).

Conclusion: We found that both intra-atrial and inter-atrial electromechanical conduction times were longer in CAS patients. This suggests that CAS patients are at risk for AF in their follow-up.

Keywords: Carotid artery stenosis, atrial fibrillation, atrial conduction time

INTRODUCTION

Stroke is one of the top three causes of death in developed societies. About 80% of strokes have an ischemic origin, and 15-20% of ischemic strokes are linked to carotid artery stenosis (CAS).[1,2] Atherosclerotic stenosis of the internal carotid artery (ICA) is found in 1-2% of adults in the general population, although >10% of those between the ages of 60 and 79 are affected.[3,4]

Atrial fibrillation (AF) is a commonly encountered clinical arrhythmia that causes hemodynamic disturbances, frequent hospitalizations, and thromboembolic events, affecting 1-2% of the general population.[5] Although the exact mechanisms causing AF are not fully understood, atherosclerotic risk factors such as hypertension (HT), diabetes mellitus (DM), advanced age, endothelial dysfunction, and increased oxidative stress play significant roles in the pathogenesis of AF.[6] As is known, there are similar risk factors in the pathogenesis of coronary artery disease (CAD), just like in AF.[7] Therefore, the risk of developing new AF may increase in these patients.

Atrial conduction time (ACT) represents the interval between sinus impulses and atrial mechanical contraction. As an alternative to invasive electrophysiological measurements, it can be analyzed non-invasively by measuring with tissue Doppler echocardiography imaging (TDI).[8] It has been shown that the prolongation of intraatrial and interatrial conduction times, known as atrial electromechanical delay (EMD), is associated with a higher risk of AF.[9,10] At the same time, it has been shown that P-wave dispersion (PD)

Corresponding Author: Şaban Keleşoğlu, dr.s.k@hotmail.com

and maximum P-wave (Pmax) duration can be noninvasive electrocardiographic indicators of AF.[10,11]

To our knowledge, there is no study evaluating atrial conduction abnormalities in patients with CAD using non-invasive tests such as TDI and ECG. In this study, we aimed to determine the correlation of atrial conduction abnormalities between surface electrocardiographic and TDI measurements in the CAS patient group.

METHODS

The study was conducted with the permission of Erciyes University Faculty of Medicine Clinical Researches Ethics Committee (Date: 24.11.2021, Decision No: 2021/767). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The retrospective study included 217 patients diagnosed with extracranial ICA stenosis at our institution between March 2022 and July 2023. Patients with symptomatic CAS, a history of ischemic or hemorrhagic stroke, a history of CAD, segmental or global wall motion abnormalities, evidence of moderate to severe heart valve disease on echocardiography, structural heart disease, permanent or paroxysmal AF, bundle branch block or any conduction disorder on ECG, those who underwent pacemaker implantation, patients with known thyroid disease and chronic obstructive pulmonary disease, and those with electrolyte imbalances that could affect atrial EMD were excluded from the study. Asymptomatic individuals with risk factors such as HT, diabetes, or a family history of cardiovascular disease, who underwent carotid artery ultrasound for the purpose of subclinical disease screening, and in whom critical stenosis (>70%) was detected in either the right or left carotid artery, were included. Patients with risk factors but normal carotid Doppler findings formed the control group. We studied patients with 70% or more carotid stenosis on Doppler ultrasonography (DUSG) in the study for CAAG. So patients with asymptomatic severe CAD were included in the study. 141 patients who did not meet the inclusion criteria were excluded from the study. Finally, 76 patients were analyzed. Asymptomatic severe CAS was defined as patients with 70-99% stenosis detected by carotid digital subtraction angiography (DSA) in the right and/or left extracranial ICA without a transient ischemic attack or stroke in the past 6 months. According to current guidelines for CAS, patients were started on medical treatment and underwent revascularization therapy. Before the planned treatments, all patients underwent detailed transthoracic echocardiographic examinations.

Detailed medical history, physical examination, 12-lead electrocardiography (ECG), complete blood count, and serum biochemistry were obtained from all patients. The presence of classical cardiovascular risk factors such as HT, DM, and hyperlipidemia was evaluated. DM, HT, and hyperlipidemia were defined as previously described.[12]

Electrocardiography

ECG recordings were performed with at least 3 QRS complexes for each derivation, at a speed of 25 mm/sec, with an amplitude of 1 mV, and in standard 12 derivations using a 3-channel simultaneous Philips brand machine ECG device. During the recording, patients were allowed to breathe comfortably, but they were not permitted to speak. The P-wave durations in all derivations were manually measured using calipers and magnifying lenses to reduce measurement errors.

The beginning of the P-wave was taken as the point where the isoelectric line intersects with the P-wave. The endpoint was taken as the intersection of the isoelectric line and the endpoint of the P-wave. The longest P-wave and the longest ACT were considered as the maximal P-wave duration. The difference between the longest P-wave (Pmax) and the shortest P-wave (Pmin) was accepted as PD (PD=Pmax-Pmin). All calculations were evaluated separately in a single-blind manner by two cardiology specialists who were unaware of the patients' clinical characteristics, and the average of these two values was accepted as PD and maximum P-wave duration.

Echocardiography

Conventional echocardiography was performed with 2-dimensional, M-mode, pulsed wave, continuous, color Doppler and tissue Doppler imaging using Philips Epiq 7 ultrasound system (Philips, Andover, Mass., USA). Simultaneous ECG recording was done. All patients were in sinus rhythm at the time of examination. Conventional echocardiographic images were obtained from the parasternal and apical views according to the guidelines of the American Society of Echocardiography.[14] Left ventricular (LV) diameters and wall thickness were measured from the para- sternal views by M-mode echocardiography. The Teichholz method was used for the calculation of LV ejection fraction. The left atrial area and diameter were measured from the parasternal long axis view. Mitral inflow velocities were measured from apical views.

Atrial Electromechanical Time Measurement

TDI was performed using transducer frequencies of 3.5–4.0 MHz. The spectral pulsed Doppler signal filters were adjusted until a Nyquist limit of 15–20 cm/s was obtained. The minimal opti- mal gain was used. Myocardial TDI velocities [peak systolic (S'), early diastolic (E') and late diastolic velocities (A')] were measured with spectral pulsed Doppler from the apical 4-chamber view. The ultrasound beam slope did not exceed 15% to acquire the optimal angle of imaging. The monitor sweep speed was adjusted at 50–100 mm/s to optimize the spectral display of myocardial velocities. Atrial EMD was defined as the time interval from the onset of atrial electrical activity (P-wave on surface ECG) to the beginning of mechanical atrial contraction (late diastolic A-wave). All values were averaged over 3 consecutive beats. Atrial EMD was measured from the lateral mitral annulus and called 'PA lateral', from the septal mitral annulus, called 'PA septal', and from the right ventricle tricuspid annulus, called 'PA tricuspid'. Interatrial EMD was calculated as the difference between PA lateral and PA tricuspid, intra-atrial EMD was calculated as the difference between PA septum and PA tricuspid, and left-atrial EMD was calculated as the difference between PA lateral and PA septum.[8]

Statistical Analysis

Statistical analyzes were performed using SPSS Statistics Package version 21.0 (SPSS Inc, Chicago, IL, USA) for Windows. The distribution characteristics of the data were determined by using Kolmogorov–Smirnov test. Independent Sample t test was used for Parametric scale variables. Mann–Whitney U test was used for nonparametric scale variables. The $\chi 2$ test was used for univariate analysis of the categorical variables. The variables were given as means±SD; categorical variables were defined as percentages. Correlation analyses were per- formed using Pearson's coefficient of correlation and Spearman coefficient of correlation. A probability value of $p<0.05$ was considered significant, and 2-tailed p values were used for all statistics.

RESULTS

The baseline characteristics of the patients and the control group are presented in Table 1. The average ages of the CAS group and the control group were 63.4±6.4 and 62.5±7.4, respectively. There was no significant difference between the patients and the control group in terms of demographic parameters such as age, gender, HT, DM, and smoking. Among the baseline blood parameters, the low-density lipoprotein cholesterol (LDL-C) level was significantly higher compared to the control group, while the high-density lipoprotein cholesterol (HDL-C) level was significantly lower (p=0.048, p=0.026, respectively). Other blood parameters were similar between the groups.

The CAS group had significantly longer Pmax and PD values compared to the control group. (Pmax 104.72±6.03 and 93.06±7.26 ms, p<0.001; PD 48.55±6.72 and 38.50±8.12 ms, p<0.001) However, the Pmin values did not show a significant difference (Table 2).

Echocardiographic and atrial electromechanical time parameters are shown in Table 3. LV systolic and diastolic diameters, interventricular septum and LV posterior wall thickness, and LV ejection fraction were similar in both groups. No significant difference was observed between the groups in the left atrium diameters and the parameters indicating LV diastolic functions, namely isovolumetric relaxation time and deceleration time.

In the TDI examination, the atrial EMD parameters (PA lateral, PA septum) were significantly longer in the CAS group compared to the control group. (77.88±5.13 vs 65.53±9.11 ms; p<0.001; 63.77±3.95 vs 54.56±7.13 ms; p<0.001 respectively). The tricuspid PA, on the other hand, was similar in both groups (46.16±6.08 vs 43.40±6.96 ms: p=0.090).

Both interatrial (PA lateral–PA tricuspid) and intraatrial (PA septal–PA tricuspid) EMD times were found to be longer in the CAS group compared to the control group (31.72±7.39 vs 22.13±8.67 ms; p<0.001; 17.61±7.76 vs 11.16±7.76 vs 11.16±7.04 ms; p<0.001, respectively). Left atrial EMD (PA lateral-PA septal) was similar between the two groups. (14.11±6.58 vs 10.96±8.60 ms: p=0.098) (Figure 1).

In the correlation analysis, a positive relationship was found between interatrial EMD and Pmax and PD (r=0.617, p<0.05 and r=0.308, p<0.05, respectively). Similarly, a similar relationship was observed between intra-atrial EMD and Pmax and PD (r=0.333, p<0.05 and r=0.372, p<0.001, respectively) (Figure 2).

DISCUSSION

In our study, three important findings identified in patients with CAS can be listed as follows:

Table 1. Baseline clinical and demographic features of the study groups

Variables	Control group (n=54)	Carotis artery disease (n=76)	p-value
Age (years)	62.56±7.48	63.44±6.40	0.609
Male/female	17/13	21/15	0.891
HT	11 (36.6%)	17 (47.2%)	0.388
DM	7 (23.3%)	11 (30.55%)	0.512
Smoke	7 (23.3%)	11 (30.55%)	0.512
Systolic blood pressure, mm Hg	120.23±12.30	119.13±10.14	0.693
Diastolic blood pressure, mm Hg	73.16±6.42	74.47±7.48	0.455
Glucose (mg/dl)	93.70±14.10	101.94±24.35	0.106
Creatinine (mg/dl)	0.84±0.20	0.95±0.27	0.059
Total cholesterol (mg/dl)	197.38±48.49	204.05±36.65	0.527
HDL cholesterol (mg/dl)	47.35±8.32	42.22±9.70	0.026*
LDL cholesterol (mg/dl)	115.70±39.19	132.83±29.64	0.048*
TG (mg/dl)	168.05±86.81	171.05±71.37	0.878
AST (U/L)	18.83±6.13	22.47±13.39	0.175
ALT (U/L)	18.36±7.69	18.83±13.80	0.870
WBC (10^3/uL)	7.69±1.49	7.75±2.20	0.904
Hemoglobin (g/dl)	13.90±1.67	17.80±20.97	0.315
Platelet (x10^3/mm^3)	259.26±65.19	258.68±76.05	0.974

Data are expressed as mean±standard deviation for normally distributed data and percentage (%) for categorical variables. DM: Diabetes mellitus, HT: Hypertension, WBC: White blood cell, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Table 2. Electrocardiographic characteristics of the study population

Variables	Control group (n=54)	Carotis artery disease (n=76)	p-value
Heart rate (min)	77.33±10.90	80.05±2.31	0.149
Pmax (ms)	93.06±7.26	104.72±6.03	p<0.01
Pmin (ms)	54.56±3.53	56.16±3.67	0.077
PD (ms)	38.50±8.12	48.55±6.72	p<0.01

Pmax: Maximum P-wave duration, Pmin: Minimum P-wave duration, PD: P-wave dispersion, Min: Minute, ms: Millisecond

Table 3. Echocardiography characteristics of the study population

Variables	Control group (n=54)	Carotis artery disease (n=76)	p-value
LA Diameter, cm	3.36±0.32	3.50±0.26	0.057
LVEDD, cm	4.72±0.32	4.75±0.45	0.747
LVESD, cm	3.00±0.35	3.06±0.32	0.458
IVSD, cm	1.06±0.11	1.07±0.16	0.847
PWD, cm	1.04±0.08	1.07±0.15	0.370
LVEF, %	66.96±4.44	64.69±4.74	0.051
PA lateral, ms	65.53±9.11	77.88±5.13	**p<0.01**
PA septum, ms	54.56±7.13	63.77±3.95	**p<0.01**
PA tricuspid, ms	43.40±6.96	46.16±6.08	0.090
PA lateral-PA tricuspid (Inter-atrial delay)	22.13±8.67	31.72±7.39	**p<0.01**
PA septal-PA tricuspid (Intra-atrial delay)	11.16±7.04	17.61±7.76	**p<0.01**
PA lateral- PA septal (Left-atrial delay)	10.96±8.60	14.11±6.58	0.098
Mitral E, cm/S	7.6±1.17	7.30±1.25	0.328
Mitral A, cm/S	5.95±1.61	6.27±1	0.324
DT, ms	170.76±23.04	165.61±29.8	0.442
IVRT, ms	86.7±10.33	89.13±8.83	0.305
(S') cm/s	11±3.26	11.02±2.47	0.969
(E') cm/s	13.66±3.4	12.52±2.64	0.131
(A') cm/s	9.46±2.6	10.94±2.26	0.017*

LA: Left atrium, LVEDD: LV end-diastolic dimension, LVESD: LV end-systolic dimension, IVSD: Interventricular septum thickness, PWD: Posterior wall thickness, LVEF: LV ejection fraction, DT: Deceleration time, IVRT: Isovolumic relaxation time, Interatrial delay: PA lateral-PA tricuspid, Intra-atrial delay: PA septum-PA tricuspid, Left-atrial delay: PA lateral-PA septum, S': Systolic velocity from the mitral annulus, E': Early diastolic velocity from the mitral annulus, A': Late diastolic velocity from the mitral annulus

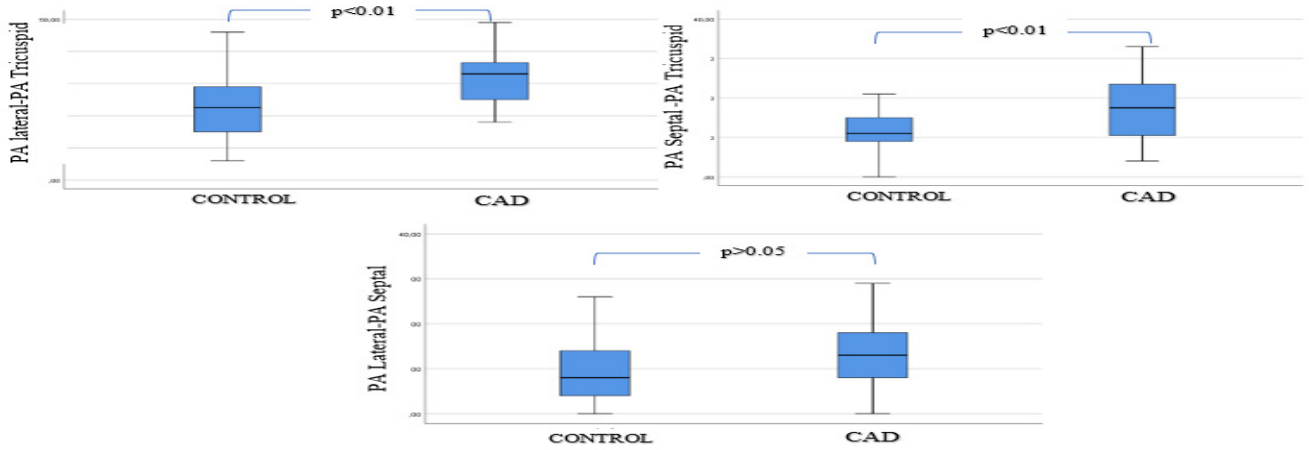

Figure 1. Change PA lateral-PA tricuspid, PA septal-PA tricuspid, PA lateral-PA septal between study groups

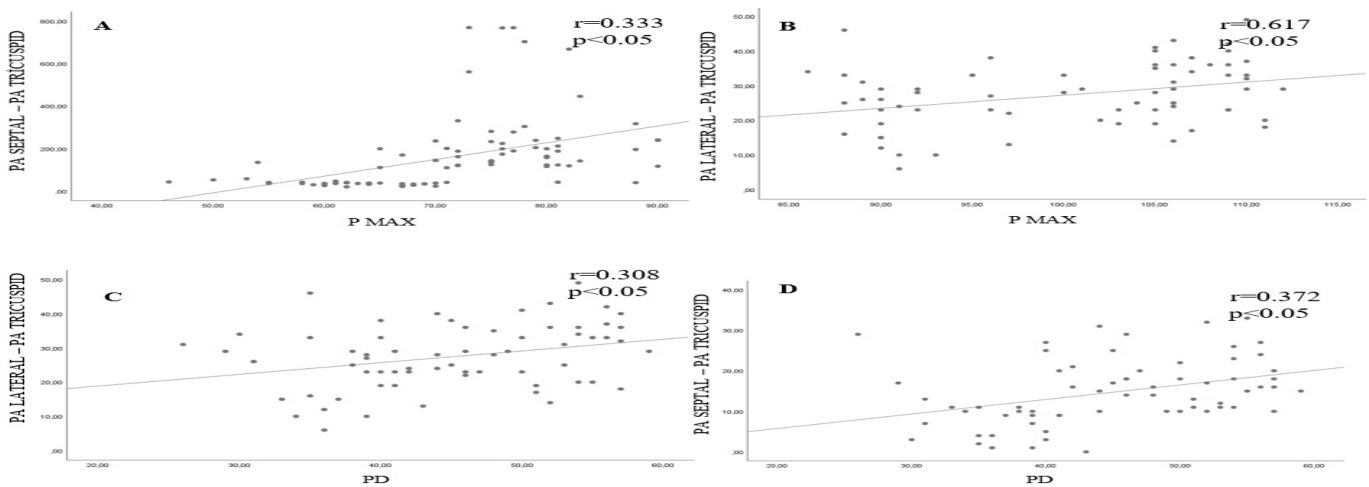

Figure 2. Correlation between PA septal-PA tricuspid and Pmax duration. (**A**) Correlation between PA lateral-PA tricuspid and Pmax duration. (**B**) Correlation between PA lateral-PA tricuspid and PD duration. (**C**) Correlation between PA septal-PA tricuspid and PD duration (**D**)
Pmax: Maximum P-wave duration, PD: P-wave dispersion

(1) The PD and Pmax durations being longer in the 12-lead surface ECG compared to the control group; (2) The duration of both intraatrial and interatrial electromechanical conduction being longer as detected by TDI; (3) The PD and Pmax durations showing a significant correlation with the intraatrial and interatrial electromechanical coupling durations.

AF is the most common arrhythmia in the community, causing an increase in cardiovascular mortality and morbidity.[15] AF is important to recognize early because it leads to stroke and thromboembolism in patients, increasing the risk of mortality and morbidity, thereby reducing the quality of life. In addition to all these effects, it also imposes a significant financial burden on society. Hospital stays, procedure and medication costs, along with absenteeism costs, lead to significant expenses.[16] Therefore, it is important to determine which patients with CAS are at higher risk for developing AF.

When we evaluate the literature, there are conflicting results regarding the prevalence of AF in patients with CAS. As is known, atherosclerotic vascular diseases are an important risk factor for the development of AF and are often found together. Adamsson Eryd et al.[17] in their study, demonstrated that both carotid atherosclerosis and high carotid artery intima-media thickness (IMT) are associated with an increased risk of AF occurrence over an average follow-up period of 15 years. They even claimed that carotid IMT showed a similar effect to HT and heart failure, which are among the strongest risk determinants for AF development. Willeit et al.[18] in their study, found a higher risk of AF development in individuals with carotid atherosclerosis compared to those without. Supporting all these studies, the Rotterdam study, although differing in details, also showed that the presence of carotid atherosclerosis measured ultrasonographically could predict the future risk of AF.[19] Luo et al.[20] claimed that AF was associated with a higher recurrence rate after radiofrequency catheter ablation in patients with carotid atherosclerosis.

In a study conducted by Chen LY et al.[21] on young patients with lone AF, they found a significant association between high carotid IMT and carotid-femoral pulse wave velocity and the development of AF. In addition to Pmax and PD, which can be easily measured with an ECG, the evaluation of Atrial EMD with TDI is one of the precursors to the risk of developing new-onset AF. Therefore, in this study, we aimed to assess the risk of AF in patients with CAS by examining the aforementioned parameters.

Pmax and PD have been used to predict the risk of AF in patients with paroxysmal AF, mitral stenosis, aortic stenosis, dilated cardiomyopathy (DCM), acute myocardial infarction, atherosclerotic heart disease, ischemia with no obstructive arteries, primary hyperparathyroidism and angina.[22-28] Atrial EMD, on the other hand, has been found to be longer in individuals with paroxysmal AF and mitral stenosis compared to controls, and this condition has been reported to be associated with PD.[29] Additionally, it has been shown in previous studies that atrial EMD increases in many clinical disorders such as DM, HT, and non-ischemic DCM.[30-34] In conclusion, both atrial EMD and Pmax and PD have frequently been used as non-invasive markers to predict the risk of AF development.

In our study, the average PD and Pmax values were found to be significantly longer in patients with CAS compared to the control group. Moreover, the intraatrial and interatrial EMD were significantly longer and showed a significant correlation with PD and Pmax durations. Some plausible reasons can be suggested for the increased risk of AF in patients with CAS. One of these is closely related to carotid atherosclerosis and wall thickening, coronary atherosclerosis, and microvascular damage.[35-37] This condition can lead to hypoperfusion and ischemia of the atrium, and subsequently to fibrosis. An increase in atrial fibrosis can cause prolonged intraatrial/interatrial conduction times and non-homogeneous propagation of sinus impulses.[38] On the other hand, with advancing age, the atherosclerotic process can affect the carotid arteries as well as the aorta and other peripheral vessels, leading to an increase in systolic load and aortic stiffening. The changes that occur at the end of this process may lead to ventricular remodeling as well as atrial remodeling, and over time, all these changes in the heart may have laid the groundwork for the prolongation of the aforementioned parameters.[39-41]

It is known that the increase in the left atrial diameter is significant in the development of AF.[42] However, in the study by Tükek et al., it has been reported that in patients with paroxysmal AF, PD is prolonged even when the atrial diameter is normal.[43] In our study, no significant difference was observed between the patient and control groups in terms of left atrial diameter.

Current guidelines recommend echocardiography and 24-72 hour rhythm Holter monitoring in patients with 50-99% ICA stenosis who have recently experienced a transient ischaemic attack or stroke.[44]

CONCLUSION

In our study, we demonstrated that intraatrial and interatrial EMD, Pmax, and PD, which are techniques predicting the risk of future AF development, were significantly longer in asymptomatic patients with CAS compared to controls. Since atrial EMD is increased in paroxysmal AF and is considered a predictor of new-onset AF, patients with severe CAD who have not yet experienced an ischemic stroke should also be investigated for paroxysmal AF, and if necessary, rhythm Holter monitoring should be considered for this patient group.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was conducted with the permission of Erciyes University Faculty of Medicine Clinical Researches Ethics Committee (Date: 24.11.2021, Decision No: 2021/767).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

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

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Echocardiographic evaluation of subclinical ventricular dysfunction in polycystic ovary syndrome: the role of isovolumic acceleration and myocardial performance index

Doğaç Okşen[1], Muzaffer Aslan[2], Yunus Emre Yavuz[2], Necip Nas[3]

[1]*Department of Cardiology, Faculty of Medicine, Altınbaş University, İstanbul, Turkiye*
[2]*Department of Cardiology, Faculty of Medicine, Siirt University, Siirt, Turkiye*
[3]*Department of Internal Medicine, Faculty of Medicine, Siirt University, Siirt, Turkiye*

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ABSTRACT

Aims: Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with increased cardiovascular risk and potential subclinical ventricular dysfunction. Isovolumic acceleration (IVA) is a novel echocardiographic parameter sensitive to myocardial contractility and less influenced by preload and afterload, making it a promising tool for early detection of cardiac dysfunction. This study aimed to assess the utility of IVA and other echocardiographic markers in identifying subclinical ventricular dysfunction in PCOS patients.

Methods: This cross-sectional study analyzed echocardiographic parameters of 59 PCOS patients and 60 age-and BMI-matched controls. Echocardiographic measurements included IVA, myocardial performance index (MPI), and additional indices of right and left ventricular function.

Results: PCOS patients exhibited significantly higher BMI and fasting glucose levels ($p<0.001$) than controls. Left ventricular MPI was elevated in the PCOS group (0.56±0.11 vs. 0.46±0.07, $p=0.004$). Right ventricular IVA was significantly lower in PCOS patients (2.77±0.69 vs. 3.98±1.01, $p=0.030$), while tricuspid acceleration time was prolonged ($p<0.001$). A positive correlation between IVA and MPI was observed in the PCOS group ($R=0.453$, $p<0.001$). Multivariate analysis identified PCOS as an independent predictor of subclinical left ventricular dysfunction (OR=0.211, $p<0.001$).

Conclusion: PCOS is associated with subtle alterations in cardiac function, particularly affecting right ventricular systolic function as detected by IVA. These findings highlight IVA's potential as a non-invasive marker for early cardiovascular risk assessment in PCOS patients, emphasizing the importance of regular cardiac monitoring in this population.

Keywords: Polycystic ovary syndrome, tissue derived Doppler, isovolumic acceleration, myocardial performance index, subclinical ventricular dysfunction

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder among women of reproductive age, characterized by hyperandrogenism, chronic anovulation, and polycystic ovarian morphology.[1,2] In addition to reproductive complications, PCOS is closely linked with various metabolic and cardiovascular disturbances, such as insulin resistance, dyslipidemia, and hypertension. These risk factors predispose PCOS patients to cardiovascular disease (CVD), and recent research indicates a heightened risk for ventricular dysfunction.[3,4] Understanding the cardiovascular implications of PCOS is crucial, as these patients are often asymptomatic but may have underlying subclinical cardiac dysfunction that could lead to adverse outcomes if left unaddressed.

Isovolumic acceleration (IVA) has emerged as a promising echocardiographic marker for assessing systolic function, particularly in detecting subclinical right and left ventricular systolic dysfunction.[5] IVA provides a non-invasive measurement of myocardial contractility that is less affected by preload and afterload variations, making it suitable for evaluating subtle changes in cardiac function. Studies involving other metabolic and endocrine disorders have demonstrated the utility of IVA in identifying early ventricular dysfunction, suggesting its potential as a prognostic tool.[6,7] However, literature on the role of IVA in PCOS patients remains limited, leaving an important gap in understanding subclinical cardiovascular alterations in this population.

Despite the established cardiovascular risks associated with PCOS, comprehensive studies focusing on IVA and other echocardiographic parameters in this patient group are lacking. The primary aim of this study was to investigate the

Corresponding Author: Doğaç Okşen, dogacoksen@gmail.com

utility of IVA as an echocardiographic marker for detecting subclinical right and left ventricular systolic dysfunction in patients with PCOS Through a comparative analysis of echocardiographic data from PCOS patients and a matched control group, this study seeks to determine the significance of IVA as an early diagnostic marker for cardiac dysfunction, offering valuable insights for the preventive management of cardiovascular health in this at-risk population.

METHODS

Ethics

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The study was conducted with the permission of Siirt University Ethics Committee (Date: 13.12.2022, Decision No: 2022/12/01/01).

Study Design and Patient Selection

This study was conducted as a retrospective, non-randomized, cross-sectional analysis at Siirt Training and Research Hospital. Eligible PCOS patients and matched controls were identified from institutional medical records between 2020 December and 2022 December. This study design facilitated the comparison of echocardiographic parameters between the two groups, offering insights into the prevalence of subclinical ventricular dysfunction among PCOS patients.

Written informed consent was waived for this retrospective analysis, as patient data were anonymized and securely stored to ensure confidentiality. However, all efforts were made to uphold the rights and privacy of the study participants, consistent with institutional guidelines.

PCOS Diagnosis and Inclusion/Exclusion Criteria

PCOS was diagnosed according to the Rotterdam criteria, requiring at least two of the following: oligo- or anovulation, hyperandrogenism (clinical or biochemical), and polycystic ovarian morphology confirmed via ultrasonography.[8] Inclusion criteria comprised women aged 18-40 with a confirmed PCOS diagnosis and no prior history of CVD, diabetes, or other significant comorbidities affecting cardiac function. Exclusion criteria included congenital heart disease, hypertension, diabetes mellitus, known cardiac arrhythmias, use of medications influencing heart rate or rhythm, and any structural abnormalities detected on initial echocardiographic evaluation. Control subjects were matched to the PCOS group based on age and body-mass index (BMI) and were free from PCOS or any other endocrine disorders.

Echocardiographic Assessment

Echocardiographic evaluations were conducted by certified cardiologists blinded to the participants' clinical statuses. Examinations adhered to standardized protocols, utilizing the Philips Affinity 50 echocardiography system with transducer S4-2 operates within a frequency range of 2 to 4 MHz. Parameters assessed included left ventricular end-diastolic diameter (LVED), left ventricular end-systolic diameter (LVES), interventricular septum (IVS) thickness, and posterior wall (PW) thickness, as outlined in **Table 1**. Left ventricular ejection fraction (LVEF) was calculated using the modified Simpson method. Additional parameters indicative of subclinical dysfunction, such as mitral E and A wave velocities, E/A ratio, and isovolumic relaxation and contraction times (IVRT and IVCT), were measured.

Right ventricular function was assessed using tricuspid annular plane systolic excursion (TAPSE) and mean pulmonary artery pressure (meanPAP), which are markers of right ventricular systolic function. IVA was calculated for both ventricles as an index of myocardial contractility independent of preload and afterload. The myocardial performance index (MPI), a combined marker of systolic and diastolic function, was measured for both ventricles. Doppler parameters, including acceleration time (AT) and ejection time (ET) for the mitral and tricuspid valves, as well as derived parameters like isovolumic velocity (IVV) and systolic velocity (Sa), were recorded (**Figure 1**). To ensure accuracy and reproducibility, all measurements were averaged over three cardiac cycles.

Statistical Analysis

Data analysis was conducted using SPSS version 22, with a significance threshold set at $p<0.05$. Normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed data were expressed as mean±standard deviation (SD), while median and interquartile range (IQR) were used for non-normally distributed data. Differences between the PCOS and control groups were evaluated using independent t-tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data. Categorical variables were analyzed using Chi-square tests. Correlation analyses between echocardiographic parameters and clinical markers were performed using Pearson correlation coefficients for parametric data and Spearman's rank correlation for non-parametric data.

A multivariate logistic regression model was constructed to identify independent predictors of subclinical left ventricular dysfunction. Variables with $p<0.10$ in univariate analysis were included in the multivariate model to adjust for potential confounders. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to quantify associations.

RESULTS

The demographic and clinical characteristics of the study population showed that the BMI in the PCOS group was significantly higher than in the control group (30.64±4.92 vs. 25.52±2.50, $p<0.001$). Additionally, fasting glucose levels were notably elevated in the PCOS group compared to the controls (115.28±24.86 vs. 97.18±13.47, $p<0.001$). In terms of the lipid profile, triglyceride (TG) levels were significantly higher in the PCOS group (191.13±82.78 vs. 140.26±36.19, $p<0.001$). No significant differences were observed between the groups for other parameters, such as age, diastolic blood pressure (DBP), systolic blood pressure (SBP), heart rate, HDL-C, total cholesterol, LDL-C, and creatinine ($p>0.05$) (**Table 2**).

When examining left ventricular echocardiographic findings, mitral AT was significantly longer in the PCOS group than in the control group (30.61±6.21 vs. 25.0±4.58, $p=0.006$). The MPI of the left ventricle was also higher in the PCOS group (0.56±0.11 vs. 0.46±0.07, $p=0.004$). Other echocardiographic measurements, including LVED, LVES, IVS thickness, PW

Table 1. Left ventricle echocardiographic findings

Variable	PCOS (n:59)	Control group (n:60)	p value
LVED, mm	45.28±3.55	44.23±2.85	0.124
LVES, mm	26.83±3.37	26.01±3.03	0.611
IVS, mm	10.42±1.45	10.31±1.34	0.551
PW, mm	9.74±1.28	9.63±1.17	0.594
LVEF, %	63.98±2.61	63.38±2.80	0.243
LA, long axis, mm	31.49±4.08	30.75±4.90	0.131
E mitrale, cm/sec	11.05±3.49	10.61±3.37	0.872
A mitrale, cm/sec	12.10±2.65	11.73±2.65	0.945
E/A ratio	0.95±0.37	0.94±0.38	0.852
Sa mitrale, cm/sec	10.25±2.40	10.30±2.46	0.787
IVV mitrale, cm/sec	8.71±3.56	8.96±3.32	0.516
AT mitrale, msec	30.61±6.21	25.0±4.58	0.006
IVA, m/sec2 AA/AC	2.89±1.11	3.85±1.35	0.455
IVRT mitrale, msec	78.22±14.84	68.85±13.93	0.569
IVCT mitrale, msec	59.32±11.96	54.61±10.62	0.114
ET mitrale, msec	247.52±31.21	266.61±30.58	0.927
MPI LV	0.56±0.11	0.46±0.07	0.004

Abbreviations: LVED: Left ventricular end-diastolic diameter, LVES: Left ventricular end-systolic diameter, IVS: Interventricular septum, PW: Posterior wall, LA: Left atrium, E/A: Ratio of early to late ventricular filling velocities, Sa: Systolic annular velocity, IVV: Isovolumic velocity, IVRT: Isovolumic relaxation time, IVCT: Isovolumic contraction time, ET: Ejection time, MPI: Myocardial performance index, IVA: Isovolumic acceleration

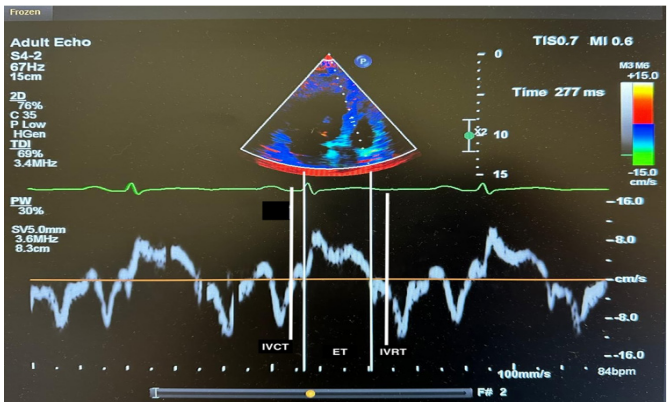

Figure 1. Tissue Doppler imaging demonstrating isovolumic contraction time (IVCT), ejection time (ET), and isovolumic relaxation time (IVRT) in left ventricular systolic function assessment

thickness, LVEF, left atrial diameter, mitral E and A wave velocities, E/A ratio, Sa, IVV, isovolumic relaxation and contraction times, and ET, did not show statistically significant differences between the groups (p>0.05) (Table 1). Regarding right ventricular echocardiographic findings, significant differences were observed in two parameters. Tricuspid AT was significantly longer in the PCOS group compared to the controls (35.01±8.14 vs. 29.40±4.18, p<0.001). Additionally, IVA of the tricuspid valve was lower in the PCOS group, indicating reduced myocardial contractility in these patients (2.77±0.69 vs. 3.98±1.01, p=0.030) (Table 3). No statistically significant differences were observed between groups for other right ventricular parameters, including TAPSE, mean pulmonary artery pressure (meanPAP), tricuspid E and A velocities, E/A ratio, Sa, IVV, IVRT, IVCT, and ET (p>0.05).

A significant positive correlation was found between IVA and MPI in the PCOS group (R=0.453, p<0.001), suggesting an association between these parameters specific to PCOS patients. This correlation was absent in the control group (R=-0.085, p=0.357), indicating a distinct pattern in PCOS patients (Table 4). In multivariate logistic regression analysis, the presence of PCOS emerged as an independent predictor of subclinical left ventricular dysfunction (OR=0.211, 95% CI: 0.091-0.490, p<0.001). Although IVA was included in the model, it did not reach statistical significance (OR=1.382, 95% CI: 0.609-1.132, p=0.240). The model had a moderate explanatory power for predicting left ventricular dysfunction, as indicated by an R-squared value of 0.357 (p=0.007) (Figure 2, Table 5).

DISCUSSION

The primary aim of this study was to investigate the utility of IVA as an echocardiographic marker for detecting subclinical right and left ventricular systolic dysfunction in patients with PCOS. Given the known cardiovascular risks associated with PCOS, our research holds clinical significance by exploring non-invasive methods for early cardiovascular assessment

Table 2. Distribution of demographic and clinical characteristics in patient and control groups

Variable	PCOS (n:59)	Control group (n:60)	p value
Age, years	46.42±10.58	47.83±9.18	0.315
BMI, kg/m²	30.64±4.92	25.52±2.50	<0.001
DBP, mmHg	88.76±6.77	81.40±9.22	0.059
SBP, mmHg	135.0±16.21	131.41±14.55	0.269
Heart rate, beats/min	76.44±12.06	76.73±12.98	0.450
Fasting glucose, mg/dl	115.28±24.86	97.18±13.47	<0.001
HDL-C, mg/dl	41.64±12.23	51.78±11.56	0.637
Tg, mg/dl	191.13±82.78	140.26±36.19	<0.001
Total-C, mg/dl	203.91±47.15	199.06±44.13	0.326
LDL-C, mg/dl	116.96±44.13	112.31±27.30	0.305
Creatinine, mg/dl	0.75±0.15	0.73±0.13	0.306

Abbreviations: PCOS: Polycystic ovary syndrome, BMI: Body-mass index, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, HDL-C: High-density lipoprotein cholesterol, Tg: Triglycerides, Total-C: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol

Table 3. Right ventricle echocardiographic findings

Variable	PCOS (n:59)	Control group (n:60)	p value
TAPSE, mm	27.49±3.52	28.11±3.37	0.643
meanPAB, mmHg	14.47±3.22	15.48±3.65	0.204
E tricuspite, cm/sec	11.22±3.01	11.15±3.03	0.938
A tricuspite, cm/sec	12.88±3.37	12.80±3.40	0.923
E/A ratio	0.91±0.34	0.91±0.34	0.935
Sa tricuspite, cm/sec	12.64±2.45	12.56±2.50	0.859
IVV tricuspite, cm/sec	11.03±4.19	11.00±4.16	0.970
AT tricuspite, msec	35.01±8.14	29.40±4.18	<0.001
IVA tricuspite AN/AO, m/sec^2	2.77±0.69	3.98±1.01	0.030
MPI RV	0.53±0.09	0.53±0.08	0.917
IVCT tricuspite, msec	65.71±10.85	65.76±10.76	0.940
IVRT tricuspite, msec	67.40±16.55	67.56±16.46	0.967
ET tricuspite, msec	250.86±25.86	251.21±25.78	0.994

Abbreviations: LVED: Left ventricular end-diastolic diameter, LVES: Left ventricular end-systolic diameter, IVS: Interventricular septum, PW: Posterior wall, LA: Left atrium, E/A: Ratio of early to late ventricular filling velocities, Sa: Systolic annular velocity, IVV: Isovolumic velocity, IVRT: Isovolumic relaxation time, IVCT: Isovolumic contraction time, ET: Ejection time, MPI: Myocardial performance index, IVA: Isovolumic acceleration

Table 4. Correlation between IVA and MPI levels

Variable	PCOS	Control
MPI LV	R=0.453/p=<0.001	R=-0.085/p=0.357
IVA	R=0.363/p=0.035	R=-0.119/p=0.131

Abbreviations: MPI: Myocardial performance index, LV: Left ventricule, IVA: Isovolumic acceleration

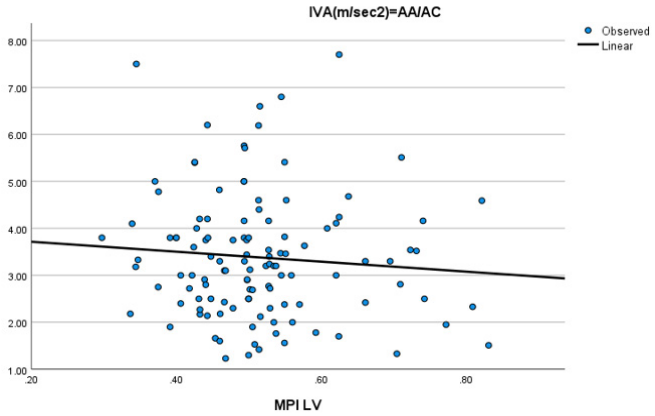

Figure 2. Scatter plot showing the relationship between left ventricular myocardial performance index and isovolumic acceleration
R square 0.357 p= 0.007

Table 5. Multivariate logistic regression analysis of echocardiographic parameters for subclinical LV dysfunction in patients with PCOS

Variable	OR (95% confidence interval)	p value
IVA	1.382 (0.609-1.132)	0.240
PCOS	0.211 (0.0910.490)	<0.001

Abbreviations: LV: Left ventricule, IVA: Isovolumic acceleration, PCOS: Polycystic ovary syndrome

in this high-risk population. The findings suggest that PCOS patients exhibit subtle changes in myocardial performance, which could be detected early with IVA, potentially guiding preventive strategies.

PCOS is associated with subclinical left ventricular dysfunction, primarily influenced by metabolic abnormalities such as insulin resistance and obesity. Studies indicate that patients with PCOS exhibit significant impairments in LV function, detectable through various echocardiographic techniques, including MPI and speckle tracking echocardiography.[9] For instance, the presence of a presystolic wave (PSW) has been identified as an independent predictor of subclinical LV dysfunction, with a prevalence of 54.7% among PCOS patients. Additionally, GLS measurements reveal reduced LV function in PCOS patients compared to healthy controls, correlating negatively with insulin levels.[10-12] GLS, a more advanced and validated echocardiographic parameter, is highly sensitive in detecting subtle left ventricular systolic dysfunction, even in patients with preserved ejection fraction. It provides a comprehensive assessment of myocardial deformation and has been shown to outperform conventional echocardiographic indices, such as LVEF and MPI, in identifying early myocardial impairment.[11,12] Furthermore, a systematic review highlights that while diastolic dysfunction is more pronounced, systolic function remains relatively preserved.[13] These findings underscore the importance of early detection and management of cardiac involvement in women with PCOS to mitigate cardiovascular risks. Machine learning algorithms have been effectively utilized to predict adverse outcomes in PCOS patients, indicating a need for early detection of associated risks, including cardiovascular health.[14,15] Additionally, studies on other conditions, such as Cushing's syndrome, demonstrate that advanced echocardiographic techniques like speckle tracking can identify subclinical myocardial dysfunction, suggesting that similar methodologies could be applied to PCOS patients to assess ventricular function.[16] Furthermore, the integration of predictive models in hormonal imbalance management highlights the importance of personalized healthcare strategies, which could also encompass cardiovascular risk assessments in women with PCOS.[17] Thus, while direct evidence is sparse, the intersection of these findings underscores the potential for developing predictive tools for monitoring cardiac health in PCOS patients.

The MPI serves as a crucial measure of cardiac function, integrating both systolic and diastolic performance, particularly relevant in patients with PCOS. Research indicates that women with PCOS exhibit subclinical left ventricular dysfunction, which can be assessed using MPI; a study found that the presence of a PSW was significantly correlated with higher MPI values, indicating poorer cardiac performance.[9] Additionally, PCOS is associated with insulin resistance, which negatively impacts cardiopulmonary functional capacity, further complicating cardiac health in these patients.[18] The MPI, defined as the sum of isovolumetric contraction and relaxation times divided by ET, provides a non-invasive assessment of heart efficiency, with higher

values indicating worse cardiac function.[19,20] In our study, left ventricular MPI was significantly higher in patients with PCOS, consistent with the literature. However, right ventricular MPI was not significant. The lack of significant differences in right ventricular MPI between PCOS patients and controls might be explained by several underlying mechanisms. The unique myocardial structure of the right ventricle, characterized by thinner walls and higher adaptability to preload and afterload changes, may delay the manifestation of detectable dysfunction.[5] Additionally, while increased arterial resistance and microvascular dysfunction are commonly observed in PCOS, their effects on right ventricular function may not be as pronounced or consistent as on the left ventricle. Lastly, heterogeneity among PCOS phenotypes may contribute to variability in cardiac involvement, with some subgroups showing more prominent left ventricular changes while right ventricular dysfunction remains subclinical.[12]

IVA time, particularly peak myocardial acceleration during isovolumic relaxation (pIVA), has emerged as a significant prognostic marker in various cardiac conditions, especially chronic heart failure (CHF). Studies indicate that lower pIVA levels correlate with increased rates of rehospitalization due to worsening heart failure, demonstrating its predictive value even when adjusted for other parameters like LVEF and E/E' ratios.[21,22] Additionally, IVA has been shown to be a preload-independent indicator of left ventricular contractility, aiding in the identification of subclinical systolic dysfunction in hypertensive and obese patients.[23] Furthermore, right ventricular IVA has also been linked to adverse outcomes in heart failure patients, suggesting that both left and right ventricular assessments can enhance risk stratification and management strategies.[24] Overall, these findings underscore the utility of IVA metrics as valuable tools in cardiac prognostication. Research indicates that ICV serves as a load-independent measure of systolic function, providing valuable insights into myocardial remodeling and treatment response, especially following therapies like sacubitril/valsartan.[25] In a study involving 651 HFrEF patients, baseline ICV was shown to enhance predictive models for LV reverse remodeling, outperforming traditional measures like LVEF alone. Additionally, the systematic review of prognostic models for heart failure highlighted the importance of various functional markers, including ICV, in assessing patient outcomes.[26] Overall, ICV's ability to predict structural and functional cardiac changes underscores its potential as a critical tool in the management of heart failure.

Bringing IVA and PCOS together in this study, we aimed to bridge a gap in the literature. Although IVA has been studied in various metabolic disorders, its application in PCOS has been limited. Our findings revealed significant alterations in IVA among PCOS patients, indicating potential subclinical myocardial impairment specific to this population. While IVA provided meaningful insights, certain parameters did not show significant differences, which could be attributed to sample size limitations or intrinsic variability in PCOS phenotypes. The lack of consistent findings in some echocardiographic parameters across studies suggests that the cardiovascular impact of PCOS may differ widely among individuals, necessitating further investigation.

The absence of significant changes in left ventricular IVA could be attributed to several factors. One potential explanation is the heterogeneity of PCOS phenotypes, with varying degrees of metabolic and cardiovascular involvement influencing myocardial function differently. The heterogeneity of PCOS phenotypes might influence the degree of cardiac involvement, with some subgroups exhibiting more pronounced right ventricular changes due to shared pathophysiological mechanisms such as hyperandrogenism, insulin resistance, and chronic low-grade inflammation. Additionally, the left ventricle's adaptive response to metabolic stressors might delay the onset of detectable systolic dysfunction when using IVA. These observations are supported by studies suggesting that the right ventricle, with its thinner wall structure and different loading conditions, may respond more sensitively to systemic and pulmonary circulatory changes often seen in PCOS.

Despite the non-significant findings for left ventricular IVA, the significant correlation between right ventricular IVA and MPI underscores IVA's potential as a marker for right-sided subclinical dysfunction. This aligns with research highlighting the importance of comprehensive right heart evaluation in conditions involving metabolic syndrome and endocrine disorders, where right ventricular alterations might precede left ventricular changes.

Limitations

This study has several limitations that should be acknowledged. First, the cross-sectional design limits our ability to establish causation or track progression of subclinical cardiac dysfunction in PCOS patients over time. Longitudinal studies would be necessary to better understand the temporal relationship between PCOS and cardiac alterations. Second, the sample size may limit the generalizability of our findings, as a larger, more diverse cohort would provide a clearer representation of IVA's diagnostic utility across different PCOS phenotypes. Third, while IVA is a useful tool for assessing myocardial contractility, it may not capture all aspects of cardiac function; incorporating other advanced imaging modalities, such as cardiac MRI, could enhance the precision of future studies. The absence of global longitudinal strain (GLS) evaluation represents a notable limitation of this study. One limitation of this study is the lack of assessment for intra-observer and inter-observer variabilities in echocardiographic measurements, which may affect the reproducibility and consistency of the results. Additionally, potential confounders like lifestyle factors and genetic predispositions, which were not controlled for in this study, might influence cardiac function and should be considered in future research. Furthermore, addressing potential multicollinearity among predictors would enhance the robustness and reliability of the findings, providing a more comprehensive understanding of the factors influencing left ventricular function in PCOS patients.

CONCLUSION

Our findings suggest that IVA is a valuable tool for assessing right ventricular systolic function in PCOS patients, whereas left ventricular IVA may not be as sensitive for early dysfunction detection in this population. The significant association between right ventricular IVA and MPI supports its potential role in early risk stratification.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was conducted with the permission of Siirt University Ethics Committee (Date: 13.12.2022, Decision No: 2022/12/01/01).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Evaluating the triglyceride glucose index as a novel method for assessing insulin resistance in Turkish women with polycystic ovary syndrome

Burçak Cavnar Helvacı, Sema Hepşen, Burcu Candemir, Erman Çakal

Department of Endocrinology and Metabolism, Ankara Etlik City Hospital, Ankara, Turkiye

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ABSTRACT

Aims: Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, characterized by insulin resistance (IR), hyperandrogenism, and polycystic ovary morphology. IR is a significant contributor to the pathogenesis and long-term complications of PCOS, but current gold-standard methods for assessing IR are often impractical for routine clinical use. This study aimed to evaluate the performance of the triglyceride glucose (TyG) index in identifying IR among Turkish women with PCOS and to assess its variability across different PCOS phenotypes.

Methods: This single-center, retrospective study included 247 patients diagnosed with PCOS according to the 2003 Rotterdam criteria. IR was assessed using both the TyG index and HOMA-IR. The study analyzed demographic and clinical data, including fasting plasma glucose, triglycerides, and various metabolic parameters. ROC curve analysis was used to determine the optimal TyG index cutoff for detecting IR.

Results: The mean age of participants was 24.09±5.53 years, with a mean BMI of 28.12±6.38 kg/m². The study identified a mean HOMA-IR of 3.46±1.82 and a mean TyG index of 4.51±0.26. A significant positive correlation was found between HOMA-IR and the TyG index ($r=0.370$, $p<0.001$). The optimal TyG index cutoff for detecting IR was 4.44, with a sensitivity of 70% and a specificity of 60% (AUC=0.693, $p=0.035$). The TyG index effectively identified IR across different PCOS phenotypes, though HOMA-IR revealed significant differences between some phenotypes.

Conclusion: This study is the first to demonstrate the effectiveness of the TyG index for predicting IR in Turkish women with PCOS and to explore its variability among phenotypes. The TyG index, based on fasting plasma glucose and triglyceride levels, offers a practical, cost-effective alternative to traditional methods for evaluating IR in PCOS.

Keywords: Triglyceride glucose index, insulin resistance, polycystic ovary syndrome, homeostatic model assessment of insulin resistance

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age and the leading cause of anovulatory infertility.[1] It is marked by ovulatory dysfunction, hyperandrogenism, and polycystic ovary morphology (PCOM). PCOS is a multifaceted condition that arises in those who have a genetic susceptibility, influenced by environmental factors from the prenatal period onward. Changes in Gonadotropin-releasing hormone (GnRH) dynamics, resulting from the interaction of genetic and environmental factors, increase both the amplitude and frequency of luteinizing hormone (LH) pulses, as well as serum LH concentrations. Increased LH levels affect ovarian steroidogenesis, shifting it towards androgen production and resulting in a pause in follicle development. Additionally, insulin resistance (IR) and hyperinsulinemia contribute to increased ovarian androgen synthesis. The primary clinical findings of the disease are related to hyperandrogenemia (HA) and IR.[2] The diagnostic criteria for PCOS are established by three major groups; The National Institutes of Health/National Institute for Child Health and Human Diseases (NIH/NICHD) (1990), The European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) (2003), The Androgen Excess-PCOS Association (2006).[3-5] PCOS is a lifelong disorder that can present with varying phenotypes. Even within the same patient, different phenotypes may appear at different times. Based on these diagnostic criteria, four distinct phenotypes were first defined and continue to be accepted today.[6] Phenotype frequency varies across different ethnic backgrounds. Additionally, the frequency can be influenced by whether the study population is drawn from clinic patients or the general community.

Corresponding Author: Burçak Cavnar Helvacı, burcakcavnar@gmail.com

IR is a cornerstone of both the pathogenesis and clinical findings of PCOS. Most women with PCOS display elevated levels of insulin, either at rest or in response to glucose, along with IR, regardless of body-mass index (BMI). IR has been observed in 50–70% of women with PCOS who maintain a normal BMI, and this percentage may be even greater among those who are obese.[7] The prevalence of prediabetes and type 2 diabetes mellitus (DM) among PCOS patients is reported to be 35–40%.[6] Each year, 2% of women with normoglycemia and 16% of women with prediabetes and PCOS progress to type 2 diabetes.[8,9] Therefore, PCOS is considered an independent risk factor for type 2 diabetes, and it is recommended that patients be regularly evaluated for glucose homeostasis.[6] Additionally, PCOS may be linked to various other metabolic conditions, including metabolic syndrome, dyslipidemia, and hepatic steatosis.[10] Therefore, early identification of IR and its variation across different phenotypes may be important. Although the hyperinsulinemic-euglycemic clamp (HIEC) is considered the gold standard for assessing IR, it is not suitable for clinical practice. Previous studies have shown that the homeostatic model assessment for IR (HOMA-IR) correlates with the HIEC.[11] However, measuring insulin levels is not typically included in routine examinations. The triglyceride glucose (TyG) index, calculated from fasting triglyceride (TG) and blood glucose (FBG) levels, offers a straightforward and cost-effective method for detecting IR. A systematic review concluded that the TyG index had a sensitivity of 96% and a specificity of 99% when the HIEC and HOMA-IR were used as reference tests.[12]

In our research, we sought to examine how effectively the TyG index identifies IR, and to evaluate its variation among different PCOS phenotypes.

METHODS

Ethics

The study was conducted with the permission of the Scientific Researches Evaluation and Ethics Committee of Ankara Etlik City Hospital (Date: 12.06.2024 Decision No: AESH-BADEK-2024-575). The study was conducted in accordance with the principles of the Helsinki Declaration.

Study Design

The study is a single-center, retrospective study conducted at the Endocrinology and Metabolism Department outpatient clinic of Ankara, in Turkey.

Demographic data and patients' clinical history were reviewed. We examined FPG, TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), follicle- stimulating hormone (FSH), LH, total testosterone (with LCMS), insulin at fasting status levels. The serum levels of LH, FSH, and insulin were assessed using chemiluminescence techniques (Siemens, New York, USA). Blood samples were collected from participants following an eight-hour fasting period during the follicular phase of menstrual cycle.

Calculated parameters related to IR: TyG index: ln[fasting Tg (mg/dl)×FPG (mg/dl)/2]; HOMA-IR: [FPG (mg/dl)]×[Fasting insulin (mIU/L)] / 405. IR was defined as a HOMA-IR value ≥2.5.

Study Participants

The study included adult patients with newly diagnosed PCOS who had not yet undergone any treatment related to the condition. Patients were diagnosed based on the 2003 Rotterdam criteria, which require at least two of the following three items: (I) oligo-ovulation and/or anovulation, (II) clinical and/or biochemical hyperandrogenism, and (III) polycystic ovaries.[4] Before diagnosing PCOS, PCOS-mimicking conditions were excluded. We assessed IR using both TyG index and HOMA-IR. We also examined differences in IR between PCOS phenotypes. We classified PCOS phenotypes based on the Rotterdam criteria as follows: A (oligo-ovulation, hyperandrogenism, polycystic ovaries), B (oligo-ovulation, hyperandrogenism), C (hyperandrogenism, polycystic ovaries), and D (oligo-ovulation, polycystic ovaries).

Exclusion Criteria

1. Women who had breastfed in last year
2. Pregnancy
3. Malignancy
4. DM
5. Using of medications that may affect glucose homeostasis
6. Non-euthyroid status

Patients who met the study criteria and agreed to participate were given detailed information about the study.

Statistical Analysis

The data were input into an Excel spreadsheet (Microsoft, Redmond, Washington) for analysis. Statistical analyses were performed using IBM SPSS Statistics software. To assess the normal distribution of the variables, we used both analytical methods (the Shapiro-Wilk test) and visual techniques, such as histograms. Normal continuous variables were expressed as means±standard deviations. Those with skewed distributions were expressed as medians with minimum and maximum values. To compare differences between two groups, we used either the independent sample Student's t-test or Mann-Whitney U test. To compare differences between three or more groups, we used one-way ANOVA. A significance level of 5% (type-I error) was used to assess statistical significance. Pearson correlation tests were conducted to investigate the relationships between variables and assess their significance.

RESULTS

The study included 247 patients from September 2022 to September 2024. The mean age of the patients was 24.09±5.53 [SD] years. Two (0.8%) patients with a diagnosis of primary hypothyroidism were receiving levothyroxine sodium treatment and were euthyroid. One (0.4%) patient had epilepsy, and one (0.4%) had accompanying depression. The mean BMI of the patients was 28.12±6.38 kg/m^2. Waist and hip measurements were available for 150 (60.7%) patients. The

mean waist measurement of these 150 patients was 89.24±14.48 cm, while the mean hip measurement was 106.12±15.57 cm. The mean waist/hip ratio was 0.84±0.07. Of the patients, 89 (36%) had obesity, 100 (40.5%) had a normal weight, and 58 (23.5%) were overweight (Table 1). One hundred thirty two (53.4%) patients were classified as phenotype A, 4 (1.6%) as phenotype B, 61 (24.7%) as phenotype C, and 50 (20.2%) as phenotype D.

Table 1. Baseline characteristics of the study participants

Patient number, n	**247**
Age (year)	24.09±5.53
BMI (kg/m²)	28.12±6.38
Waist circumference (cm, n=150)	89.24±14.48
Hip circumference (cm, n=150)	106.12±15.57
Waist/hip ratio	0.84±0.07
Weight classification, n (%)	100 (40.5%) normal weight 58 (23.5%) overweight 89 (36%) obese
Prediabetes, n (%)	21 (8.5%)
IR (according HOMA-IR), n (%)	163 (66%)
Dyslipidemia, n (%)	49 (19.8%)
Basal FSH (IU/L)	5.42±2.02
Basal LH (IU/L)	10.79±7.01
Basal total testosterone (ng/dl)	59±29.9
Fasting glucose (mg/dl)	85.41±9.37
Fasting insulin (mIU/L)	16.36±7.98
HOMA-IR	3.46±1.82
Total cholesterol (mg/dl)	177±33
LDL-C (mg/dl)	106±27
Triglyceride (mg/dl)	112±59
HDL-C (mg/dl)	51±13
TyG index	4.51±0.26

BMI: Body-mass index, IR: Insulin resistance, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, HOMA-IR: Homeostatic model assessment for IR, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TyG: Triglyceride glucose

The mean values were as follows: FSH 5.42±2.02 IU/L, LH 10.79±7.01 IU/L, TT 59±29.9 ng/dl, and prolactin 15.79±12.91 ng/ml. The mean FPG was 85.41±9.37 mg/dl, while the mean fasting insulin level was 16.36±7.98 µU/ml. The mean HOMA-IR was 3.46±1.82. The mean values were as follows: total cholesterol 177±33 mg/dl, LDL-C 106±27 mg/dl, triglycerides 112±59 mg/dl, and HDL-C 51±13 mg/dl. The mean TyG index was 4.51±0.26. In repeated measurements, dyslipidemia was detected in 49 patients (19.8%), while prediabetes was detected in 21 patients (8.5%). Based on HOMA-IR, 163 (66%) patients had IR while 84 (34%) did not (Table 1). The distribution of baseline characteristics by phenotype is shown in Table 2.

When patients were categorized into normal weight, overweight, and obese groups, significant differences were observed in HOMA-IR and the TyG index ($p<0.001$, $p<0.001$). When normal weight and overweight patients were compared separately with obese patients, significant differences were found in both HOMA-IR and the TyG index ($p<0.001$ for all comparisons). However, when overweight patients were compared directly with obese patients, a significant difference was observed in HOMA-IR, but no difference was found in the TyG index ($p<0.001$, $p=0.10$). When comparing different phenotypes, a significant difference was observed in HOMA-IR ($p<0.001$), but no significant difference was found in the TyG index ($p=0.87$). When evaluating the phenotypes separately, a significant difference in HOMA-IR was found only between phenotypes A and C ($p=0.024$). There was no significant difference in age, BMI, waist/hip ratio, FSH, LH, TT, TK between phenotypes ($p=0.95$, $p=0.18$, $p=0.27$, $p=0.32$, $p=0.65$, $p=0.78$, $p=0.17$). There was no statistically significant difference in IR among PCOS phenotypes when assessed using the TyG index as a reference ($p=0.86$).

The correlation analysis revealed a significant positive relationship between HOMA-IR and the TyG index ($p<0.001$, $r=0.37$) (Figure). A significant positive correlation was found between the TyG index and BMI ($p<0.001$, $r=0.36$), the TyG index and waist/hip ratio ($p<0.001$, $r=0.24$), the TyG index and fasting insulin level ($p<0.001$, $r=0.31$), the TyG index and total cholesterol (TC) ($p<0.001$, $r=0.55$), and the TyG index and LDL-C ($p<0.001$, $r=0.45$). A significant negative correlation was also found between TyG index and HDL-C ($p<0.001$, $r=-0.21$). No statistically significant correlation was found between the TyG index and FSH, LH, or TT ($p=0.06$, $p=0.11$, $p=0.72$). The correlations of HOMA-IR and the TyG index with biochemical and anthropometric parameters are shown in Table 3.

ROC curve analysis identified an optimal cutoff value for the TyG index at 4.44, with a sensitivity of 0.70 and a specificity of 0.60 for identifying IR (AUC=0.693, $p=0.035$). All patients were categorized into two groups according to the TyG index cutoff values: group 1 (TyG index<4.44) and group 2 (TyG index≥4.44). We analyzed fasting IR-related metabolic parameters between the two groups (Table 4).

DISCUSSION

IR is a key factor in the pathogenesis and progression of long-term complications in individuals with PCOS. Hyperinsulinemia, in turn, plays a significant role in exacerbating hyperandrogenism and reproductive disorders. The dynamic euglycemic clamp technique, although recognized as the gold standard for measuring insulin sensitivity, is both costly and complicated. Moreover, it may not be available in all countries, making it less feasible for use in outpatient clinic settings. Therefore, there is a need to develop a simpler, more practical, and cost-effective method for assessing IR. Such a method would enable more accurate and personalized diagnosis, treatment, and prognosis for individuals with PCOS. Numerous studies have examined indirect tests, such as HOMA-IR for evaluating IR in PCOS.[13] Our study is among the first to investigate the effectiveness of the TyG index in assessing IR in PCOS patients within the Turkish population and to explore its variability across different phenotypes.

The TyG index has been recognized as an effective alternative to insulin testing for evaluating IR. This recommendation is supported by various studies that have highlighted the TyG index's effectiveness in evaluating IR within the general population.[12,14-16] Subsequently, studies have emerged demonstrating the utility of the TyG index for diagnosing IR in patients with PCOS. First study was published by Kwon et al.[17] and they evaluated 172 Korean PCOS patients. They reported a strong correlation between the TyG index and HOMA-IR ($r=0.524$). Their analysis identified an optimal

Table 2. Baseline characteristics according to phenotypes

	Phenotype A	Phenotype B	Phenotype C	Phenotype D
Patient number, n	132	4	61	50
Age (year)	24.15±5.80	23.50±3.31	23.79±5.33	24.34±5.30
BMI (kg/m²)	28.90±6.70	26.93±3.97	27.67±6.52	26.71±5.24
Waist circumference (cm)	89.14±14.31 (n=84)	N/A (n=0)	91.50±15.47 (n=38)	86.50±13.56 (n=28)
Hip circumference (cm)	106.01±13.45 (n=84)	N/A (n=0)	106.26±13.95 (n=38)	102.71±11.52 (n=28)
Waist/hip ratio	0.83±0.07	N/A (n=0)	0.85±0.09	0.83±0.06
Weight classification Normal weight, n (%) Overweight n (%) Obese n (%)	 47 (35.6) 32 (24.2) 53 (40.2)	 2 (50) 1 (25) 1 (25)	 28 (45.9) 12 (19.7) 21 (34.4)	 23 (46) 13 (26) 14 (28)
Prediabetes, n (%)	10 (7.5%)	3 (75.0%)	5 (8.1%)	3 (6.0%)
IR (According to HOMA-IR), n (%)	97 (73.5%)	4(100%)	34 (55.7%)	28 (56.0%)
Dyslipidemia, n (%)	28 (21.2%)	0	13 (21.3%)	8 (16.0%)
Basal FSH (IU/L)	5.36±2.20	4.50±1.60	5.28±1.85	5.85±1.72
Basal LH (IU/L)	10.55±6.87	14.74±7.03	10.63±7.73	11.25±6.51
Total testosterone (ng/dl)	58.06±29.80	56.53±8.75	62.39±24.90	57.54±34.46
Fasting glucose (mg/dl)	85.71±9.39	99.00±15.14	84.68±9.79	84.42±7.54
Fasting insulin (mIU/L)	50.18±12.52	65.50±27.24	51.33±10.95	55.52±16.08
HOMA-IR	3.69±1.80	5.60±1.83	3.06±1.78	3.18±1.73
Total cholesterol (mg/dl)	178.34±34.83	202.75±35.89	171.10±34.28	181.19±29.86
LDL-C (mg/dl)	109.12±28.06	128.75±33.39	99.39±28.02	107.97±24.20
Triglyceride (mg/dl)	113.93±60.52	100.25±42.57	112.47±64.86	107.46±52.56
HDL-C (mg/dl)	50.18±12.52	65.50±27.24	51.33±10.95	55.52±16.08
TyG index	4.52±0.25	4.56±0.17	4.50±0.29	4.50±0.22

N/A: not available

BMI: Body-mass index, IR: Insulin resistance, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, HOMA-IR: Homeostatic model assessment for IR, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TyG: Triglyceride glucose

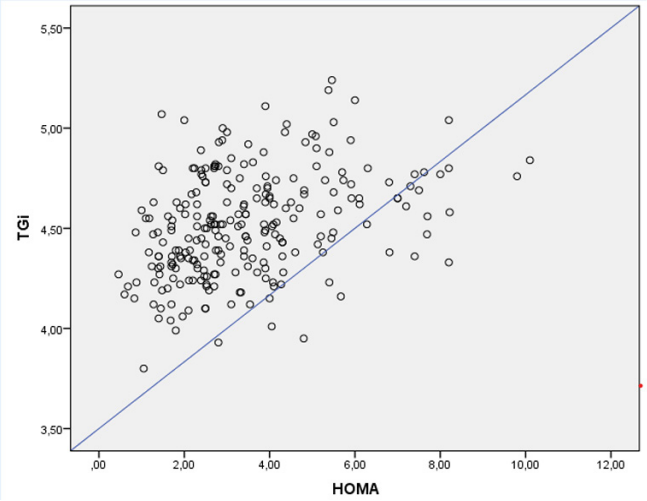

Figure. Correlations between TyG index and HOMA-IR
TyG: Triglyceride glucose, HOMA-IR: Homeostatic model assessment for insulin resistance

Table 3. Correlation analysis

	TyG index (p-value, r-value)	HOMA-IR (p-value, r-value)
BMI	p<0.001, r=0.362	p<0.001, r=0.546
Waist/hip ratio	p<0.001, r=0.248	p<0.001, r=0.273
Fasting plasma insulin	p<0.001, r=0.318	p<0.001, r=0.955
TC	p<0.001, r=0.550	p=0.09
LDL-C	p<0.001, r=0.455	p<0.001, r=0.201
HDL-C	p<0.001, r=0-0.219	p<0.001, r=-0.306
FSH	p=0.06	p<0.001, r=-0.171
LH	p=0.11	p=0.22
Total testosterone	p=0.72	p=0.26

Statistical significance was established when p<0.05, BMI: Body-mass index, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone

Table 4. Comparison of metabolic parameters between the two groups divided according to the cutoff value of triglyceride and glucose index

	Group 1 (IR-) (n=97)	Group 2 (IR+) (n=150)	p value
BMI (kg/m²)	25.49±5.39	29.82±6.41	p<0.001
Waist/hip ratio (cm)	0.82±0.08 (n=64)	0.84±0.07 (n=86)	p=0.13
Fasting glucose (mg/dl)	82.55±8.23	87.26±9.63	p<0.001
Fasting insulin (µU/ml)	13.43±6.77	18.24±8.15	p<0.001
HOMA-IR	2.74±1.44	3.93±1.89	p<0.001
Total cholesterol (mg/dl)	158.7198±26.65	189.43±32.77	p<0.001
LDL-C (mg/dl)	93.87±23.88	115.17±26.68	p<0.001
Triglyceride (mg/dl)	126.07±57.73	143.92±56.08	p<0.001
HDL-C (mg/dl)	62.74±13.83	52.36±10.65	p<0.001

Statistical significance was established when p<0.05. BMI: Body-mass index, IR: Insulin resistance, HOMA-IR: Homeostatic model assessment for IR, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol

TyG cutoff value of 8.126, with a sensitivity of 0.807 and a specificity of 0.683, for detecting IR. Similarly, our study also demonstrated a correlation between HOMA-IR and the TyG index (r=0.370). However, our study determined that the optimal cutoff value for the TyG index in detecting IR in PCOS patients was 4.44. In their study, the mean HOMA-IR was 2.28±2.21, whereas in our study, it was 3.46±1.82. The fact that IR was higher in our study population may have caused the difference. Kheirollahi et al.[18] investigated the TyG index in women with PCOS in Iran, IR was detected by HOMA-IR in 9.83% of the patients. In our study, this rate was found to be 66%. This discrepancy could be attributed to differences in patient characteristics, such as BMI, which was 26.62±4.19 kg/m² in their study compared to 28.12±6.38 kg/m² in ours. Additionally, their study used a HOMA-IR cutoff of ≥2.63,

which might also contribute to the variation in detection rates. Similarly, their study also demonstrated a correlation between HOMA-IR and the TyG index (r=0.233). They identified the optimal cutoff point for the TyG index in predicting IR as 4.65, with a sensitivity of 63% and a specificity of 60%. The variability in the correlation strength between the TyG index and HOMA-IR across studies may be attributed to differences in cutoff values and population characteristics. Studies utilizing higher TyG cutoff points, such as 8.126 and 8.51, demonstrated stronger correlations with HOMA-IR.[17,20] In contrast, the lower cutoff values in our study (4.44) and Kheirollahi et al's study (4.65) were associated with weaker correlations.[18] This discrepancy could stem from differences in fasting insulin levels (16.36 μU/mL in this study vs. 9.98 μU/mL in Kwon et al's study) and BMI scores (28.12 kg/m² in this study vs. 26.62 kg/m² in Iranian study).[17,18] Higher fasting insulin and BMI levels may result in elevated HOMA-IR scores, thereby weakening the correlation with the TyG index. Furthermore, ethnic variations, such as the lower BMI and IR typically observed in Far Eastern populations, could also influence these findings. These factors emphasize the importance of considering population-specific metabolic characteristics when evaluating the TyG index as a marker for IR.

In a retrospective cross-sectional study by Yang et al.[19] the correlation between the TyG index and metabolic syndrome (MS) was explored. MS was identified in 32.5% of the subjects with PCOS, and the study demonstrated a strong association between the TyG index and MS in these women. Obesity, hyperglycemia, and dyslipidemia were diagnosed in 33.8%, 20.9%, and 33.1% of women with PCOS, respectively. In our study, the rates were 36% for obesity, 8.5% for hyperglycemia, and 19.8% for dyslipidemia. They discovered that the TyG index was independently linked to risk factors for metabolic syndrome in women with PCOS, such as hyperglycemia, obesity, and dyslipidemia. Similarly, we found a significant positive correlation between the TyG index and BMI, waist/hip ratio fasting insulin level, TK and, LDL-C. Zheng et al.[20] published a study assessing whether the TyG index is superior to other indices for diagnosing IR in patients with PCOS. The TyG index achieved the highest area under the ROC curve for predicting IR in patients with PCOS, as determined by HOMA-IR, with a value of 0.781 (95% CI: 0.693–0.853, p<0.001). At a cutoff point of 8.51, the TyG index demonstrated a sensitivity of 63.2% and a specificity of 87.0%. We did not evaluate other lipid ratios in defining IR. However, in our study, the TyG index demonstrated comparable effectiveness to HOMA-IR in identifying IR in patients with PCOS.

Głuszak et al.[21] examined whether there are hormonal, biochemical and metabolic differences between PCOS phenotypes. In both their study and ours, phenotype A was the most common (60.2%, 53.4%). In both their study and ours, no significant differences were observed between the subtypes regarding age, weight, height, waist to hip ratio, and BMI. However, while their study found no difference in HOMA-IR among the groups, our study revealed no difference in the TyG index between phenotypes. In contrast, we did find a significant difference in HOMA-IR between phenotypes A and C (p=0.024). In the study by Pehlivanov et al.[22] phenotype A was the most common, occurring in 58.6% of the cases. In contrast to our findings, their study reported that groups A and B were more obese and had higher levels of IR. However, this difference may be attributed to the smaller sample size in their study.

Limitations

This study has several limitations, such as its retrospective design and reliance on existing patient records, which could introduce selection bias and limit the applicability of the findings. Prospective cohort studies with larger sample sizes and longitudinal follow-up are needed to assess the predictive value of these indices for long-term metabolic outcomes in PCOS.

CONCLUSION

In conclusion, this study is the first to illustrate the utility of the TyG index for predicting IR in Turkish women with PCOS and to explore its variability among different phenotypes. Given the significance of carbohydrate homeostasis in the pathogenesis and complications of PCOS, the TyG index—based on fasting plasma glucose and triglyceride levels—emerges as an effective, easy-to-use, and cost-efficient method for evaluating IR during follow-up.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was conducted with the permission of the Scientific Researches Evaluation and Ethics Committee of Ankara Etlik City Hospital (Date: 12.06.2024 Decision No: AESH-BADEK-2024-575).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Clinical outcomes of topical epidermal growth factor in diabetic foot ulcers

Levent Demir[1], Murat Kahraman[2], İbrahim Altun[2], Mustafa Avcı[3], Selahattin Kılıç[4], Sena Aslan[5]

[1]*Department of Underwater and Hyperbaric Medicine, Kayseri City Training and Research Hospital, Kayseri, Turkiye*
[2]*Department of Orthopedics and Traumatology, Kayseri City Training and Research Hospital, Kayseri, Turkiye*
[3]*Department of Emergency Medicine, Antalya Training and Research Hospital, University of Health Sciences, Antalya, Turkiye*
[4]*Department of Family Medicine, Kayseri City Training and Research Hospital, Kayseri, Turkiye*
[5]*Department of Podology, Kayseri City Training and Research Hospital, Kayseri, Turkiye*

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ABSTRACT

Aims: This study aims to evaluate the clinical outcomes of topical epidermal growth factor (EGF) in the treatment of diabetic foot ulcers (DFUs) in outpatient settings. It also seeks to provide guidance on the use of topical EGF, which is eligible for reimbursement for treating DFUs in our country.

Methods: A retrospective analysis involved 55 patients with DFUs who received topical EGF treatment. Patients received training on EGF application and were monitored for healing outcomes. Data were collected from medical records, including demographic information, wound characteristics, and laboratory results. Statistical analysis was performed using IBM SPSS version 25, employing chi-square, Pearson's correlation, and ANOVA tests to evaluate healing rates and associated factors.

Results: The study found that 70.9% of patients achieved complete wound closure within an average of 15.44 weeks. Healing rates were significantly higher for non-plantar wounds (83.8%) compared to plantar wounds (44.4%). Factors such as age, body weight, and body-mass index (BMI) were identified as influencing healing outcomes, with higher weights and BMI correlating with lower healing rates. Mild skin irritation was the only adverse effect reported.

Conclusion: Topical EGF demonstrates promising potential for enhancing the healing of DFUs in outpatient settings, achieving a healing rate comparable to specialized diabetic foot centers. The findings underscore the importance of considering patient-specific factors, such as obesity and adherence to treatment recommendations, to optimize healing outcomes. Further research with larger, multi-center studies is necessary to validate these results and improve access to effective treatments for patients with DFUs.

Keywords: Diabetic foot ulcers, epidermal growth factor, wound healing

INTRODUCTION

Diabetic foot ulcers (DFUs) are a common and severe complication of diabetes mellitus. The lifetime risk of foot ulcers is between 19% and 34%, and this figure is rising due to increased longevity and the medical complexity of individuals with diabetes.[1] These DFUs are a significant cause of morbidity, leading to prolonged hospital stays, increased healthcare costs, and, in severe cases, amputations. The pathogenesis of DFUs is multifactorial, involving peripheral neuropathy, peripheral vascular disease, and immune dysfunction. The chronic nature of these ulcers poses a challenge to effective management and healing.[2]

Growth factors are proteins that play a crucial role in the regulation of cellular processes, including proliferation, migration, and differentiation. They are essential for wound healing, as they promote the repair and regeneration of damaged tissues.[3] In the context of DFUs, growth factors can help overcome the impaired healing response associated with diabetes mellitus. Among these, epidermal growth factor (EGF) has garnered attention for its potential to enhance wound healing when applied topically.[4] EGF is a polypeptide that stimulates cell growth, proliferation, and differentiation by binding to its receptor, EGFR, on the cell surface. This interaction activates intracellular signaling pathways that promote epithelial cell migration and proliferation, essential processes in wound healing. EGF also enhances angiogenesis, the formation of new blood vessels, which is critical for supplying nutrients and oxygen to the healing tissue.[5] Topical EGF can be administered in various forms, including creams, gels, and sprays. The frequency of application varies, but it is

Corresponding Author: Levent Demir, drldemir@yahoo.com

typically applied once or twice daily to the wound bed after thorough cleaning.[6] Studies have suggested that debridement and infection control for ulcers should be performed before initiating EGF treatment.[7] Topical EGF is commonly used for wounds classified as Wagner stages 1 and 2.[7] For high-grade wounds, intralesional EGF can be utilized[8], although this treatment tends to be costly.[9] Prompt treatment of wounds in the early stages significantly reduces the risk of amputation, improves quality of life, and decreases healthcare costs for individuals with DFUs.[10]

This study aims to report the results of topical EGF gel application in outpatients with DFUs. The study also aims to offer guidance on the use of topical EGF, which is eligible for reimbursement for treating DFUs in our country.

METHODS

The study was conducted with the permission of Kayseri City Hospital Non-interventional Clinical Researches Ethics Committee (Date: 05.11.2024, Decision No: 237). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The retrospective evaluation includes patients admitted to our clinic between August 2022 and October 2024, who were followed as outpatients for one year period and received only wound cleansing and topical EGF application due to DFU. The medical records of 104 patients who met the inclusion criteria were reviewed. A data recording form was created with the ethics committee's approval. Data obtained through retrospective screening of the hospital automation system was recorded on this form, including age, gender, height, and body weight. Body-mass index (BMI), comorbidities, and past medications were also recorded. The patients' medical histories were recorded, including details such as the location of the wound, the grading of the wound, results from any cultures taken, hemogram values, blood levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), HbA1c value, and the duration of the wound healing process. The blood results obtained just before the start of treatment were evaluated. The wounds were categorized as plantar or non-plantar to assess the treatment's effect on different wound locations. Wagner staging, commonly used in studies involving topical EGF, was utilized to determine wound stages. Neuropathy data on the data record form were obtained during the first specialist examination when the diagnostic Semmes Weinstein monofilament and pinprick test were conducted. The initial specialist assessment identified several symptoms of poor circulation in the feet, including pale or cyanotic skin color, hair loss, and thinning of the skin. These observations were noted on the data record form as signs of circulatory disorders. Topical EGF was applied only to uninfected wounds. The treatment with topical EGF was started on infected and necrotic wounds after addressing the infection and surgically removing all dead tissue. This treatment started for grade 3 patients with abscesses or osteomyelitis after their infections were effectively treated through surgical or medical interventions. Patients who had previously undergone surgery were also noted. Patients were advised to avoid weight-bearing on the affected foot and to use double crutches for offloading. Patients aged 18 and older who were admitted to our clinic for DFUs and were prescribed topical EGF treatment, along with accessible patient information required for the study through the hospital automation system, were included in the study. Patients under 18 years old, those with incomplete information, those who used different wound care products during follow-up, and those who did not finish the follow-up were excluded from the study (**Figure 1**).

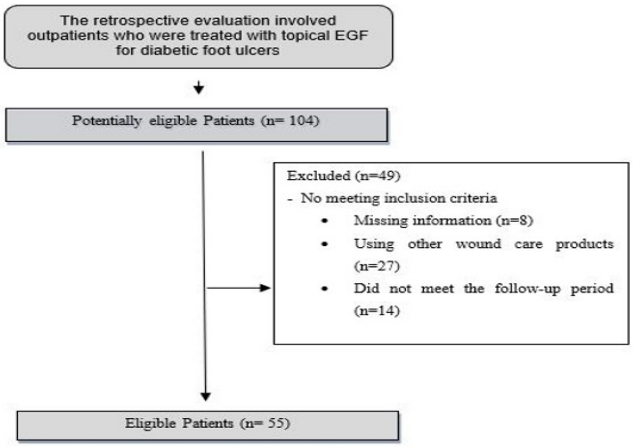

Figure 1. Study flow diagram

All patients received 10 minutes of theoretical training and 5 minutes of practical training on how to apply EGF to their wounds from the study director, a specialist physician on hyperbaric medicine, before participating in the study and starting treatment. The training included seeing each patient and their relative apply EGF to the wound site as the study director prescribes at least twice. After completing the training, all participating patients were prescribed topical EGF and instructed to apply a thin layer of the medication to their wounds twice a day, as recommended in the product's package insert. The dosage of the drug administered was 150 micrograms of recombinant EGF per gram. Complete closure of the wound was considered an indicator of successful healing. The criterion for failure was the absence of complete closure of the wound within one year. The study evaluates the rate and duration of wound healing while examining the correlation between wound healing and variables such as demographic data, laboratory results, and wound location.

Statistical Analysis

The data collected in the study were analyzed using the IBM SPSS version 25 statistical software package (SPSS Inc., Chicago, IL, USA). The normality of the data was evaluated using the Kolmogorov-Smirnov test. Descriptive statistics were calculated using the Chi-square test. Pearson's correlation test was applied for normally distributed data, while Spearman's rho test was used for non-normally distributed data. $p<0.05$ was considered significant at a 95% confidence interval to determine statistical significance. The difference between independent groups was compared using the t-test. ANOVA test was used for data from more than two groups. The study evaluates the rate and duration of wound healing. Also, the correlation between wound healing and variables such as demographic data, laboratory results, and wound location is examined.

RESULTS

A total of 55 patients who were followed up for one year and met the inclusion criteria were included in the study. A total of two patients (3.63%) had wounds classified as Wagner stage 1, while 39 patients (70.9%) had stage 2 wounds, and 14 patients (25.5%) had deep, stage 3 wounds with healed infection. All patients exhibited varying degrees of peripheral neuropathy. The median age of the patients was 65 years, with a mean hemoglobin level of 12.5±1.8 g/dl. The median white blood cell count was 9.41, the CRP level was 13.0 mg/L, and the HbA1c level was 9.15 (Table 1). Complete wound closure was achieved in 39 patients (70.9%) within a mean of 15.44±8.35 weeks (Figure 2). However, the healing rate for non-plantar wounds was 83.8%, while the healing rate for plantar wounds was only 44.4%. The healing rate in patients with plantar lesions was significantly lower (p=0.003). Other factors that affected healing included age, weight, and BMI (Table 2).

Aside from mild skin irritation in two patients, no serious adverse events were reported during treatment, which did not necessitate treatment interruption.

Table 1. Demographic data, laboratory values, and examination results of the patients

Variable	Mean±SD	Median	Min-max
Height (cm)	170.88±8.41		155-188
Weight (kg)	79.41±13.11		60-110
Recovery time (week)	15.44±8.35		4-43
Wound duration (week)	25.06±28.32		2-144
Hb (g/dl)	12.5±1.8		9.6-17.7
BMI[a] (kg/m²)		27.20	21-43
Age (years)		65	37-82
WBC (10^3/μL)		9.41	5.07-16.93
CRP (mg/dl)		13.0	0.5-129
ESR (mm/h)		19.50	2-99
HbA1c (%)		9.15	6.1-13.9
	n	**%**	
Gender			
Male	40	72.7	
Female	15	27.3	
Plantar location of the lesion			
Yes	18	32.7	
No	37	67.3	
Signs of circulatory disorders			
No	16	29.1	
Yes	39	70.9	
Wagner phase			
1	2	3.6	
2	39	70.9	
3	14	25.5	
Treatment result			
Healed	39	70.9	
Not healed	16	29.1	
Operation			
No	47	85.5	
Yes	8	14.5	

Abbreviations: Hb: Haemoglobin, BMI: Body-mass index, WBC: White blood count, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, HbA1C: Haemoglobin A1C

DISCUSSION

Our study found that 70.9% of patients with DFUs treated as outpatients healed with only wound cleaning and a prescribed topical EGF gel. The healing rate is nearly identical to the one-year results from specialized diabetic foot centers.[11,12] It is not always feasible for patients with diabetic foot conditions to access diabetic foot centers. There are many barriers to access to diabetes foot care services for people with diabetes

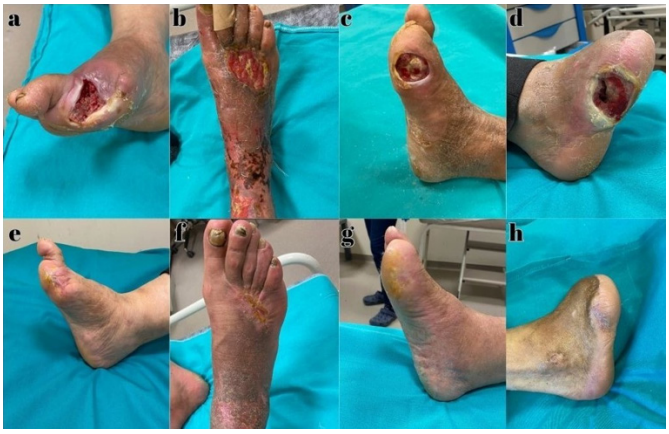

Figure 2. Pre-treatment images (**a–d**) and post-treatment images (**e–h**) of patients with DFUs receiving a topical EGF jel

Table 2. Factors affecting recovery

Variable	Recovery+(n=39)	Recovery−(n=16)	p
Age (years)	65.41±9.50	58.00±10.74	0.013[β]
Gender			
Female	10	5	0.671[&]
Male	29	11	
Height (kg)	169.87±9.09	168.75±8.49	0.666[*]
Weight (cm)	78.41±12.81	88.38±11.30	**0.008**[*]
BMI (kg/m²)	26.98±3.55	31.03±5.66	**0.005**[β]
Plantar location of the lesion			**0.003**[&]
Yes	8	10	
No	31	6	
Signs of circulatory disorders			0.821[&]
Yes	28	11	
No	11	5	
Wagner phase			0.142[#]
1	2	0	
2	29	10	
3	8	6	
Hb (g/dl)	12.43±1.76	13.02±1.97	0.283[*]
WBC (10^3/μL)	9.91±2.78	11.03±5.76	0.853[β]
CRP (mg/dl)	23.85±33.37	23.56±27.32	0.753[β]
ESR (mm/h)	28.62±25.25	24.06±21.67	0.767[β]
HbA1c (%)	9.37±2.15	13.42±18.52	0.690[β]

*Independent sample T-test. & Phi correlation. # ANOVA test. β Mann-Whitney U Test. Hb: Haemoglobin, BMI: Body-mass index, WBC: White blood count, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, HbA1C: Haemoglobin A1C

mellitus.[13] Facilitating access to quality treatment is crucial for individuals at diabetic foot centers and those unable to access such facilities. Meta-analyses of clinical trials have provided robust evidence supporting the efficacy of EGF in DFUs.[14-16] Enhancing access to effective treatments like EGF is vital for improving healing outcomes for patients with DFUs, especially those unable to reach specialized diabetic foot centers.

Our study's findings show that participants who did not heal had significantly higher weight and BMI compared to those who achieved healing. There is currently no information in the literature regarding the relationship between the efficacy of topical EGF in DFUs and body weight. However, some studies have identified a negative correlation between serum EGF levels and BMI.[17,18] Furthermore, several obesity-related factors may lead to poor wound healing outcomes.[19] In obesity, fat cells grow larger without an increase in blood vessels, delaying angiogenesis. This leads to hypoxia due to insufficient oxygen, which can damage blood capillaries

and increase infection risk. Hypoxia also impairs essential collagen synthesis for wound healing. Additionally, vascular issues hinder immune cell recruitment and prolong inflammation. At the same time, nutritional deficiencies further complicate the healing process.[20] Additionally, wounds with a pathological inflammatory state and a high proteolytic microenvironment were considered to create an unfavorable environment for growth factors and their receptors.[21] Chronic low-grade inflammation in obesity may reduce treatment efficacy by affecting EGF and EGFR.[22,23] For these reasons, it may be posited that obesity was associated with non-recovery in our study.

Our study results showed that the age of those who recovered was significantly higher than that of those who did not recover. Two studies examined the relationship between the efficacy of topical topical EGF and age and found no significant difference.[24,25] However, it is known that the synthesis of growth factors declines with age. This reduction in EGF levels can slow the cell cycle and impair skin repair, negatively affecting the skin's ability to heal wounds effectively.[26] Therefore, using EGF in our study may provide a more targeted therapeutic strategy for treating wounds in elderly patients who are already deficient in endogenous growth factors.

In the present study, the location of the wounds was identified as plantar in 32.7% of the patients. The healing rate of plantar wounds was 44.4%, statistically significantly lower than that of non-plantar wounds. There is no information in the literature about the significant relationship between the efficacy of topical EGF and wound location in DFUs. An old study has demonstrated that topical growth factor is ineffective for plantar DFUs.[27] In a different study, topical growth factors significantly improved more than standard treatment in patients receiving a total contact cast for plantar DFUs.[28] In our study, patients were advised to refrain from weight-bearing on the affected foot. Patients often show poor adherence to these interventions, which affects their daily activities, partly due to the effectiveness of available offloading techniques.[29] In our study, patient non-compliance with offloading recommendations may have decreased the effectiveness of topical EGF for plantar wounds. Using topical EGF for plantar wounds without a total contact cast or effective offloading may not achieve the desired healing results.

Topical EGF is typically used for Wagner grade 1 and grade 2 wounds.[7,30] In our study, a significant proportion of participants (25.5%) had a non-infected grade 3 wound, unlike other studies. This study is the first to investigate the use of topical EGF in Wagner grade 3 wounds, and we found no significant differences in the results based on wound grade. Our findings suggest that topical EGF can also effectively treat non-infected deep ulcers.

Physicians treating DFUs often encounter considerable challenges, such as healing difficulties, complex management, and high costs. Administering this EGF gel to patients with clean, uninfected DFUs may provide an effective strategy for overcoming these challenges. In recent years, there has been a growing emphasis on involving the patient as a member of the care team and encouraging self-care for this complex condition.[31,32] Improved outcomes can be achieved by teaching appropriate outpatients how to apply topical EGF through a short training session, as it is easy to apply. A comparative analysis of patients treated as outpatients in a non-specialist setting and those treated at a diabetic foot center showed no significant differences in outcomes for patients with Wagner grades 2 and 3. However, the diabetic foot center demonstrated a higher improvement rate for patients with Wagner grade 4.[33] Effective treatment of DFUs relies on strong collaboration between patients and healthcare providers, with interventions tailored to individual needs across primary care and specialist settings.[34] The results of our study offer a solution for the follow-up care of clean grade 2 and grade 3 DFUs in outpatient settings at both primary care and diabetic foot centers. Given the challenges many patients encounter when accessing diabetic foot centers, it is essential for other outpatient clinics serving wound patients to adopt effective strategies that enable patients to engage in their treatment without facing financial burdens.[35]

Limitations

The study's retrospective nature limits causal inferences, as historical data may lead to biases in participant selection and recording of results, and caution is required in interpreting results. Furthermore, factors that may influence healing, such as the measurement of the wound's width and depth, could not be assessed due to the retrospective nature of this study. The fact that the study was conducted in a single center limits the generalisability of the findings, as differences in treatment protocols and patient demographic characteristics in different institutions may affect the results. The sample size may need further expansion to assess the success of this method reliably and in a generalizable manner.

CONCLUSION

Topical EGF shows promising potential for enhancing the healing of DFUs in outpatient settings, achieving a 70.9% healing rate similar to specialized diabetic foot centers. Healing success was influenced by body weight, BMI, and wound location, with higher body weights and plantar wounds leading to lower healing rates. These findings highlight the need to consider patient-specific factors, like obesity and adherence to treatment recommendations, to optimize outcomes. Further research with larger, multi-center studies is necessary to validate these results. Incorporating topical EGF into standard care for DFUs could improve access to effective treatment, especially for patients facing challenges in obtaining specialized care. By educating patients and encouraging self-care, healthcare providers can enhance healing outcomes and improve the quality of life for individuals with diabetes.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was conducted with the permission of Kayseri City Hospital Non-interventional Clinical Researches Ethics Committee (Date: 05.11.2024, Decision No: 237).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process
Externally peer-reviewed.

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

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

MEDICINE PALLIATIVE CARE

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Factors affecting pregnancy rates in IVF patients with low ovarian reserve: the role of anti-Müllerian hormone and antral follicle count

Mehmet Çok[1], Işıl Turan Bakırcı[2], Gökhan Bolluk[2], Zübeyde Aytufan[3], Nadiye Köroğlu[4], İsmail Özdemir[5]

[1]*Department of Obstetrics and Gynecology, Başakşehir Çam and Sakura City Hospital, İstanbul, Turkiye*
[2]*Department of Perinatology, Başakşehir Çam and Sakura City Hospital, İstanbul, Turkiye*
[3]*Department of Obstetrics and Gynecology, Medical Park Göztepe Hospital, İstanbul, Turkiye*
[4]*Department of Obstetrics and Gynecology, In vitro Fertilization Unit, Acıbadem Atakent Hospital, İstanbul, Turkiye*
[5]*Obstetrics and Gynecology Specialist, Private Practice Physician, İstanbul, Turkiye*

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ABSTRACT

Aims: This study evaluated in vitro fertilization (IVF) pregnancy rates in patients with low ovarian reserve (LOR), compared pregnancy rates between patients with very low and low anti-Müllerian hormone (AMH) levels, and identified factors affecting pregnancy outcomes.

Methods: We analyzed 311 IVF cycles in 217 women with LOR. Patient selection followed the Bologna criteria for poor ovarian response. We compared the pregnant (n=22) and non-pregnant (n=289) groups for AMH, antral follicle count (AFC), oocyte parameters, and clinical outcomes. Multivariate logistic regression identified the independent predictors of pregnancy success.

Results: Pregnant patients showed higher AFC (4.6±2.4 vs 3.4±2.3, p=0.008) and AMH values (0.6±0.2 vs 0.4±0.3 ng/ml, p=0.024). Patients with AMH ≤0.5 ng/ml had higher cycle cancellation rates (26.1% vs. 4.2%, p<0.001), and clinical pregnancy rates remained similar between the AMH groups (6% vs. 8.3%, p=0.421). Multivariate analysis identified AFC (OR: 1.32, 95% CI: 1.08-1.62, p=0.007) and oocyte count (OR: 1.28, 95% CI: 1.05-1.56, p=0.015) as independent predictors of pregnancy success.

Conclusion: In our clinic, AMH levels predicted ovarian response, but not pregnancy outcomes, in patients with LOR. AFC and oocyte count were better predictors of successful IVF.

Keywords: Low ovarian reserve, anti-Müllerian hormone, infertility, pregnancy rate, in vitro fertilization

INTRODUCTION

Infertility affects approximately 15% of reproductive-age couples, and low ovarian reserve (LOR) presents a significant challenge in contemporary fertility treatment. Current data suggest that LOR accounts for nearly one-third of infertility cases among women seeking assisted reproductive technology, highlighting its growing clinical significance in reproductive medicine.[1,2] LOR, characterized by a reduced number of ovarian follicles and diminished oocyte quality, can be attributed to a range of factors including age, genetics, and environmental factors.[3] In the realm of assisted reproductive techniques, in vitro fertilization (IVF) offers hope to couples struggling with LOR, although the factors influencing successful pregnancy outcomes in these patients remain unclear.[3]

The role of anti-Müllerian hormone (AMH) in evaluating ovarian reserves has evolved significantly over the past decade. Although AMH serves as a well-established marker for assessing ovarian reserve, its predictive value for IVF success remains a subject of ongoing debate.[3,4] Recent meta-analyses have revealed that while AMH demonstrates a strong correlation with oocyte yield, its utility in predicting live birth rates appears to be limited.[5,6] Moreover, studies have shown considerable variability in pregnancy outcomes among patients with similar AMH levels, suggesting the involvement of additional factors beyond this single marker in determining reproductive success.[7]

This study aimed to evaluate IVF pregnancy rates in patients with LOR, compare pregnancy rates between patients with very low and low AMH levels, and identify the factors affecting pregnancy outcomes. Additionally, the roles of AMH and antral follicle count (AFC) in predicting IVF success were investigated.

METHODS

This study was approved by the University of Health Sciences Kanuni Sultan Süleyman Training and Research Hospital

Corresponding Author: Işıl Turan Bakırcı, isilturan@yahoo.com

Clinical Researches Ethics Committee (Date: 27.05.2020, Decision No: 2020-36). The research was conducted in accordance with the principles outlined in the 1964 Declaration of Helsinki and its subsequent revisions, as well as the ethical guidelines established by the relevant institutional and national research committees.

We conducted a retrospective analysis of 217 women aged 18-40 years who underwent IVF treatment at our center between 2018 and 2020. Patient selection strictly adhered to the Bologna criteria for poor ovarian response, requiring at least two of the following features: advanced maternal age or other POR risk factors, previous poor ovarian response (≤3 oocytes with conventional stimulation), or abnormal ovarian reserve testing (AFC <7 or AMH <1.1 ng/ml).

The exclusion criteria were tubal factors, male factor infertility with a total progressive motile sperm count below 5 million, history of recurrent pregnancy loss, and presence of uterine abnormalities. All included patients underwent a thorough baseline evaluation, including hormonal assessment, transvaginal ultrasonography, and standard preoperative screening.

The diagnosis of LOR was based on serum AMH levels <1.2 ng/ml and an AFC <7, as observed on ultrasonography. AMH levels were measured using a standardized assay, and the results were used to categorize patients into two groups. AMH levels were categorized based on the commonly used threshold values in the literature.[5] Patients with AMH levels ≤0.5 ng/ml were classified as the 'very low AMH' group, while those with AMH levels >0.5 ng/ml were classified as the 'low AMH' group. This categorization was made to compare the ovarian response and pregnancy outcomes between patients with different AMH levels. The husbands' spermiograms were obtained from the hospital's urology clinic and evaluated using the WHO 2010 criteria, which assess volume, viability, sperm count, total sperm count, total progressive motile sperm count (TPMSC), morphology, pH, and viscosity.[6]

Our standardized IVF protocol included initial ovarian stimulation with gonadotropins (Merional/Gonal-f) at doses ranging from to 150-450 IU administered either intramuscularly or subcutaneously, with the starting dose determined by patient age, BMI, and previous response history. Monitoring included regular transvaginal ultrasound assessment every 2-3 days and serum estradiol measurements when clinically indicated.

GnRH antagonist (cetrotide 0.25 mg) was introduced when leading follicles reached 12–14 mm in diameter. Trigger criteria included at least two follicles ≥17 mm, with final oocyte maturation induced using Ovitrelle 250mcg. Oocyte retrieval was performed 36 h post-trigger under ultrasound guidance.

Embryology procedures followed standardized laboratory protocols, and ICSI was performed in all cases because of the limited number of oocytes. Embryo transfer was conducted under ultrasound guidance on day 2-5 based on embryo development and patient characteristics.

Statistical Analysis

Data analysis was performed using IBM SPSS v22.0. We assessed normality using the Kolmogorov-Smirnov test and applied appropriate parametric or non-parametric tests accordingly. Continuous variables were compared using the Student's t-test or Mann-Whitney U test, while categorical variables were analyzed using the chi-square or Fisher's exact test, as appropriate.

Multivariate logistic regression analysis identified independent predictors of pregnancy success, with variables showing $p<0.1$ in the univariate analysis included in the model. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated, with $p<0.05$. Power analysis indicated that our sample size provided 80% power to detect a 15% difference in pregnancy rates between groups.

RESULTS

Our analysis included 311 IVF cycles in 217 patients with LOR. Table 1 presents the demographic and clinical characteristics of the study population, with participants showing a mean age of 34.7±5 years, mean AMH levels of 0.48±0.3 ng/ml, and mean AFC of 3.5±2.3, representing typical characteristics of an LOR population. Initial gonadotropin doses averaged 342±103 IU, with a mean stimulation duration of 7.3±3.2 days.

Table 1. Descriptive data, laboratory and treatment results of the women participating in the study

LOR (n=311)	
	mean±SD
Age (years)	34.7±5
Duration of infertility (years)	4.7±3.6
BMI (kg/m²)	26.4±5
FSH (mIU/ml)	12.2±8.3
LH (mIU/ml)	7.3±4.5
E2 (pg/ml)	52.2±36.8
TPMSC (million)	72.3±73
AMH (ng/ml)	0.48±0.3
AFC (n)	3.5±2.3
Initial dose (IU)	342±103
Number of hMG days (n)	7.3±3.2
Total dose of gonodotropin (IU)	2887±1537
hCG day in cycle	9.4±2.2
hCG day E2 (pg/ml)	1030±708
Number of oocytes (n)	2.8±2.4
Number of MII oocytes (n)	2±2.1
Number of ICSI oocytes (n)	2±2.1
2PN (n)	1.7±1.6
Fertilization rate (%)	65.8±37.1
Embryo transfer day (day)	2.9±0.6
Number of embryos transferred (n)	1.3±0.4
B-HCG (mIU/ml)	27.3±143.2
	n (%)
IUI attempt	
Present	78/311 (25.1)
Absent	233/311 (74.9%)

Data are given as mean±SD and percentage. LOR: Low ovarian reserve, BMI: Body-mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TPMSC: Total progressive motile sperm count, AFC: Antral follicle count, AMH: Anti-Müllerian hormone, IUI: Intrauterine insemination, hMG: Human menopausal gonadotropin, hCG: Human chorionic gonadotropin, MII: Metaphase II, E2: Estradiol, ICSI: Intracytoplasmic sperm injection, OPU: Oocyte pick-up

In Table 2, a comparison between the pregnant (n=22) and non-pregnant (n=289) groups revealed significant differences across several key parameters. The pregnant group demonstrated notably higher AFC (4.6±2.4 vs 3.4±2.3, p=0.008) and AMH values (0.6±0.2 vs 0.4±0.3 ng/ml, p=0.024). Treatment outcomes also differed significantly, with successful cycles yielding higher oocyte counts (4.1±3.2 vs 2.7±2.3, p=0.003), more MII oocytes (3.5±2.8 vs 1.9±2.0, p=0.001), and increased numbers of 2PN embryos (2.6±2.3 vs 1.6±1.5, p=0.003). Table 2 also shows that the baseline FSH, LH, and estradiol levels did not differ significantly between the groups.

Table 2. Descriptive data, laboratory and treatment results of clinically pregnant (n:22 cycles) and non-pregnant women (n:289 cycles)

	Pregnancy (+) (n=22)	Pregnancy (-) (n=289)	p
	mean±SD	mean±SD	
Age (years)	35.3±4.3	34.7±5.1	0.539
Duration of marriage (years)	6.4±5.4	5±3.8	0.389
Duration of infertility (years)	5.8±4.9	4.7±3.5	0.474
BMI (kg/m^2)	26.6±6.5	26.4±4.9	0.912
IUI (n)	0.4±0.8	0.4±0.8	0.811
TPMSS (million)	89±100	71±70	0.594
Total AFC (n)	4.6±2.4	3.4±2.3	0.008
AMH (ng/ml)	0.6±0.2	0.4±0.3	**0.024**
FSH (mIU/ml)	10.4±3.9	12.4±8.6	0.765
LH (mIU/ml)	6.4±2.5	7.4±4.6	0.558
Basal E2 (pg/ml)	53.8±20	52.1±37.8	0.171
Initial dose (IU)	306±113	345±102	0.136
Number of HMG days (n)	7.1±3.8	7.4±3.2	0.716
Gonadotropin total dose (IU)	2761±1224	2896±1559	0.834
hCG day in cycle	10±1.6	9.3±2.3	0.095
hCG day E2 (pg/ml)	1585±796	996±691	**0.013**
Number of oocytes (n)	4.1±3.2	2.7±2.3	0.003
Number of MII oocytes (n)	3.5±2.8	1.9±2	**0.001**
Number of ICSI oocytes (n)	3.5±2.7	1.9±2	**0.001**
2PN (n)	2.6±2.3	1.6±1.5	**0.003**
Fertilization rate (%)	80.6±23.4	64.2±38.1	0.123
Embryo transfer day (day)	2.9±0.4	2.9±0.6	0.641
Number of embryos transferred (n)	1.5±0.5	1.3±0.4	0.073

Data are given as mean±SD and percentage. LOR: Low ovarian reserve, BMI: Body-mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TPMSS: Total progressive motile sperm count, AFS: Antral follicle count, AMH: Anti-Müllerian hormone, IUI: Intrauterine insemination, HMG: Human menopausal gonadotropin, HCG: Human chorionic gonadotropin, E2: Estradiol, ICSI: Intracytoplasmic sperm injection, OPU: Oocyte pick-up

Further stratification of outcomes by AMH level is presented in Table 3. While the very low AMH group (≤0.5 ng/ml) experienced significantly higher cycle cancellation rates compared to the low AMH group (>0.5 ng/ml) (26.1% vs 4.2%, p<0.001), those who proceeded to embryo transfer achieved comparable clinical pregnancy rates (6% vs 8.3%, p=0.421) and similar live birth rates per cycle (3.5% vs 3.4%, p=0.392). Table 3 also demonstrates that the total gonadotropin doses and stimulation duration were similar between the AMH groups.

Multivariate logistic regression analysis was performed to identify the independent predictors of pregnancy success. Table 4 presents the results. AFC and oocyte count were significant predictors of pregnancy success, with AFC showing a 32% increase in the odds of pregnancy (OR: 1.32, 95% CI: 1.08-1.62, p=0.007) and oocyte count showing a 28% increase in the odds of pregnancy (OR: 1.28, 95% CI: 1.05-1.56, p=0.015). Other variables, including AMH level and age, were not significantly associated with pregnancy outcomes.

Table 3. Descriptive data, laboratory and treatment results of very low AMH levels and low AMH levels

	AMH≤0.5 (n=165)	AMH>0.5 (n=144)	p value
	mean±SD	mean±SD	
Age (years)	35±5.2	34.5±4.7	0.454
Duration of infertility (years)	4.9±4	4.6±3.1	0.501
BMI (kg/m^2)	26.6±5	26.2±5	0.268
IUI (n)	0.2±0.7	0.5±0.8	**0.001**
TPMSS (million)	73.6±71	70.8±75	0.469
Total AFC (n)	2.6±1.9	4.5±2.4	**<0.001**
FSH (mIU/ml)	14.3±9.7	9.8±5.4	**<0.001**
LH (mIU/ml)	8.2±5.4	6.3±2.8	**<0.001**
Basal E2 (pg/ml)	51.6±38.2	53±35.2	0.447
Initial dose (IU)	352±111	330±93.1	**0.026**
Number of HMG days (n)	7.2±3.3	7.5±3.1	0.160
Gonodotropin total dose (IU)	2931±1799	2835±1163	0.931
hCG days in cycle (n)	9.1±2.5	9.6±1.9	**0.039**
hCG day E2 (pg/ml)	777.4±615.9	1307.8±702.9	**<0.001**
Number of oocytes (n)	1.9±1.6	3.6±2.7	**<0.001**
Number of MII oocytes (n)	1.4±1.3	2.6±2.5	**<0.001**
Number of ICSI oocytes (n)	1.4±1.4	2.6±2.5	**<0.001**
2PN (n)	1.5±1.1	1.9±2	0.417
Fertilization rate (%)	71.8±37.7	61.1±36.1	**0.010**
Embryo transfer day (n)	2.8±0.5	2.9±0.6	0.320
Number of embryos transferred (n)	1.2±0.4	1.3±0.5	0.400
Clinical pregnancy rate per cycle (%)	6%	8.30%	0.421
Clinical pregnancy rate per embryo transfer (%)	14%	13.40%	0.439
Live birth rate per cycle (%)	3.50%	3.40%	0.392
Live birth rate per embryo transfer (%)	8.40%	5.60%	0.240
	n (%)	n (%)	
Cycle outcome			<0.001
Cycle cancellation for lack of response	43 (26.10)	6 (4.20)	
OPU negative	8 (4.80)	4 (2.80)	
Embryo transfer	71 (43.00)	89 (61.81)	
OPU, no embryo development	43 (26.10)	45 (31.25)	

Data are given as mean±SD and percentage. LOR: Low ovarian reserve, BMI: Body-mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TPMSS: Total progressive motile sperm count, AFS: Antral follicle count, AMH: Anti-Müllerian hormone, IUI: Intrauterine insemination, hMG: Human menopausal gonadotropin, hCG: Human chorionic gonadotropin, E2: Estradiol, ICSI: Intracytoplasmic sperm injection

Table 4. Multivariate logistic regression analysis of factors affecting pregnancy success in IVF patients with low ovarian reserve

	OR	95% CI	p value
Antral follicle count	1.32	1.08-1.62	0.007
Oocyte count	1.28	1.05-1.56	0.015
AMH (ng/ml)	1.15	0.95-1.40	0.150
Age (years)	0.98	0.92-1.04	0.500

AFC: Antral follicle count, AMH: Anti-Müllerian hormone, OR: Odds ratio, CI: Confidence interval

DISCUSSION

Our findings highlight the complex relationship between ovarian reserve markers and IVF outcomes in patients with LOR. The observation that AMH effectively predicts ovarian response but not pregnancy outcomes aligns with previous studies suggesting that additional factors beyond ovarian reserve markers play a critical role in determining reproductive success.[7-9] Although AMH is a valuable tool for estimating oocyte retrieval during ovarian stimulation, its ability to predict live birth rates remains limited, as evidenced by the considerable variability in pregnancy outcomes among patients with similar AMH levels.[10,11] This discrepancy underscores the importance of integrating multiple predictive factors to assess the fertility potential.

In agreement with previous research, we found a positive correlation between the number of retrieved oocytes and live birth rate.[9] In our study, the pregnant group demonstrated significantly higher oocyte counts, AFC, and AMH levels than the non-pregnant group, reaffirming the prognostic value of these parameters in IVF outcomes. However, the predictive utility of AMH and AFC in achieving pregnancy remains a topic of debate, with some studies highlighting their limitations in directly influencing pregnancy success.[12,13]

The inconsistency in the predictive value of AMH and AFC reflects the multifaceted nature of the LOR and its impact on oocyte and embryo quality. While our findings align with those of prior studies suggesting that reduced oocyte numbers in LOR cases do not necessarily compromise oocyte or embryo quality[14], others have reported that even slight elevations in AMH levels can be associated with higher pregnancy rates.[15,16] In our study, multivariate logistic regression analysis identified AFC and oocyte count as significant predictors of pregnancy success in patients with a LOR. These findings are consistent with those of previous studies that have highlighted the importance of AFC and oocyte yield in predicting IVF outcomes.[17,18] AFC, which reflects the number of recruitable follicles, has been widely recognized as a reliable marker of ovarian reserve and the response to stimulation.[19] Similarly, a higher oocyte count has been associated with an increased chance of fertilization and embryo development, ultimately leading to higher pregnancy rates.[9] AMH, while useful in predicting ovarian response, was not significantly associated with pregnancy outcomes in our study, aligning with reports suggesting its closer relation to oocyte yield rather than embryo quality or implantation potential.[5] However, other studies have reported conflicting results, indicating that even small increases in AMH levels may be associated with higher pregnancy rates.[10] These discrepancies may be attributed to differences in patient populations, laboratory protocols, or thresholds used to define low AMH levels. In our study, age, often associated with diminished ovarian reserve, did not significantly affect pregnancy outcomes, likely due to the narrow age range of the population or the predominant role of AFC and oocyte count in determining success. Our findings underscore the importance of incorporating multiple predictive factors, including AFC and oocyte count, in the assessment of IVF success in patients with a LOR. Future studies should focus on refining stimulation protocols and optimizing laboratory conditions to improve the outcomes in this challenging patient population. These conflicting findings highlight the complexity of LOR and the need for a nuanced approach for patient assessment and treatment planning.

Notably, our study observed significant differences in cycle cancellation rates between the very low and low AMH groups, with patients in the very low AMH group (AMH ≤0.5 ng/ml) experiencing higher cancellation rates (26.1% vs. 4.2%, $p<0.001$). Despite these challenges, the clinical pregnancy rates (6% vs. 8.3%, $p=0.421$) and live birth rates per cycle (3.5% vs. 3.4%, $p=0.392$) were comparable between the two groups. This finding underscores the potential for successful pregnancies even in patients with very low AMH levels, emphasizing the importance of individualized treatment strategies.

Our results also highlight the critical need for patient counseling regarding the potential for poor treatment response and increased cycle cancellation risk in patients with low AMH levels. Optimizing IVF outcomes in LOR cases requires not only effective stimulation protocols but also improvements in laboratory conditions and embryologist expertise. Consistent with previous literature, clinical pregnancy rates in our study ranged from 7% to 15% in LOR patients, with rates of 6% for AMH ≤0.5 ng/ml and 8.3% for AMH >0.5 ng/ml.[20] However, live birth rates per cycle remained consistent across both AMH groups, underscoring the need for further research to improve the success rates in this population.

Limitations

Our study had several limitations. The retrospective design and small sample size of the pregnancy group limited the ability to establish causality and may have affected the reliability of statistical analyses. Variations in patient characteristics, such as age and infertility, could have influenced the outcomes and reduced their generalizability. Protocol changes during the study, including adjustments to gonadotropin dosing and trigger timing, may have introduced treatment inconsistencies. Moreover, the learning curve of our embryology team, as a newly established center, likely contributed to the variability in the results. Finally, this single-center study limits the broader applicability of our findings to other clinics with different patient populations and protocols.

CONCLUSION

In conclusion, AMH levels alone may not be sufficient to predict pregnancy or live birth rates, highlighting the need for a more comprehensive approach for IVF treatment planning in patients with LOR. AFC and oocyte count, along with other dynamic markers, should be incorporated into patient assessments to improve the predictive accuracy and outcomes. To address the challenges in LOR management, future studies should focus on key areas such as refining laboratory protocols and enhancing embryologists' expertise to optimize embryo development. Additionally, investigating genetic and molecular markers to improve the prediction of ovarian response, exploring metabolomic profiles of follicular fluid as novel biomarkers, and examining endometrial receptivity factors are critical steps. Personalized stimulation protocols tailored to individual patient characteristics combined with lifestyle interventions may also contribute to improved

treatment success. Integrating these parameters into a cohesive and personalized treatment framework, supported by prospective multicenter trials, holds great potential for advancing IVF planning and achieving better outcomes in this challenging patient population.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the University of Health Sciences Kanuni Sultan Süleyman Training and Research Hospital Clinical Researches Ethics Committee (Date: 27.05.2020, Decision No: 2020-36).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The relationship between thyroid functions, vitamin B12, and lipid profiles across different BMI categories in adults

Vasfiye Demir Pervane[1], Özden Gökdemir[2], Olgu Aygün[3], Sevgi Ceylan Gündoğdu[1], Pakize Gamze Erten Bucaktepe[1], Tahsin Çelepkolu[1]

[1]*Department of Family Medicine, Faculty of Medicine, Dicle University, Diyarbakır, Turkiye*
[2]*Department of Family Medicine, Faculty of Medicine, İzmir University of Economics, İzmir, Turkiye*
[3]*Department of Family Medicine, Bozyaka Training and Research Hospital, University of Health Sciences, İzmir, Turkiye*

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ABSTRACT

Aims: Obesity is a multifactorial condition characterized by abnormal or excessive fat accumulation that adversely impacts health and disrupts various metabolic processes. This study aimed to assess thyroid function tests, vitamin B12 levels, and lipid profiles in normal, overweight, and obese adults, and to elucidate the correlation with body-mass index (BMI) values.

Methods: This study was planned as a retrospective descriptive cross-sectional study. Within the scope of the study, age, gender, occupation, history of chronic diseases, smoking, alcohol use and drug use; weight, height, BMI, blood pressure; TSH, free T3, free T4, total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglyceride, fasting blood glucose, vitamin B12, folate, ferritin, serum iron level, iron binding capacity and whole blood parameter values were retrieved and recorded by reviewing health records retrospectively. Patients were grouped according to BMI values, and the relationships between obesity, sociodemographic characteristics and blood parameters were analyzed.

Results: A total of 539 patients were analyzed, with 63.6% identified as women. The average age of the patients was 36.88±13.75 years (range: 13-79), and the average BMI was 26.92±6.37 kg/m². Analysis revealed that 40% of patients were classified as normal weight, 30.1% as overweight, 26.1% as obese, and 3.8% as underweight based on BMI criteria. The classification of obesity indicates that class 1 obesity accounts for 59.7%, while class 2 and class 3 obesity each represent 20.1% of the total cases. The obesity rate was 72.1% in women and 27.9% in men, with a statistically significant difference observed between genders and BMI groups ($p<0.001$). The prevalence of B12 deficiency was 1.2%, and no significant association was observed among BMI groups. The study identified a statistically significant difference in total cholesterol ($p<0.001$), HDL ($p=0.001$), LDL ($p<0.001$), VLDL ($p<0.001$), triglycerides ($p<0.001$), and BMI groups. Conversely, no significant relationship was observed between B12 values and TSH ($p=0.430$), fT3 ($p=0.462$), or fT4 ($p = 0.279$).

Conclusion: In conclusion, our findings indicate that BMI significantly influences the lipid profile of individuals; however, it does not exhibit a direct relationship with B12 levels or thyroid functions. Given the fact that obesity elevates cardiometabolic risks, particularly through heightened lipid levels, it is essential to monitor not only obese individuals but also those at risk for it as well, to reduce obesity and prevent its onset.

Keywords: BMI, obesity, vitamin B12, lipid, TSH, thyroid hormones

INTRODUCTION

Obesity is a complex condition characterized by abnormal or excessive body fat accumulation, adversely affect. Obesity typically results from an imbalance between energy intake and expenditure; nonetheless, genetic, environmental, psychological, and socioeconomic factors significantly contribute to its development.[1,2] The World Health Organization (WHO) classifies individuals with a body-mass index (BMI) of 25-29.9 kg/m² as overweight and individuals with a BMI of ≥30 kg/m² as obese according to the BMI, which is the most widely used criterion to evaluate obesity.[2] The global prevalence of obesity has increased rapidly in recent years. According to 2022 data, more than 1 billion people worldwide are obese, and approximately 880 million of these people are adults, and 159 million are children.[3,4] The effects of obesity on energy metabolism, hormonal balance, and absorption of nutrients lead to changes in metabolic and endocrine parameters. Thyroid hormones have a critical role in the regulation of energy metabolism. Various studies have shown that especially thyroid-stimulating hormone (TSH) levels show a positive correlation with BMI in obese individuals, and this has a significant effect on thyroid

Corresponding Author: Vasfiye Demir Pervane, vasfiyyedemir@hotmail.com

functions.[5] This suggests that thyroid dysfunction in obese individuals may worsen metabolic balance.

Vitamin B12 is crucial for DNA synthesis, hematopoiesis, and the functioning of the nervous system. Factors such as obesity, alterations in dietary habits, insufficient intake, and the use of proton pump inhibitors can contribute to an increased risk of vitamin B12 deficiency. This deficiency can result in elevated homocysteine levels, thereby heightening the risk of cardiovascular disease.[6]

Obesity induces dyslipidemia through its impact on lipid metabolism. Obese individuals often exhibit elevated triglyceride and low-density lipoprotein (LDL) cholesterol levels, alongside reduced high-density lipoprotein (HDL) cholesterol levels. Atherogenic dyslipidemia refers to these lipid profile changes and constitutes a modifiable risk factor for cardiovascular diseases.[7]

This study evaluates thyroid function tests, vitamin B12 levels, and lipid profiles in normal, overweight, and obese adults, aiming to determine their relationship with BMI values. In particular, we hypothesised that increasing BMI leads to significant changes in thyroid function (especially TSH levels), vitamin B12 levels and lipid profile parameters and that these changes reflect obesity-related metabolic disorders.

METHODS

The study was approved by the Dicle University Non-interventional Ethics Committee (Date: 05.08.2014, Decision No: 306), and conducted by the Declaration of Helsinki. Patient data were anonymized by confidentiality principles.

This study was planned as a retrospective descriptive cross-sectional study. This study analyzed the records of patients aged 18 years and older who visited the Family Medicine, Endocrinology, and Dietary outpatient clinics at Dicle University Faculty of Medicine Hospitals from January 1, 2014, to December 31, 2014. Participants admitted to the outpatient clinics with recorded weight and height measurements, along with conducted investigations, were included in the study. Age, gender, occupation, history of chronic diseases, smoking, alcohol and drug use, weight, height, BMI, blood pressure, TSH, free T3, free T4, total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides, fasting blood glucose, vitamin B12, folate, ferritin, serum iron level, iron binding capacity, and whole blood parameter values were retrospectively scanned in the health records and entered into the data collection form. No additional laboratory or imaging methods were used, and only existing patient records were considered. Exclusion criteria were individuals aged <18 years and patients with incomplete or inaccurate laboratory results.

BMI calculations of the patients were made according to WHO, calculated by dividing their body weight (in kilograms) by their height (in meters squared), recorded, and classified. BMI value <18.5 kg/m^2 was categorized as underweight, 18.5-24.9 kg/m^2 as normal weight, BMI 25-29.9 kg/m^2 as overweight, and BMI ≥30 kg/m^2 as obese. In obesity classification, Obesity class I represents patients with a BMI of 30-34.9 kg/m^2, Obesity class I BMI 35-39.9 kg/m^2, and Obesity class I BMI ≥40 kg/m^2. The following reference ranges and units were used for laboratory parameters (Table 1).

Blood tests for hemoglobin, hematocrit, mean cell volume, and platelets were performed using the Sysmex XN-1000 SA-01 device with the laser method; glucose, total iron-binding capacity, iron, HDL, LDL, VLDL, total cholesterol, and triglycerides were analyzed using the Beckman Olympus AU5800 autoanalyzer with the photometric method; ferritin, folate, TSH, FT4, FT3, and vitamin B12 were measured using the Beckman DxI 600 device with the electrochemiluminescence method; and HbA1c was analyzed using the BIO-RAD Variant II device.

Statistical Analysis

Data were analyzed with IBM SPSS Statistics Version 23 software.

- The conformity of the data to normal distribution was evaluated with the Kolmogorov-Smirnov test.
- The Kruskal-Wallis H test was used to compare non-normally distributed data in three or more groups, and Dunn's test was used for multiple comparisons.
- One-way analysis of variance (ANOVA) was used to analyze the normally distributed data in three or more groups, and the Tukey test was used for multiple comparisons.
- The relationships between variables that did not fit the normal distribution were evaluated by Spearman's rho correlation.
- Quantitative data were presented as mean±standard deviation and median (minimum-maximum); categorical data were presented as frequency and percentage.
- The significance level was accepted as $p<0.05$ in all statistical analyses.

RESULTS

The data of 539 patients were analyzed, 63.6% of the patients were women and 36.4% were men. The mean age of the patients was 36.88±13.75 (13-79)/year, the mean height was 165.45±8.32 cm, the mean weight was 73.99±18.64 kg, and the mean BMI was 26.92±6.37 kg/m^2. Descriptive statistics of quantitative variables are given in Table 2.

The analysis revealed that the prevalence of chronic diseases among patients was 59.7%. Hypertension prevalence was 29.9%, diabetes 50.3%, hyperlipidemia 6.4%, coronary artery disease 10.8%, hyperthyroidism 7.6%, and hypothyroidism 29.9% among chronic diseases. The proportion of individuals who did not use medication regularly was 56.8%, whereas the proportion of those who did was 43.2%.

The classification based on BMI values revealed that 40% of patients were of normal weight, 30.1% were overweight, and 26.1% were classified as obese, while 3.8% fell into the underweight category. The classification of obesity indicates that class 1 obesity accounts for 59.7%, class 2 obesity for 20.1%, and class 3 obesity for 20.1%.

Table 1. Reference ranges and units were used for laboratory parameters

Glucose: 74-106 mg/dl	Triglyceride: 50-180 mg/dl	Vitamin B12: 211-911pg/ml
TIBC: 1550-3550 µg/L	Total cholesterol: 112-200 mg/dl	Hb: 12.9-14.2 g/dl
Iron: 600-1800 µg/L	HDL: 37-79 mg/dl	Htc: 37.7-53.7%
HbA1c: 4.3-6.1%	LDL: 60-160 mg/dl	MCV: 81.1-91.6 fL
TSH: 0.35-5.5 µIU/ml	VLDL: 10-32 mg/dl	Plt: 155-366 10e3/uL
FT4: 0.89-1.76 ng/dl	Ferritin: 11-306.8 µg/L	
FT3: 2.3-4.2 pg/ml	Folate: 5.9-24.8 µg/L	

Abbreviations: TIBC: Total iron binding capacity, HbA1c: Hemoglobin A1c, TSH: Thyroid-stimulating hormone, FT4: Free T4, FT3: Free T3, Vit. B12: Vitamin B12, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volum, Plt: Platelet, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein

Table 2. Descriptive statistics of quantitative variables

	Mean±SD*	Median (min-max)
Age (years)	36.888±13.751	35 (13-79)
Length (cm)	165.454±8.32	165 (145-195)
Weight (kg)	73.994±18.641	72 (30-210)
BMI value (kg/m^2)	26.916±6.367	25.9 (13.8-59.2)
SBP (mmHg)	112.936±18.533	110 (80-200)
DBP (mmHg)	65.483±12.654	60 (40-120)
TSH (µIU/ml)	5.195±22.744	1.54 (0.01-248)
FT4 (ng/dl)	17.011±9.979	15.9 (1.52-129.2)
FT3 (pg/ml)	5.089±3.292	4.745 (0.27-49.8)
Blood sugar (mg/dl)	115.476±63.815	96 (32-672)
Triglyceride (mg/dl)	148.449±101.424	119 (35-1110)
Total cholesterol (mg/dl)	196.656±116.952	184 (38-2221)
HDL (mg/dl)	45.207±12.563	43 (3-96)
LDL (mg/dl)	115.759±37.764	110 (27-299)
VLDL (mg/dl)	31.125±27.178	24 (7-313)
Ferritin (µg/L)	58.904±70.109	28.2 (1.46-464)
Folate (µg/L)	9.074±6.771	8.415 (2.6-89.6)
B12 (pg/ml)	325.492±154.612	291 (26.38-1244)
Iron (µg/L)	74.294±39.718	69 (8-215)
TIBC (µg/L)	269.281±79.772	271 (26-527)
Hemoglobin (g/dl)	13.929±1.925	14 (7.8-18.75)
Hematocrit (%)	42.207±5.126	42 (26.36-59.17)
MCV	84.984±7.24	86 (30-107)
Platelet x1000	274.162±72.888	264 (32.6-658.9)

Abbreviations: SD: Standard deviation, Min: Minimum, Max: Maximum, BMI: Body-mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TSH: Thyroid-stimulating hormone, FT4: Free T4, FT3: Free T3, HDL; High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, Vit. B12: Vitamin B12, TIBC: Total iron binding capacity, MCV: Mean corpuscular volum

The comparison of BMI groups, age, and vitamin B12 deficiency is presented in Table 3. Obesity prevalence was identified at 72.1% in women and 27.9% in men, with a statistically significant difference observed between gender and BMI groups (p<0.001). The prevalence of B12 deficiency was determined to be 1.2%, with no significant correlation identified among BMI groups.

The quantitative data of the patients were compared across groups based on BMI, as presented in Table 4. A statistically significant difference was observed across age, blood pressure values (systolic and diastolic), blood sugar, blood lipids, blood glucose, ferritin, iron, iron binding capacity, B12 value, hemoglobin, hematocrit, MCV, platelet level, and BMI groups (p<0.001 for all values). Obese patients exhibited higher age, blood pressure values (systolic and diastolic), blood glucose, and blood lipids (excluding HDL), while HDL levels were lower compared to non-obese patients. The overweight group exhibited elevated levels of ferritin, iron, hemoglobin, hematocrit, and MCV values.

The analysis of the correlation between BMI and quantitative variables revealed statistically significant positive correlations with systolic blood pressure (r=0.440; p<0.001), diastolic blood pressure (r=0.400; p<0.001), blood sugar (r=0.320; p<0.001), triglyceride level (r=0.381; p<0.001), total cholesterol level (r=0.227; p<0.001), LDL level (r=0.204; p<0.001), VLDL level (r=0.375; p<0.001), ferritin (r=0.265; p<0.001), iron (r=0.159; p=0.016), hemoglobin (r=0.119; p=0.009), and hematocrit level (r=0.161; p<0.001). Conversely, HDL level exhibited a negative correlation (r=-0.208; p<0.001). The correlation analysis revealed no statistically significant results between BMI and other quantitative variables (Table 5).

In addition, in the correlation analysis of smoking with quantitative variables, significant relationships were found between HDL level (r=-0.144, p=0.025), ferritin level (r=0.181, p=0.035), hematocrit level (r=0.208, p<0.001), hemoglobin level (r=0.190, p<0.001) and MCV level (r=0.229, p<0.001). In the correlation analysis of alcohol use with quantitative variables, significant relationships were found between total cholesterol level (r=0.167, p=0.021), LDL level (r=0.167, p=0.029), hemoglobin level (r=0.197, p=0.003), hematocrit level (r=0.177, p=0.007) and MCV level (r=0.184, p=0.005). No statistically significant results were found in the correlation analysis between smoking and alcohol use and other quantitative variables.

DISCUSSION

This study examined a sample group of 539 individuals, revealing an obesity rate of 26.1% among participants. Obesity prevalence in women was 2.5 times greater than in men. A statistically significant correlation was observed between blood lipids and BMI categories; however, no correlation was found between thyroid function tests and B12 levels.

This study found that 40% of patients were classified as normal weight, 30.1% as overweight, and 26.1% as obese, while

Table 3. Comparison of BMI groups and categorical demographic characteristics

		Underweight	Normal	Overweight	Obese	Total	Test statistic	p
		n (%)	n (%)	n (%)	n (%)	n (%)		
Gender	F	15 (75)^{abc}	145 (69.4)^{c}	71 (45.2)^{b}	98 (72.1)^{ab}	329 (63)	30.965	**<0.001**^{x}
	M	5 (25)^{abc}	64 (30.6)^{c}	86 (54.8)^{b}	38 (27.9)^{ac}	193 (37)		
Vitamin B12 deficiency	Yes	0 (0)	3 (1.5)	1 (0.7)	2 (1.6)	6 (1.2)	0.955	0.819^{y}
	No	19 (100)	193 (98.5)	145 (99.3)	121 (98.4)	478 (98.8)		

x: Pearson Chi-square test, y: Fisher's Exact test with Monte Carlo correction, a-c: There is no difference between the rates, n (%). Abbreviations: BMI: Body-mass index, F: Female, M: Male

Table 4. Comparison of quantitative data between groups according to BMI

	Underweight	Normal	Overweight	Obese	Total	Test statistic	p
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		
Age	20.5 (15-50)[a]	29 (13-77)[b]	37.5 (15-71)[c]	43 (15-79)[c]	35 (13-79)	78.140	**<0.001**[x]
SBP (mmHg)	110 (90-120)[abc]	100 (80-200)[a]	110 (80-155)[b]	120 (90-190)[c]	110 (80-200)	36.352	**<0.001**[x]
DBP (mmHg)	60 (60-80)[abc]	60 (40-120)[a]	62.5 (45-90)[b]	75 (50-100)[c]	60 (40-120)	34.778	**<0.001**[x]
TSH (µIU/ml)	1.185 (0.01-5.31)	1.55 (0.01-207)	1.585 (0.01-181)	1.51 (0.01-248)	1.54 (0.01-248)	2.759	0.430[x]
FT4 (ng/dl)	17 (13.6-32.68)	15.98 (9.16-129.2)	15.945 (6.93-29.92)	15.5 (1.52-120)	15.9 (1.52-129.2)	3.842	0.279[x]
FT3 (pg/ml)	5.36 (4.17-25.58)	4.69 (3.23-49.8)	4.78 (2.37-18.42)	4.73 (0.27-6.19)	4.745 (0.27-49.8)	2.576	0.462[x]
Blood sugar (mg/dl)	91 (64-384)[ab]	92 (32-558)[a]	98 (67-672)[bc]	101 (70-382)[c]	96 (32-672)	43.213	**<0.001**[x]
Triglyceride (mg/dl)	70 (41-123)[a]	98 (35-1110)[a]	135 (44-481)[b]	147.5 (61-487)[b]	119 (35-1110)	45.741	**<0.001**[x]
Total cholesterol (mg/dl)	156 (104-226)[a]	179 (58-380)[a]	190 (115-2221)[ab]	194 (38-634)[b]	184 (38-2221)	18.127	**<0.001**[x]
HDL (mg/dl)	51 (45-72)[ab]	48 (15-96)[a]	42 (8-80)[c]	43 (25-88)[bc]	43 (3-96)	27.708	**<0.001**[x]
LDL (mg/dl)	78 (67-156)[a]	101 (34-299)[ab]	113.5 (42-293)[bc]	114.5 (27-228)[c]	110 (27-299)	16.130	**0.001**[x]
VLDL (mg/dl)	14 (8-25)[a]	19.5 (7-222)[a]	27 (9-128)[b]	29 (13-313)[b]	24 (7-313)	43.849	**<0.001**[x]
Ferritin (µg/L)	22.68 (4.07-87.14)[ab]	19.88 (1.46-199)[a]	67.5 (3.7-464)[b]	35. 89 (3.82-222)[ab]	28.2 (1.46-464)	23.841	**<0.001**[x]
Folate (µg/L)	7.585 (5-10.94)	8.5 (2.6-18.17)	7.96 (3.69-20)	8.995 (5.09-15.71)	8.415 (2.6-89.6)	1.659	0.646[x]
Vit. B12 (pg/ml)	262.8 (165.7-346)	291.3 (140.4-778)	288.1 (26.38-1244)	299.9 (113.9-1083)	291 (26.38-1244)	1.931	0.587[x]
Demir (µg/L)	66.5±32.667[ab]	67.382±35.044[a]	91.906±46.698[b]	69.638±34.979[a]	74.294±39.718	4.472	**0.010**[y]
TIBC	293.875±68.265[ab]	276.99±80.909[ab]	230.919±76.891[b]	294.933±66.895[a]	269.281±79.772	7.460	**<0.001**[y]
Hemoglobin	14.02 (10-16.7)[ab]	13.045 (8.52-18.75)[a]	14.85 (7.8-18.33)[b]	14 (9.86-18.25)[ab]	14 (7.8 -18.75)	22.688	**<0.001**[x]
Hematocrit	41.74 (36-48.5)[ab]	40.59 (26.36-54.93)[a]	44 (27.6-59.17)[b]	42.8 (30.4-53.5)[b]	42 (26.36-59.17)	26.340	**<0.001**[x]
MCV	86.4 (69-91.73)[ab]	85.985 (31-97.72)[ab]	86.95 (58-107)[a]	84 (30-96.98)[b]	86 (30-107)	8.864	**0.031**[x]
PlateletX1000	238.8 (186-376.7)	268.3 (43.4-513)	261 (121.6-442)	278.9 (89.3-658.9)	264 (32.6-658.9)	7.183	0.066[x]

[x]: Kruskal-Wallis H test, [y]: One-way analysis of variance, (a-c): There is no difference between groups with the same letter. Mean±standard deviation, Median (minimum-maximum). Abbreviations: BMI: Body-mass index, SD: Standard deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TSH: Thyroid-stimulating hormone, FT4: Free T4, FT3: Free T3, HDL; High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, Vitamin B12, TIBC: Total iron binding capacity, MCV: Mean corpuscular volum

Table 5. Examination of the relationship between BMI value and quantitative variables

	BMI value	
	r^x	p
Systolic blood pressure	0.440	<0.001
Diastolic blood pressure	0.400	<0.001
TSH level	0.039	0.436
FT4 level	-0.059	0.284
FT3 level	-0.086	0.165
Blood sugar	0.320	**<0.001**
Triglyceride level	0.381	**<0.001**
Total cholesterol level	0.227	**<0.001**
HDL level	-0.208	**<0.001**
LDL level	0.204	**<0.001**
VLDL level	0.375	**<0.001**
Ferritin level	0.265	**<0.001**
Folate level	-0.009	0.913
Vitamin B12 level	0.008	0.904
Iron level	0.159	**0.016**
Total iron binding capacity	-0.048	0.486
Hemoglobin level	0.119	**0.009**
Hematocrit level	0.161	**<0.001**
MCV level	-0.073	0.111
Platelet levelx1000	0.056	0.222

[x]: Spearman's rho correlation, Abbreviations: BMI: Body-mass index, TSH: Thyroid-stimulating hormone, FT4: Free T4, FT3: Free T3, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, MCV: Mean corpuscular volume

3.8% fell into the underweight category based on BMI. The classification of obesity indicates that class 1 obesity accounts for 59.7%, while both class 2 and class 3 obesity each represent 20.1% of the total cases. The obesity rate was 72.1% in women and 27.9% in men, with a statistically significant difference observed between genders and BMI groups (p<0.001). The prevalence of B12 deficiency was 1.2%, and no significant association was observed between BMI categories. The study identified a statistically significant difference in total cholesterol (p<0.001), HDL (p=0.001), LDL (p< 0.001), VLDL (p<0.001), triglycerides (p<0.001), and BMI groups. However, no significant relationship was observed between B12 values and TSH (p=0.430), fT3 (p=0.462), or fT4 (p=0.279).

It was noted that 63.6% of the participants were female. Literature indicates that women typically exhibit higher participation rates.[8,9] Analysis of the BMI distribution in our study revealed that 40% of participants were classified as normal weight, 30.1% as overweight, and 26.1% as obese. These rates corroborate research indicating a rising prevalence of obesity in developing nations, including Turkey.[1,10] Data from the Turkish Statistical Institute (TÜİK) in 2022 indicates that the obesity rate among individuals aged 15 years and older was 20.2%. This rate is lower than the 26.1% obesity rate reported in our study. This data aligns with the 2023 report from the World Obesity Federation, released in 2022, which predicts that over half of adults in Turkiye may be obese by 2035.[11] The elevated obesity rates observed in our study compared to national data may be attributed to regional variations or specific characteristics of the study population, given that Turkey is situated in the eastern Anatolia region and has a dietary pattern that includes frequent consumption of fat and meat products. This situation highlights the significance of regional strategies in combating obesity. The combined rates of Obesity in class 2 and class 3 obesity classification stand at 40.2%, highlighting the significance of severe obesity as a critical issue.

In our study, significant positive correlations were found between systolic and diastolic blood pressure and BMI. This finding is consistent with existing data in the literature. For

example, in a study conducted on sedentary women, it was determined that systolic and diastolic blood pressure showed significant positive relationships with BMI. In Mendeş and Mendeş's[13] study, a weak positive relationship was found between BMI and systolic and diastolic blood pressure in adult individuals.[12,13] These data emphasize that increasing body weight has negative effects on blood pressure, and obesity is an important risk factor in the development of hypertension. Therefore, the positive association between BMI and blood pressure suggests that obesity may impair blood pressure regulation and increase cardiovascular risks.

In our study, a significant positive association was found between BMI and blood glucose. This finding is consistent with existing data in the literature. For example, in a study conducted at Gaziantep University, it was determined that fasting blood glucose levels increased as BMI increased in obese individuals.[14] Similarly, a study conducted on university students in Istanbul showed that as anthropometric measurements such as BMI, waist circumference, and waist/height ratio increased, random blood glucose levels also increased.[15] These data support that increasing body fat leads to insulin resistance and increases blood glucose levels, and thus there is a positive relationship between BMI and blood glucose.

In the study, a significant positive correlation was found between triglyceride levels and BMI. This indicates that as BMI increases, triglyceride levels also increase. Similarly, a significant positive relationship was observed between total cholesterol levels and BMI. This finding suggests that an increase in BMI may affect total cholesterol levels. A significant negative relationship was found between HDL level and BMI. This suggests that HDL levels decrease with increasing BMI, which is a significant factor increasing cardiovascular risks. A significant positive relationship was also found between LDL levels and BMI, suggesting that higher BMI contributes to an increase in LDL levels. These findings are consistent with existing studies in the literature. For example, a study conducted in 2018 reported that there were positive and negative associations between BMI and triglyceride and HDL levels, respectively, but no significant association was found with LDL.[16-18] These results suggest that increasing BMI has significant effects on lipid profile and should be considered in terms of cardiometabolic health. In the study in which metabolic parameters of obese and coronary heart disease patients were evaluated, it was shown that both obese and coronary heart disease patients were more dyslipidaemic, with higher LDL and TG levels and lower HDL levels compared to healthy controls.[19] The risks of cardiometabolic comorbidities should not be reduced to the assessment of BMI alone. It should be kept in mind that in the presence of normal BMI but high body fat percentage, which is called normal weight obesity, lipid profile disorder may be seen more frequently compared to people without normal weight obesity, and in this case, a worse cardiometabolic profile may be encountered.[20]

In this study, no significant relationship was found between TSH level and BMI. This finding is consistent with some studies in the literature. For example, in a study conducted by Manji et al.[21] in 2006, no significant relationship was found between serum TSH or free T4 levels and BMI in euthyroid individuals. Similarly, in another study conducted in 2010, TSH levels were found to be within normal reference ranges in obese individuals.[5] However, there are also studies in the literature that found a positive correlation between TSH levels and BMI. For example, Knudsen et al.[22] in 2005 reported that even small changes in thyroid function in the general population may have an effect on BMI and the incidence of obesity. In a large population-based study conducted in Spain investigating the prevalence of abnormal thyroid function and potential modulatory factors, mean serum TSH levels in euthyroid individuals were found to be higher in those with obesity (BMI ≥30 kg/m²).[23] The impact of thyroid hormones on lipid profiles has been widely investigated. In Wang Y.'s[24] study, the role of BMI in modulating the relationship between thyroid hormones (TH) and lipid parameters in euthyroid healthy adults was examined. The findings suggest that high-normal FT3 and TSH levels, as well as low-normal FT4 levels, are associated with an unfavorable lipid profile. Moreover, BMI mediates the effect of thyroid function on lipid metabolism in euthyroid adults.[24] Given the clinical significance of the interaction between metabolism and thyroid hormones, it is crucial to determine whether obesity leads to elevated TSH levels or whether higher TSH levels contribute to obesity. In Wang X.'s[25] study, this relationship was examined, and the findings indicated that genetically predicted serum TSH levels do not directly influence BMI or obesity risk. However, TSH levels were significantly elevated as a result of genetically determined high BMI. A study evaluating the association between TSH levels and metabolic profiles in obese individuals demonstrated that obesity complicated by mildly elevated TSH is associated with higher fasting insulin levels, more severe chronic low-grade inflammation, and lower HDL levels compared to obesity with normal TSH levels.[26] In Marzullo's[27] study, the impact of metabolic phenotype on thyroid function in obesity was evaluated. The findings indicated that FT4 levels were inversely associated with BMI, insulin resistance, and triglyceride levels, while showing a direct correlation with HDL-cholesterol levels. Additionally, TSH was found to be associated with FT4, total cholesterol, and BMI. Significant predictors of FT4 included BMI, TSH, and age.[27]

In clinical practice, ferritin is used for diagnosing iron deficiency; however, it can also serve as a marker of inflammation. In Khan's[28] study, the role of ferritin in overweight and obese individuals was investigated, either as an indicator of inflammation or iron deficiency. The findings revealed that ferritin levels were highest in obese patients and showed a strong positive correlation with BMI. The study concluded that ferritin serves as a marker of inflammation rather than iron status in overweight and obese individuals.[28] In our study, a positive correlation was found between BMI and ferritin levels. In a nationwide population-based study conducted in South Korea, serum ferritin levels were found to be positively associated with metabolically obese normal-weight individuals.[29] In a study conducted among the adult population in the United States, a linear positive association was observed between BMI and serum ferritin levels.[30] In a study evaluating the relationship between the percentage of visceral fat mass and serum ferritin levels, a significant

negative association was found between visceral fat mass and serum ferritin. Additionally, serum ferritin showed a significant positive correlation with BMI and the waist-to-hip ratio.[31]

In our study, significant positive correlations were found between alcohol consumption and total cholesterol, LDL, hemoglobin, hematocrit, and MCV levels. These findings are consistent with studies in the literature showing the effects of alcohol consumption on various hematologic and biochemical parameters. Similarly, in a study conducted by Demir and Özsoy[33], it was found that liver enzymes as well as lipid profiles were affected in patients with alcohol use disorder; especially when total cholesterol and LDL levels increased. In a study conducted by Kulu[34], MCV values were found to be significantly higher in individuals with alcohol use disorder compared to the control group. In addition, it was determined that hemoglobin levels were higher but platelet levels were lower in alcohol addicts compared to the control group.[33-34]

Limitations

This study utilized data obtained retrospectively from the hospital registration system. Some individuals were excluded from the study due to incomplete records or incorrect entries. The potential use of non-standardized methods for measuring clinical and laboratory values in individuals is acknowledged as a limitation that may influence the results. The cross-sectional design of the study presents a significant limitation, as it precludes the evaluation of causal relationships between BMI and biochemical parameters. A relationship exists between obesity and changes in lipid profiles; however, the direction of this relationship and the underlying mechanisms remain unexplained.

The study did not evaluate critical variables that may influence obesity, including socioeconomic status, dietary habits, physical activity levels, and other environmental factors. The study was limited to individuals admitted to a single center. This restricts the applicability of the results to the broader population and raises concerns about the validity of the findings for individuals in different regions.

While the study incorporated lifestyle habits like smoking and alcohol consumption, it lacked comprehensive data regarding the frequency and duration of these behaviors. This constituted a limitation in the comprehensive evaluation of the impacts of smoking and alcohol on biochemical parameters. The study was limited to data from a specific timeframe, preventing the longitudinal analysis of changes in weight, blood values, and other individual parameters over time. This constrains the assessment of the outcomes from a dynamic viewpoint.

A commission established through a real collaboration involving 56 leading experts from a wide range of disciplines, representing high-income, middle-income, and low-income countries, announced on January 14, 2025, a new definition and diagnostic framework that clarifies when obesity is a risk factor (preclinical obesity) and when it is a disease on its own (clinical obesity).[35] The new, evidence-based definition distinguishes "clinical obesity," a chronic, systemic disease condition directly caused by excess fat, from "preclinical obesity," a condition of excess fat with no current organ dysfunction or limitations in daily activities but with an increased future health risk. Since our study was planned and data were collected prior to the establishment of this definition, patients were categorized according to BMI and evaluated by comparing with laboratory parameters. A specific assessment regarding clinical or preclinical obesity was not provided.

Finally, the study did not assess factors that may influence obesity, such as psychological stress, cortisol levels, or other hormonal parameters. All these limitations stand out as important issues that should be accounted for when interpreting the study findings.

The strength of this study is that the relationships between obesity and important metabolic and biochemical parameters such as thyroid function, vitamin B12 levels, and lipid profile were comprehensively evaluated with a large sample group. A detailed analysis of different BMI categories provided a better understanding of the effects of obesity on biochemical parameters. In addition, the fact that the data used in the study were obtained from the hospital registration system allowed the evaluation of data obtained under real-life conditions and made it possible to directly reflect the results in clinical practice.

CONCLUSION

This study assessed the associations between obesity and several biochemical and metabolic parameters, yielding significant findings. Obesity elevates cardiometabolic risks through the rise in blood glucose, triglycerides, total cholesterol, LDL cholesterol, and VLDL cholesterol levels, while simultaneously reducing HDL cholesterol levels as BMI increases. Additionally, elevated ferritin levels have been noted in obese individuals, suggesting potential inflammation or alterations in iron metabolism. Nonetheless, no significant correlation was identified between BMI and TSH, FT3, and FT4 levels, suggesting that the discussion regarding the impact of obesity on thyroid function remains unresolved.

The data obtained in the study once again emphasize the negative effects of obesity on metabolic health and reveal the importance of strategies to combat obesity. It is concluded that metabolic and biochemical changes associated with obesity should be regularly monitored, and individualized treatment plans should be established in health policies and clinical practices. In the future, more comprehensive and prospective studies should be conducted to investigate the biochemical effects and cause-effect relationships of obesity in more detail.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was approved by the Dicle University Non-interventional Ethics Committee (Date: 05.08.2014, Decision No: 306).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Advancements and challenges in Hashimoto's thyroiditis research: a bibliometric analysis

Pınar Alarslan

Department of Endocrinology and Metabolism, Tepecik Training and Research Hospital, University of Health Sciences, İzmir, Turkiye

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ABSTRACT

Aims: Hashimoto's thyroiditis (HT) is the most common autoimmune thyroid disorder and a leading cause of hypothyroidism worldwide. This bibliometric analysis aims to provide a comprehensive overview of HT research trends, key contributors, and emerging themes by evaluating 1.309 articles published between 2004 and 2023. The study seeks to identify gaps, highlight advancements, and offer insights to guide future research efforts.

Methods: Data were collected from the Web of Science Core Collection database using the keyword "Hashimoto" and filtered by the "endocrinology metabolism" category. Bibliometric analysis was conducted using VOSviewer to visualize publication trends, keyword relationships, and collaboration networks. Citation metrics and publication outputs were analyzed to identify influential contributors and emerging research themes.

Results: The analysis revealed a steady growth in publications, peaking in 2021, with notable contributions from leading institutions and journals such as thyroid and Journal of Clinical Endocrinology & Metabolism. Dominant themes included autoimmune mechanisms, thyroid dysfunction, and the gut-thyroid axis. Emerging areas, such as microbiota-targeted interventions and personalized medicine, offer promising avenues for advancing HT research. However, regional disparities and conflicting findings in treatment strategies, including dietary interventions and supplementation, underscore the need for more robust studies.

Conclusion: HT research has achieved significant progress in understanding its pathogenesis and clinical management. Future efforts should focus on multidisciplinary, large-scale studies that integrate advanced technologies and address regional research gaps. By fostering global collaborations and embracing innovative approaches, the scientific community can improve patient outcomes and advance the field of HT research.

Keywords: Hashimoto's thyroiditis, autoimmune thyroid disease, bibliometric analysis, thyroid dysfunction

INTRODUCTION

Hashimoto's thyroiditis (HT), known as chronic lymphocytic thyroiditis in some circles, is an autoimmune condition that results in hypothyroidism as a result of the bombarding of lymphocytic infiltration followed by the damage of the normal functioning thyroid capsules. The HT was first described by Dr. Hakaru Hashimoto in 1912 and it was this disease which brought a revolution in the field of autoimmune endocrinology.[1] With the passage of time, some advancement in the understanding of histology and immunology have made its characteristics well defined, for example, thyroid-specific antibodies such as thyroglobulin antibodies (TgAb) and thyroid peroxidase antibodies (TPOAb) became the signature biomarkers of the disease.[2] However more than a hundred years of investigation did not obtain its cause which seems even more complex because of genetic, environmental and immune factors prevailing together.[3]

HT is the most prevalent autoimmune thyroid disease across the globe, with internal thyroid deficiency being its main downstream effect, hypoparathyroidism. Its occurrence shows a striking prevalence among women with the female to male ratio of nearly four to one but spreads the most between the ages of 30-50.[4,5] Furthermore, widespread areas with socio-economic disparities and increased age have a higher chance of occurrence, especially in areas where iodine intake is satisfactory.[6] HT is habitually present in combination with other autoimmune disorders such as type 1 diabetes mellitus, systemic lupus erythrematosus and rheumatoid arthritis and they are parts of autoimmune polyglandular syndromes.[7] This interconnectedness of autoimmune disorders raises more biomedic research questions in the domain of genetics and immunology of the patients with multiple autoimmune diseases.

Corresponding Author: Pınar Alarslan, drpinaralarslan2024@gmail.com

HT is known to be the most prevalent autoimmune disease and one of the contributors of endocrine disorders across the world. However, the range and breadth of scholarship on HT do not allow for the complete resolution of doubts concerning its etiology, diagnosis and therapy. The results obtained are intended to illuminate future HT research by diagnosing the main issues concerning HT, analysing areas of interest and focus, and knowledge maps construction. The significance of the problem is determined by the possibility of expanding the knowledge of the impact of HT on the public health and elaborating more efficient approaches to the management of this condition. The results of the analysis should also help in the identification of research gaps and areas of emphasis.

In this context, it is sought to contribute useful information towards the scientific progression of HT studies and to motivate interdisciplinary point of view.

METHODS

Since this research is a bibliometric study, it did not require ethics committee approval. It is conducted with the institution's permission.

Data for this bibliographic analysis were obtained from the Web of Science (WoS) Core Collection platform, an authoritative resource containing high-quality, peer-reviewed scientific articles from various regions. The study focused on publications on HT and examined studies conducted between January 1, 2004 and December 31, 2023. The search was conducted using the keyword "Hashimoto" and applying the "topic" filter. Articles were selected from those included in the "endocrinology metabolism" category within WoS Categories.

As a result of the initial search, 1.678 articles were identified as focusing on HT. The titles, abstracts, and keywords of the articles were examined in detail; duplicate records were removed. Peer-reviewed articles that met the inclusion criteria were analyzed and 1.309 scientific articles were included in the final data set. The first 10 articles were independently evaluated by two researchers, and disagreements regarding selection were resolved through discussion.

Data collection was conducted between January and March 2023. The following information was collected for each article: article title, author names, publication year, journal name, journal impact factor, citation counts, country of affiliation of authors, institution name, and frequently used phrases. The obtained data were verified by two independent observers, and differences were resolved through consensus.

Bibliographic analysis was performed using VOSviewer (version 1.6.11, Leiden University, The Netherlands) to visualize research trends, keyword relationships, and collaboration networks. The main areas of focus of the analysis are:

- Annual publication trends
- Journal-specific publication trends
- Citation analysis (authors, titles, journals, and publication years)
- Keyword concurrency analysis
- Institutional affiliations and inter-institutional collaborations
- Country-level collaboration networks
- Author collaboration networks

Statistical Analysis

Descriptive statistics SPSS-(frequencies and percentages) were used to summarize publication numbers, citation distributions, and journal impact measures. Changes in annual article numbers were examined to assess temporal trends in publication output. Concurrency networks of the 150 most frequently used keywords were created to reveal thematic clusters and connections in the field.

Inter-institutional and international collaboration patterns were visualized using bibliometric mapping techniques. The density of collaborations was represented by the thickness of the connection lines, revealing common research focuses across institutions and countries. Cluster coefficients and connection densities were used to measure the integrity and integration of research themes.

RESULTS

Analysis of the Distribution of Articles by Year

Figure 1 illustrates the distribution of articles on the topic of "Hashimoto" over the years, based on Web of Science data.

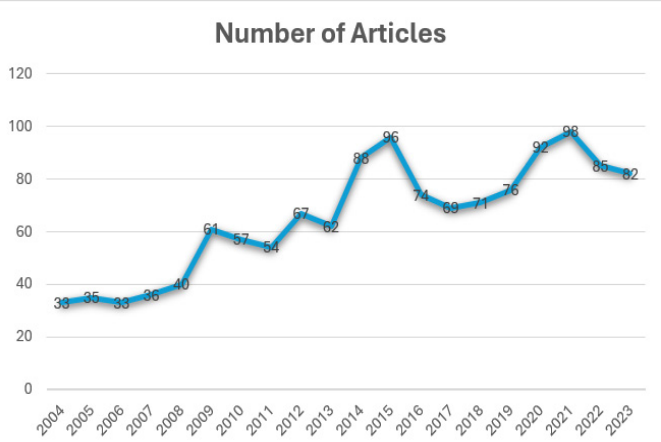

Figure 1. Yearly distribution of articles on "Hashimoto"

Figure 1 shows the year-by-year distribution of articles related to "Hashimoto." According to the data, only 33 articles were published in 2004, and this number shows a steady upward trend over the years. By 2010, the number of articles reached 57, indicating a moderate increase in interest during that period. From 2015 onward, the number of articles surpassing 96 demonstrates a growing scientific focus on the topic and increased research activity.

The year 2021 represents the peak, with approximately 98 articles published, highlighting the intense attention the topic received in scientific literature during that time. However, in the following years, a slight decline was observed, with 85 articles in 2022 and 82 in 2023. These fluctuations may reflect shifts in scientific interest or changes in research priorities during those years.

Journals Publishing the Most Articles on the Topic

The number of articles on the topic of "Hashimoto" published in journals within the Web of Science database is presented in Table 1.

Table 1. Journal names, number of publications, and percentage distributions of articles

No	Name of journal	Number of article	%
1	Thyroid	133	10.16%
2	Journal of Clinical Endocrinology Metabolism	95	7.25%
3	Frontiers in Endocrinology	87	6.64%
4	Endocrine	66	5.04%
5	Endocrine Journal	60	4.58%
6	Journal of Endocrinological Investigation	58	4.43%
7	Clinical Endocrinology	53	4.04%
8	Journal of Pediatric Endocrinology Metabolism	45	3.43%
9	European Journal of Endocrinology	37	2.82%
10	BMC Endocrine Disorders	32	2.44%
11	Other	643	49.17

Table 1 compiles data on the journals that have published the highest number of articles on "Hashimoto" in the Web of Science database. According to the data, 10.16% of the total articles (133 articles) were published in the Journal Thyroid, making it the most preferred platform for research in this field. It is followed by the Journal of Clinical Endocrinology & Metabolism with 7.25% (95 articles) and frontiers in endocrinology with 6.64% (87 articles). These journals represent key contributors to the scientific literature on the topic.

Journals with a relatively smaller share, such as endocrine (5.04%, 66 articles), Endocrine Journal (4.58%, 60 articles), and Journal of Endocrinological Investigation (4.43%, 58 articles), have also made notable contributions to the field. At the lower end of the list, BMC Endocrine Disorders accounts for 2.44% (32 articles), completing the table.

The "Other" category, which includes multiple journals not individually listed, accounts for 49.17% of the total articles (643 articles). This significant proportion indicates that research on "Hashimoto" is disseminated across a wide range of journals, highlighting the topic's broad scientific reach.

Information on Authors, Articles, Journals, Publication Dates, and Citation Counts of the Most-Cited Publications

The authors, article titles, journals, publication dates, and citation counts of the most-cited publications on the topic of "Hashimoto" within the Web of Science database are presented in Table 2.

Table 2 includes the authors, article titles, publishing journals, publication years, and citation counts of the most-cited publications on "Hashimoto." According to the data, the most-cited article was authored by Laurberg et al., published in 2010 in the journal Best Practice & Research Clinical Endocrinology & Metabolism, and received a total of 335 citations. This study examines the effects of iodine intake on thyroid diseases and serves as a significant reference point in the literature.

The second most-cited article, authored by Wartofsky and Dickey, was published in 2005 in the journal Journal of Clinical Endocrinology & Metabolism and received 299 citations. This article provides evidence for narrowing the thyrotropin reference range. The third-ranked article, authored by Lee et al., was published in 2013 in European Journal of Endocrinology and received 232 citations. This study investigates the relationship between papillary thyroid carcinoma and HT using meta-analysis.

Other articles listed in the table also address various topics, such as the pathogenesis, inflammatory processes, and immune response in HT, making significant contributions to the scientific literature. For example, a study by Figueroa-Vega et al., published in 2010, examines the increase in circulating pro-inflammatory cytokines and Th17 lymphocytes in HT and has received 193 citations.

Publication Statistics of the Most-Cited Institutions

The institutions affiliated with the authors of articles on "Hashimoto," the number of publications produced by these institutions, and the citation counts of these publications are presented in Table 3.

Table 3 highlights the most-cited institutions in the Web of Science database on the topic of "Hashimoto," along with their publication counts and total citation numbers. According to the data, the most-cited institution is the University of Messina, which has produced 46 publications and received a total of 1.237 citations, demonstrating its significant influence in this field. It is followed by the University of Pisa, which has published 33 articles with a total of 1,214 citations. These two institutions have made substantial contributions to the literature on "Hashimoto" with their high citation counts.

In third place, Johannes Gutenberg University Mainz has garnered 666 citations from 14 publications, while University of Naples Federico II ranks fourth with 21 publications receiving 575 citations, and University of Turin is fifth with 18 publications earning 573 citations. The citation impact per publication of these institutions underscores their significant influence in the field.

Other noteworthy institutions include:

- Osaka University (20 publications, 551 citations),
- Sapienza University of Rome (13 publications, 481 citations),
- Sungkyunkwan University (7 publications, 471 citations).

Additionally, Odense University Hospital has achieved 467 citations from 14 publications, while the University of Birmingham, with just 5 publications, has received 452 citations, showcasing remarkable academic impact.

Trends in Keyword Usage

Keywords act as a bridge between the article and the targeted audience in order to increase the impact of the study.

Table 2. Most cited publications: authors, journals, publication dates, and citation counts

No	Authors	Journals	Publication names	Dates	Citation
1	Laurberg P, et al.	Iodine intake as a determinant of thyroid disorders in populations	Best Practice & Research Clinical Endocrinology & Metabolism	2010	335
2	Wartofsky L and Dickey RA.	The evidence for a narrower thyrotropin reference range is compelling	Journal of Clinical Endocrinology & Metabolism	2005	299
3	Lee J. H. et al.	The association between papillary thyroid carcinoma and histologically proven Hashimoto's thyroiditis: a meta-analysis	European Journal of Endocrinology	2013	232
4	Figueroa-Vega N, et al.	Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis	Journal of Clinical Endocrinology & Metabolism	2010	193
5	Nanba T.	Increases of the Th1/Th2 cell ratio in severe Hashimoto's disease and in the proportion of Th17 cells in intractable graves' disease	Thyroid	2009	172
6	Ajjan RA and Weetman AP.	The pathogenesis of Hashimoto's thyroiditis: further developments in our understanding	Hormone and Metabolic Research	2015	167
7	Zhu CL, et al.	Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease	Journal of Clinical Endocrinology & Metabolism	2012	164
8	Manji N, et al	Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype	Journal of Clinical Endocrinology & Metabolism	2006	164
9	Köhrle J and Gärtner R.	Selenium and hyroid	Best Practice & Research Clinical Endocrinology & Metabolism	2009	161
10	Brix TH, et al	High frequency of skewed X-chromosome inactivation in females with autoimmune thyroid disease: a possible explanation for the female predisposition to thyroid autoimmunity	Journal of Clinical Endocrinology & Metabolism	2005	155

Table 3. Most cited institutions and publication distributions according to Web of science data

No	Institution name	Number of publications	Number of citations
1	University of Messina	46	1237
2	University of Pisa	33	1214
3	Johannes Gutenberg University Mainz	14	666
4	University of Naples Federico II	21	575
5	University of Turin	18	573
6	Osaka University	20	551
7	Sapienza University of Rome	13	481
8	Sungkyunkwan University	7	471
9	Odense University Hospital	14	467
10	University of Birmingham	5	452

In this case, a list of 13 keywords most frequently used with "Hashimoto" in the WOS was included, and their frequencies of appearance in the database are presented in Figure 2.

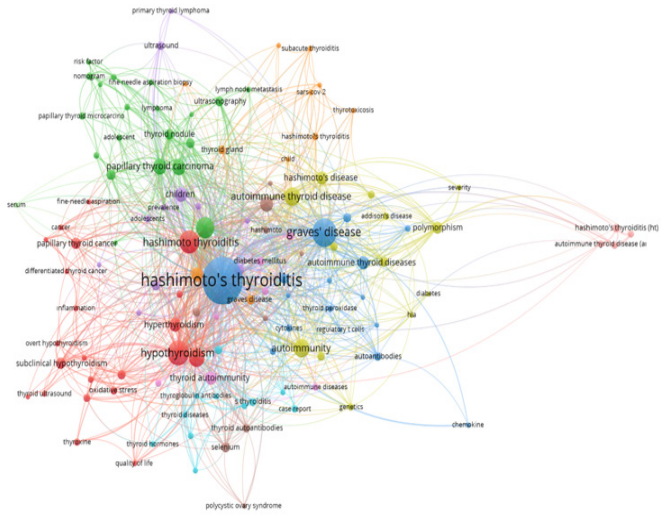

Figure 2. Frequently co-used keywords and their usage frequency

Bibliometric analysis enabled by Vosviewer software was used for multi case studies with a minimum threshold of 5 occurrences to include a keyword in the bibliometric and systematic review. The analysis included all cases where a keyword was present but appeared a minimum of 5 times in the documents being studied. This method has ensured that only the commonly used and more significant terms are considered in the analysis. From 2.100 unique keywords only 125 were within this threshold and thus were incorporated in the analysis. This means that the analysis was only on these 125 keywords and their relations.

Due to the analysis, it was possible to pinpoint the most frequently cited keywords in a particular research field and their most important connections. Also, a total of 1,043 connections and 11 different clusters among the keywords were identified. All of these results illustrate in detail interconnections that exist among keywords in the area of the study. Such analysis is beneficial in providing understanding on the terminology of the field as well as outlining major ideas for subsequent research to be conducted.

Figure 2 depicts the keywords that are in high preference in the academic articles regarding "Hashimoto" with their respective statistics of use. As per the data, 'HT' is the most used keyword achieving a total of 339 mentions. This demonstrates that the Center's research is primarily "HT" and more importantly this term is well covered in the literature.

"Graves' disease" is the second most used keyword as it was mentioned 123 times meaning that Graves' disease is a subject of many studies in relation to "HT". The third keyword which is mostly used is "hypothyroidism", with 98 moments of touch, clearly relates to "HT" which explains why it is greatly emphasized in the literature.

Other keywords, which are not so common but which are also important include \"thyroid\" (69) or Hashimoto thyroiditis (83) and autoimmune thyroiditis (54). These terms suggest attention to autoimmune thyroid diseases together with other disorders of thyroid functions in the selected research area.

The data presented in the figure portray quite visibly the major trends and areas of concentration of research activities that have been covered in the literature on "Hashimoto." Additionally, the findings also highlight the tactical aspect of selection of keywords for increasing the adherence of studies and linkage within that area. These results suggest that research on "Hashimoto" has been quite broad in nature and even encompasses more related aspects with high degree of linkages.

Analysis of Inter-Institutional Publications

The findings from the analysis of the institutions affiliated with the authors of articles on "Hashimoto" and the collaborative relationships between these institutions are visualized in Figure 3 below.

In the collaboration map created using Vosviewer software, the size of the circles represents either the number of publications by institutions or their central roles in the collaboration network. The colors of the circles indicate thematic or regional groupings. The lines between the circles illustrate the collaborative relationships between institutions, with the thickness of the lines reflecting the intensity of these collaborations. This visualization provides valuable insights for evaluating the influence of institutions in the research field, identifying strong relationships within the collaboration network, and recognizing potential opportunities for further collaboration.

Based on the figure, the key findings from the inter-institutional collaboration analysis related to "Hashimoto" are as follows:

- The graph highlights that University of Messina plays a central role in the collaboration network. With a total of 9 connections, it stands out as the institution with the highest number of collaborations. This demonstrates that the University of Messina has developed strong and extensive relationships with other institutions in "Hashimoto" research.
- Fudan University and Johns Hopkins University, each with 6 connections, hold significant positions in the collaboration network. These institutions contribute to the advancement of research in this field by fostering collaborations at both national and international levels.
- Osaka University and Ankara University, each with 3 connections, represent more limited collaboration networks but still maintain interactions with other institutions on the graph. Similarly, China Medical University, with the same number of connections, plays an active role, particularly in regional collaborations.

Overall, the intensity of the connections and the clustering structures in the graph indicate that certain institutions have become focal points in "Hashimoto" research, supporting the dissemination of knowledge through collaborative relationships in this field. Such analyses provide strategic guidance for more effectively directing academic efforts and identifying collaboration opportunities.

Author Collaboration Analysis

The patterns of collaboration that emerged between the authors of articles on "Hashimoto" in Web of Science, have been examined and the results are presented in Figure 4.

Figure 4 deals with the author's bibliometric relationships who had contributed five or more studies in one area of

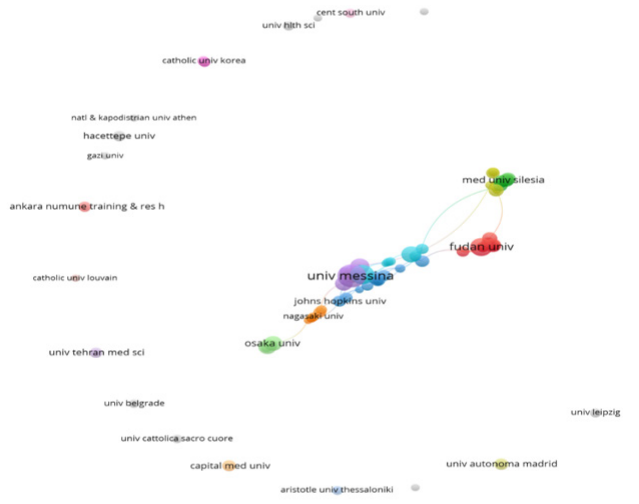

Figure 3. Bibliometric network visualization of inter-institutional collaborations

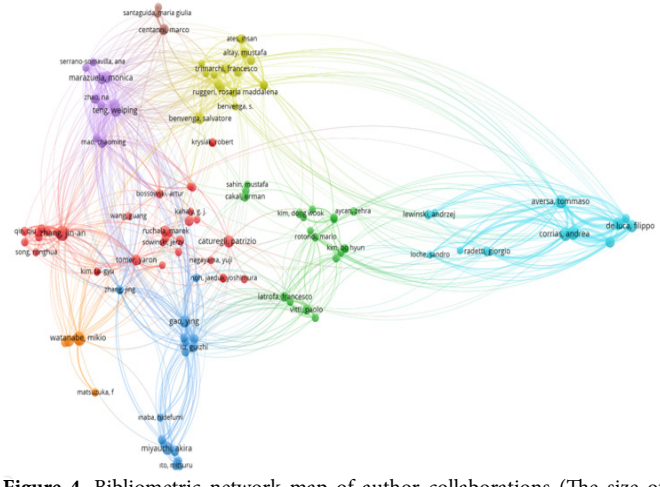

Figure 4. Bibliometric network map of author collaborations (The size of the circles represents the main authors, while the lines between the circles indicate collaborative relationships.)

research. Out of 6,789 authors who attempted to author the paper, just 121 authors who met the threshold were included in the analysis. Setting five or more works as a condition helps to capture those authors who are more focused on the synthesis of the literature and are productive enough. This also improves the credibility and significance of the results by considering only the active and productive authors.

Since this method centers on the minimal number of publications, it guarantees that the analysis is concentrated on significant scientific contributions and enhances the consistency of results. This technique disqualifies authors who have too few publications or whose cases were too atypical, and so it brings attention to the strongest authors and institutions which comprise the basic building blocks of the field. Therefore, the data analysis fully captures the cross-cutting patterns and research linkages in the region so that the analysis of the relationships of the key players in the focal literature can be done in detail. Such an approach increases the credibility of the outcomes of the study and provides good modeling of the basic scientific order in the field.[8]

The analysed data in **Figure 4** depicts that among the 121 authors, 8 unique clusters were formed. These clusters illustrate that authors with similar interests tend to form groups that work on the same ideas or concepts and use citations to establish interrelations. Each such cluster depicts coauthorship and information flow between the authors but at the same time, the cluster indicates research concentration pertaining to certain topics in the literature. Such bibliometric analyses do not only establish the leading researchers and the scientific networks established in the given field but also allow to obtain informative data about the changing picture of the discipline and its topic areas.[9]

The visualization in **Figure 4** illustrates the bibliometric connections and collaboration networks among authors working on "Hashimoto." In the visualization, each author is represented by a circle, the size of which reflects the author's contribution to the literature. The colors of the circles indicate the clusters to which the authors belong, signifying thematic or research-focused groups. The lines between the circles represent the strength of bibliometric connections, indicating the frequency of shared references and detailing the scientific relationships among authors.

The analysis of the graph reveals collaboration relationships and thematic clustering among authors in the field of "Hashimoto" research:

- The blue cluster, located in the lower middle part of the figure, represents the authors with the most extensive collaboration network in the literature. Notable authors in this cluster include Miyauchi Akira and Ito Mitsuru, who play a central role in the research area through international collaborations and high productivity. The density of connections indicates strong relationships between this cluster and other author groups.
- The red cluster, located in the left middle part of the graph, includes authors concentrated around a specific thematic framework. Zhang Jin-An and Ruchala Marek stand out in this cluster, maintaining close scientific ties with other authors within the cluster. This cluster appears to represent thematic diversity within the literature.
- The green cluster, situated in the upper right corner, represents another significant thematic group in the field. Latrofa Francesco and Vitti Paolo are prominent authors in this cluster. The density of connections indicates strong relationships both within the cluster and with other groups.
- The orange cluster, located in the lower left corner, is a smaller group but exhibits strong internal connections. Watanabe Mikio and Matsuzuka F. are notable names in this cluster, which is focused on a specific sub-theme. Despite its narrow research focus, this cluster contributes meaningfully to the literature.
- The yellow cluster, concentrated in the upper middle part of the graph, highlights authors such as Trimarchi Francesco and Ruggeri Rosaria Maddalena. This cluster represents scientific interactions among authors with a thematic research focus.
- The turquoise cluster, on the right edge, includes authors like Tommaso Aversa and Andrea Corrias, who contribute to the literature through high levels of collaboration.
- The purple cluster, situated in the upper left, includes authors such as Monica Marazuela and Weiping Teng.

Overall, the density of connections and clustering structures in the graph clearly illustrate the breadth and depth of collaboration relationships in the literature. This visualization serves as a crucial tool for understanding the leading authors and thematic groups in the field.

Country-Based Distribution of Citations

A comprehensive assessment was conducted based on countries on the articles that pertained to the topic of "Hashimoto" and are part of the WoS database. The results of this analysis are visualized in **Figure 5** and show a clear citation diffusion trend geographically.

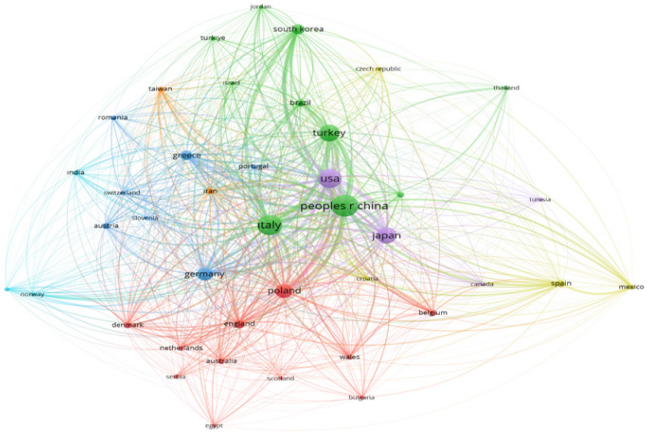

Figure 5. Analysis of citation

Figure 5 gives clear images depicting the analytical view of the mapping out of the citations distribution by country for articles in the WoS and the inter country academic collaborations. For further analysis countries which managed

to publish five or more documents were included (41 out of 72 countries that met the inclusion criteria). A total of six clusters were identified in this analysis, each defined with a particular theme of research or a common network of collaboration.

The figure depicts the distribution and the geography of the citations for research work done on the topic "Hashimoto" as well as the inter- country academic collaborations. Each circle represents a country and the area of the circumference is proportional to the area of the contribution of that country in the literature. The lines between such circles are collaborative links and graphically, the thicker the line the more the cov-alterations occurred. The colors in the figure demonstrate the arrangements of nations in groups, and the collaboration networks that are formed around specific theme of the research work done.

As seen from the figure there is a large circle at the center depicting the United States whose contribution has been great in the literature and the countries working collaboratively with other countries networks. This central role of the U.S. demonstrates its leadership and influence in Hashimoto research in the world. Among the European countries, the more advanced ones are the United Kingdom, Germany and France. These countries are more nearer to the center circle of the figure and have closer collaborative relations with each other which means that they form a well-connected scientific network in Europe. Also, the U.K. has created strong links with the U.S. which adds to its prominence in the literature. In Asia it is the countries such as Japan, China and Korea which play a more active role. These countries are prominent from the regional and international perspectives because of their strong networks with the U.S. His figure reminds us how Japanese researchers and professionals have been positioned in the international scene in terms of the geocentric model for "Hashimoto" research. Turkey can be seen at the middle of the figure as both a European continent and an Asian country giving it a unique opportunity of being a link between the two clusters. This location helps to explain Turkey's presence in the literature as well as its potential for cross border collaborations.

Such nations depicted by smaller circles which are places on the borders of this figure are, in turn, indicated as having less of an influence on the literature. It is however important to note that these nations also participate in regional collaboration networks.

To sum up, the regional collaboration networks in the field of "Hashimoto" research are shown in **Figure 5**. The US, UK and Japan are becoming quite prominent in the field as the core countries apart from Turkey and other countries in Asia that show good potential for collaboration. Thus, this sort of visualization helps the readers understand the important contributors and engagement patterns of the countries in the collaboration within the literature.

DISCUSSION

Taking into account the findings of this scientific study, it also becomes evident which specialized researchers and groups of researchers are actively participating in the development of the field and what directions and themes are becoming popular. However, there is no dispute that given the evidence these works bring, the field of inquiry needs a more in depth assessment at both its advances and challenges.

The trend of increasing concern in respect of scientific publications first noticed in 2004 and continuing up to the present time with the 170 articles registered in 2021 shows the growth in the interest in research into and development of auto immune thyroid diseases. This accords with the rise in prevalence of autoimmune thyroid disease and new methods of treatment which were reported by Tao et al. in 2023. That there has been a slight decrease in publications within the subject matter after 2021 could be due to change in interests or other imposing issues such as the COVID-19 pandemic. Such a situation may warrant the change of interests or the limited number of resources may constrain the effort in this particular area as Ahmed et al.[2] and Akamizu and Amino[6] have observed.

As reported by Hu et al.[10] there has been noticeable growth in studies about epidemiological and worldwide distribution trends, especially in parts of the world which are backwards, which denotes some improvements in diagnosis and awareness. On the other hand, Ragusa et al.[11] lamented that even though there are more scientists trying to research the disease, there remains high ignorance of the geographical and ethnical heterogeneity of the disease which remains a concern for this present study.

There are substantiating evidences which show Thyroid and Journal of Clinical Endocrinology & Metabolism as being key in the communication of meaningful research findings. In due course, they become influential in the study of HT. These findings also imply that research such as those by Zaletel and Gaberscek[3] examining the interactions of genetics and the environment are also common in these journals. However, Sawin[1] noted that A historical approach to the disease suggests that it is possible there was a relative famine of research on HT in some earlier periods.

The pair of studies of Wartofsky and Dickey (2005) on thyrotropin reference ranges and of Laurberg et al. (2010) on the role of iodine intake and thyroid diseases worked towards the development of some clinical guidelines. However, there are also some contradictory findings. For instance, Krysiak et al 2019 report limited effect of such nutritional interventions like gluten-free diet on the disease outcomes and challenge the wider impact of the nutritional interventions suggested by Ihnatowicz et al. (2020). Such contradictions emphasize the necessity of further randomized controlled interventions to definitively demonstrate the evidence supporting specific dietary recommendations.

The bibliometric analysis touches upon the major themes, which include "HT", "Graves' disease" and "hypothyroidism" This corresponds with the observations made by Pyzik et al.[12] who noticed that there is an emphasis on immune system dysfunction as a area of study. Other emerging areas such as microbiota and gut-thyroid axis are also promising. As shown by Fernandez-Garcia et al.[13] and Liu et al.[14], it is possible to target microbiota in order to regulate thyroid autoimmunity. The term "HT" appears to be the most prominent among

key words appearing in the literature and this is similar to Radetti's[4] study which attempted to investigate clinical features of pediatric Hashimoto's disease. Of course, other keywords including "Graves' disease" and "hypothyroidism" are important due to the idiopathic nature of the disease with many autoimmune phenomena. Although these works postulate many positive correlations between gut health and autoimmunity, Lerner et al.[15] cautioned that generalizations ought to be made bearing in mind some reproduction and heterogeneity limitation in study design. Such results also indicate an unproven, and quite broad approach to the gut-thyroid axis. Such well-cited studies are represented by the University of Messina and the University of Pisa. Such European institutions also to some extent contribute as seen in Ragusa et al.[11] in examining thyroid autoimmunity.

Also, other authors such as Miyauchi Akira, Ito Mitsuru also contributed towards the principles of HT pathogenesis at the clinical level and molecular level. These findings conform to the set up views emphasized by Ahmed et al.[2] and Akamizu and Amino[6] that knowledge transfer and collaboration enhances research output. But, the absence of such research works in developing countries, in the same way contributes to limiting the prospect of research.

But, certain areas such as Africa and South America are less noted in the body of literature. According to McLeod and Cooper[16] that these regions' contributions in this arena need be looked at in view of genetic and environmental influences on autoimmune thyroid diseases.

Concepts regarding the management of HT still remain a contentious domain. Research of this type e.g. Pirola et al.[17] confirms the opinion that subclinical hypothyroidism can be treated by selenium supplementation, on the contrary dispute on the fact that such measures will be useful in the long run.

The increasing number of studies on vitamin D and selenium suggests a growing interest in immunomodulatory approaches to managing HT. This finding supports the notion that research on HT has expanded beyond traditional endocrinological assessments to include nutrition and adjunctive therapeutic approaches as significant areas of investigation. Furthermore, Shin et al.[18] reported an association between low vitamin D levels and increased antithyroid peroxidase antibody titers, Nodehi et al.[19] found that vitamin D supplementation alone failed to significantly alter disease progression, and Pirola et al.[17] showed that selenium supplementation may be beneficial in some patients. Such conflicting findings highlight the need for larger, more comprehensive randomized controlled trials. This highlights the complexity of autoimmune thyroid diseases and the need for multifaceted treatment strategies.

When analyzing the available research on HT there is an ongoing theme that this is a very broad field that keeps changing with the advancements being made. There are considerable advances toward the epidemiological understanding, due care toward the pathogenesis and the disease management resulting in the paradoxes witnessed as it relates to various interventions being dietary, vitamin D, supplementation or the risk for cancer.

To solve this issue, large population-based multi-centric studies should be the key focus of further research in this area. Furthermore including new platforms such as microbiota and precision medicine can further enhance the prevention and treatment strategies. This task can be achieved through increased collaboration between different scientific disciplines and tackling local issues in the research on HT which in turn would improve the research fit for translation enablers.

Limitations

This study has some limitations. First, the analysis is restricted to the Web of Science Core Collection, excluding relevant articles from other databases. Second, it only covers publications from 2004 to 202, potentially missing earlier trends and historical perspectives. Third, using the term "Hashimoto" as a search criterion may have excluded relevant studies with different terminology. Lastly, limited research from developing countries may overlook regional differences and contributions to HT research.

CONCLUSION

Research in this field focused on HT has shown tremendous growth in the last 20 years which has increased the understanding of its diverse pathophysiology, epidemiology and treatment aspects. The findings obtained through this bibliometric analysis stresses the increasing patterns of alliances across the globe in research initiatives targeting HT as indicated by the rising number of publications, changing trends in cross border research and increasing number of international linkages. However, the findings also point out areas still faced with challenges such as the inability to explain wide variations in contribution to research from different regions to the problem of RT, certain evidences on some therapeutic approaches being contradictory, and the more recent areas such as the gut-thyroid link and other aspects requiring more focus being barely scratched.

In order to tackle those mentioned above, longitudinal studies of a multidisciplinary and multicenter nature should be the focus of future studies, as they afford the opportunity for the employment of newer diagnostic and treatment approaches, for instance, microbiota profiling and genomic analysis. Of equal importance is promoting greater involvement of gaps in regions and populations so that the findings can be more widely applied and approaches more culturally appropriate.

By bridging knowledge gaps and embracing innovative methodologies, the scientific community can advance the understanding of HT and improve clinical outcomes for patients worldwide.

ETHICAL DECLARATIONS

Ethics Committee Approval

Since this research is a bibliometric study, it did not require ethics committee approval.

Informed Consent

Since this research is a bibliometric study, it did not require informed consent.

Referee Evaluation Process
Externally peer-reviewed.

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

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Review

MEDICINE PALLIATIVE CARE

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Use and comparison of MTT, XTT and iCELLigence methods in the evaluation of drug toxicity

Ayşe Nur Coşkun Demirkalp

Vocational School of Health Services, Ahi Evran University, Kırşehir, Turkiye

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ABSTRACT

Toxicology tests are one of the fundamental methods used in biological and pharmaceutical research. These tests are used to evaluate the viability, proliferation and toxic responses of cells. The study of biological activities of cells plays a critical role in drug development, cancer research, toxicology and various biotechnological applications. Drug toxicology is an important field of research to determine the harmful effects of drugs on cellular and biological systems. In vitro tests are widely used for accurate evaluation of drugs and their toxic effects. These tests examine the effects of drugs on cell cultures, rapidly revealing their potential harmful effects in terms of time and resources. This review discusses the advantages of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and XTT (sodium 3'-[1-(phenylamino)-carbonyl]-3,4-tetrazolium]-bis (4 methoxy-6-nitro) benzene-sulfonic acid hydrate) tests, which are widely used as cytotoxicity tests, as well as the newer method iCELLigence. MTT and XTT are widely used and reliable tests that measure cell metabolism; both methods are very effective in assessing cell viability, but provide limited dynamic data. In contrast, iCELLigence is a newer technology and provides more in-depth data by monitoring the real-time responses of cells. iCELLigence continuously monitors the growth rate and morphological changes of cells, allowing for more comprehensive and sensitive results compared to traditional methods. Comparison of these methods allows determining which methodology provides more appropriate results according to different research needs.These tests are also used to define the concentration range over which more comprehensive and detailed in vitro testing can be performed to obtain meaningful data on parameters such as genotoxicity, mutation induction or programmed cell death. This review aims to compare these three methods and discuss their advantages and limitations in the assessment of drug toxicity.

Keywords: iCELLigence, MTT, XTT, drug toxicity, cytotoxicity

INTRODUCTION

Toxicology tests are important scientific studies conducted to determine the harmful effects of a chemical or biological agent on organisms. The use of these tests is widespread in many areas, especially drug development, environmental protection, food safety and control of industrial chemicals.[1,2] In the pharmaceutical industry, these tests are vital for testing the safety of new drugs, while in environmental sciences they are used to determine the effects of chemical waste on nature.[3,4] Additionally, toxicological testing of food additives is a critical tool for determining whether these substances pose harm to human health.[5] These tests are generally conducted to detect substances that may cause genetic mutations or have carcinogenic effects.[6] These tests measure the response of cancer cells to treatment and play an important role in the development of new drugs. Cytotoxicity tests determine the ability of drugs to target and kill cancer cells and their potential to harm healthy cells. They also help monitor the development of resistance to treatment and enable the development of more selective and effective treatment strategies.[2,6]

Cytotoxicity is defined as the potential of a compound to induce cell death.[7] Detailed studies on the dose and time dependent changes in toxic effects on cells can provide valuable information about necrosis, apoptosis and other mechanisms, together with observation of the effects on the cell cycle and the reversibility of these effects.[8,9] Therefore, in vitro cytotoxicity tests are important and necessary to determine, for example, the potential of a compound to cause cell death by damaging essential cellular functions. Determining the dose at which 50% of the cells are affected (IC_{50}) allows quantitative comparison of the effects of a single compound in different systems or of several compounds in different systems.[10]

MTT test, one of the common methods used in toxicity measurement, measures cell viability based on the metabolic activities of cells. MTT is reduced in living cells to form purple formazan crystals. The density of these crystals varies according to the level of viability of the cells and is usually measured spectrophotometrically.[11] Although the MTT assay is a widely used and relatively simple method in many

Corresponding Author: Ayşe Nur Coşkun Demirkalp, ayse.coskundemirkalp@ahievran.edu.tr

cell types, it has limitations such as resolution issues and the possibility of missing transient toxic effects.[12]

Similarly, the XTT test is another tetrazolium-based method used to measure the metabolic activity of cells. XTT, similar to MTT, is reduced to form soluble formazan products, which makes the measurements more reliable and sensitive. The XTT test has advantages over the MTT test, especially in cases where cell density is high.[13] The accuracy of this test is increased because it does not require the use of solvents, thus providing a clearer measurement of cell death and viability.[14,15]

Finally, iCELLigence technology monitors toxicity and cell viability in real time using the electrical impedance of cells. This technology continuously measures the morphological changes and proliferation rates of cells and detects their response to cell death through changes in electrical conductivity. In this way, electrical changes that occur when the integrity of the cell membrane is disrupted can indicate cell toxicity.[16] iCELLigence is an important tool, especially for long-term and dynamic toxicity monitoring studies.[17]

MTT ASSAY

The MTT test is based on the determination of the metabolic activities of cells, especially by the reduction of mitochondrial dehydrogenase enzymes. Mitochondrial dehydrogenases are located in the cell's energy production center and are involved in basic biochemical processes of the cell, such as oxidative phosphorylation. These enzymes are key components in cell metabolism and are necessary for maintaining cell viability.[18] Mitochondrial activity in living cells is critical for cellular energy production and metabolism. Cells produce ATP using glucose and other substrates for energy production, and some metabolic byproducts are released during this process. The MTT assay provides a measure that reflects these processes and indicates metabolic activity.[19]

The MTT compound is reduced in living cells to form a purple compound. This colorchange is considered an indicator of cellular metabolism. The MTT compound is a compound with a tetrazolium structure. Mitochondrial dehydrogenase enzymes in living cells reduce this compound. Reduced MTT changes from a yellow solution to insoluble formazan crystals with a purple-blue color. Formazan is a product of the mitochondrial activities of cells and this reaction occurs only in metabolically active cells (**Figure 1**).[20] When formazan accumulates, its absorbance value is usually measured using a spectrophotometer. This measurement serves as a biomarker of the metabolic activity of cells. High formazan concentration indicates high cellular metabolism and viability, while low concentration indicates cellular death or low metabolic activity.[21]

The results of the MTT test are usually measured in optical density (OD) values. The purple color obtained is proportional to the cells' metabolic activity; this value is used to understand whether a cell culture is healthy. In order to interpret the test correctly, appropriate control groups and standards must be used.[22]

The protocol uses a standard 96-well plate. This can be scaled up, however, to suit a different plate format. The absorbance

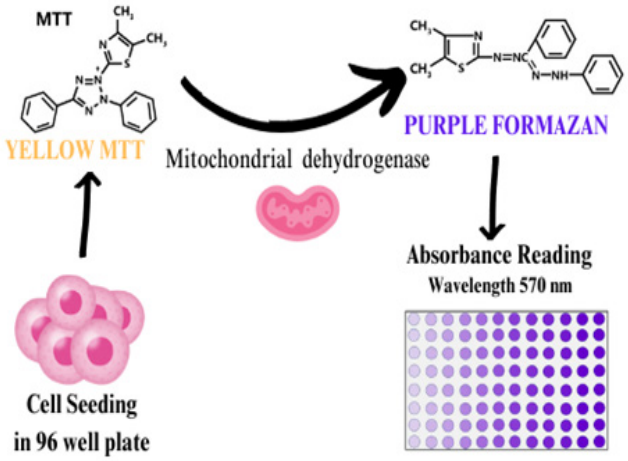

Figure 1. Conversion of MTT to formazan by mitochondrial dehydrogenase activity

of the samples at 570 nm is read using a microplate reader to determine the "half-maximum inhibitory concentrations" (IC_{50}). IC_{50} are calculated using appropriate statistical calculation methods.[11]

The cheapness of the chemical components used in the MTT test and the ease of application providesa great advantage in being a fast and economical test. In addition, the ability to work with a 96-well plate offers the opportunity to apply wide-range doses. With these aspects, it can be used on different cell types and is widely preferred in many drug researches.

In addition to all this, the MTT test only measures cellular metabolic activity, which may be insufficient to examine all aspects of cellular death or toxic effects. Additional studies may be needed for more detailed research outside of cellular metabolism. The MTT test is also limited to rapid observation of cellular responses. As a result, it lacks the ability to monitor dynamic changes.[23]

XTT ASSAY

The XTT assay is based on cellular metabolism, similar to the MTT assay, but provides more sensitive and stable results. XTT is reduced by mitochondrial dehydrogenases in living cells, creating a more stable colored compound.[24]

XTT Compound is a yellow, water-soluble tetrazolium compound. Mitochondrial dehydrogenase enzymes in living cells reduce this compound. Reduced XTT forms a orange colored insoluble formazan product in connection with the metabolic activities of living cells. Cells produce ATP in energy production, especially by using substrates such as glucose and oxygen. In this process, mitochondrial dehydrogenases of cells (e.g. NADH dehydrogenase and succinate dehydrogenase) reduce XTT compound. This reaction only occurs in metabolically active cells, because it is necessary for the continuation of mitochondrial functions and ATP production. Reduced XTT forms formazan crystals in an insoluble form. The density of these formazan crystals is proportional to cellular metabolism. A orange formazan solution is formed by the reduction of XTT. This color change is considered an indicator reflecting the cell's metabolic activity. The density of the orange formazan deposit increases

depending on the energy-producing capacity of the cells and their mitochondrial functions (Figure 2).[25,26]

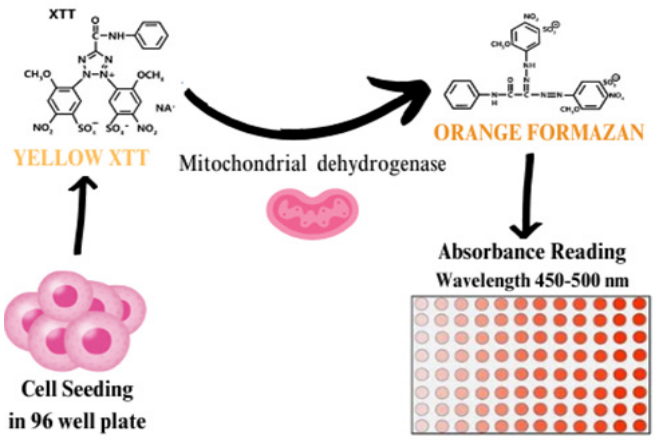

Figure 2. Conversion of XTT to formazan by mitochondrial dehydrogenase activity

The formazan formed can be separated from the solution because it is insoluble and particulate. As a result, the color intensity can be measured. Usually, the absorbance value is measured at a specific wavelength (usually 450-500 nm) using a spectrophotometer. High absorbance indicates that the cells have high metabolic activity and are alive, while low absorbance indicates that the cells have low metabolic activity or are dead.[27] As in the MTT test, IC_{50} values are calculated with the appropriate program and the results are analyzed.[28]

The XTT test provides reliable results even at low cell densities. This allows us to obtain more sensitive results. Since the XTT compound is more stable, the color change is more reliable and increases accuracy. In addition, the XTT test provides results more quickly than the MTT test. However, the XTT test is a more costly method compared to the MTT test because it requires more expensive chemical compounds and devices. In addition, just like the MTT test, the XTT test only measures metabolic activity and does not provide information about the detailed mechanisms of cell death.[29] It also limits our studies on cellular mechanisms such as MTT.[14]

iCELLigence REAL-TIME CELL ANALYSIS SYSTEM

The iCELLigence™ system is a technology that measures the electrical responses of cells. This approach analyzes the electrical properties of cells, especially changes in the electrical resistance of the cell membrane. Biological activities of cells, such as growth, proliferation, differentiation, and apoptosis, are associated with electrical changes in the cell membrane. This method is based on the principles of impedance spectroscopy and electrical impedance, which are commonly used in microelectronics. Electrical impedance is the resistance that cells exhibit to electrical current. Electrical impedance changes as the growth and metabolic activities of cells change the ion flows in their membranes, the membrane potential, and the physical properties of the cells. These changes provide information about the state of the cells. For example, as the cell proliferates, the cell membrane becomes thicker, resulting in increased electrical resistance. On the other hand, external factors that lead to cell death can cause a decrease in electrical resistance.[16,30]

iCELLigence™ continuously measures these electrical changes using a biosensor surface onto which cell cultures are placed. As the cells adhere to these surfaces, they establish an electrical connection with the surface, and the biological activities of the cells cause the electrical responses to change over time (Figure 3). These responses are then analyzed through data processing software to obtain many parameters such as cell viability, growth rate, drug responses, and toxicity levels.[31]

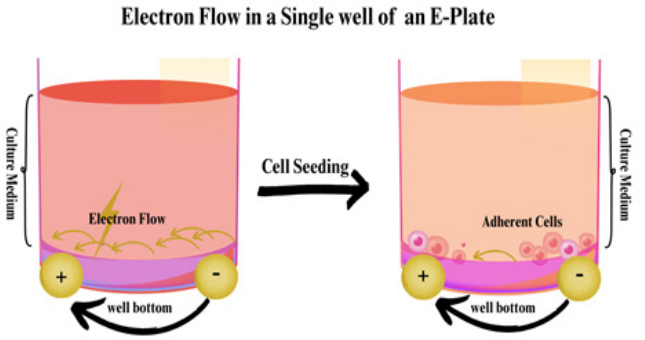

Figure 3. In the iCELLigence system, electron flow from the negative terminal to the positive terminal and the decrease of this electrical effect with cell proliferation

Cells create an electrical barrier between the cell membrane and intracellular fluids.[32] This barrier changes depending on the activity of ion channels, pumps and transport systems in the cell membrane.[33] As cells grow, the physical and electrical properties of this barrier change. iCELLigence™ analyzes cellular activities by measuring these changes.[34]

iCELLigence™ is a much newer system than the other 2 methods. The main feature that sets iCELLigence apart from the others is its real-time monitoring feature. This allows you to watch how cellular responses change over time, which gives you access to dynamic data. While its high-volume wells allow you to use more cell media, iCELLigence offers effective results with fewer cells than other tests. In addition to all this, it provides both electrical and morphological data, providing more detailed information about exactly how cellular responses occur. The ability to easily monitor cellular changes over time through the system allows us to see the onset and development of drug toxicity, which in turn allows us to understand the dynamics of drug toxicity over time. Moreover, this system not only allows us to see the data simultaneously, but can also create graphics. In Figure 4, we can see our graphic example created with the icelligence system in our previous work.[2]

In addition to all these, the system uses gold-plated plates, which are high-volume plastic or glass alternatives. However, these plates have a small sample area compared to other methods, so it may be necessary to plan the study by setting up the system multiple times to try different dose ranges. In addition, the iCELLigence system requires more expensive equipment and technologies. The system's installation and operation may be more complex than other tests.[33,34]

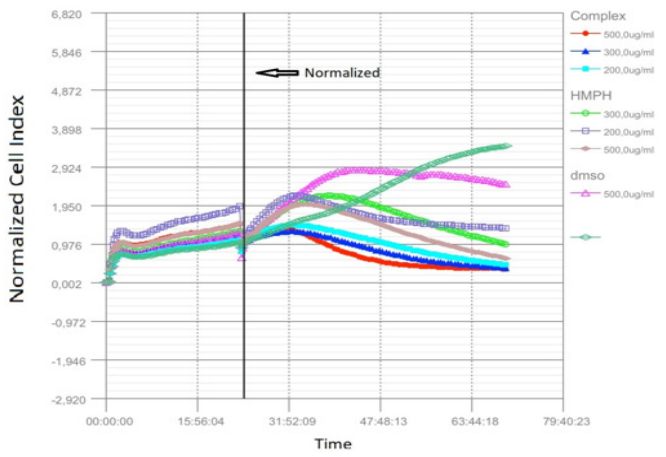

Figure 4. Example graph from iCELLigence system showing normalized cell index

DISCUSSION

Schröterová and colleagues[35] who tried different cytotoxicity assays and compared them when looking at the antiproliferative effects of selenium compounds in colon cancer cells, could not detect a cytotoxic effect except at the highest concentration of the selenium compound. Their MTT assay was more sensitive and gave mutually comparable results with a significant decrease in the measured parameters in a concentration-dependent manner.[35]

In the study conducted by Atmaca et al.[36] in which resveratrol cytotoxicity in human cancer cell lines was calculated by comparing different methods, it is clearly seen that the IC_{50} values were lower as a result of simultaneous monitoring compared to the XTT method. This shows us that the sensitivity of the real-time test is higher.

Garcia and colleagues[37] who investigated the real-time cellular analysis method as a new approach, summarized the benefits of using this method in their study as follows: Daily assessment of cell viability is possible without disturbing the cell culture and without using dyes that could negatively affect the results. In addition, an automated test can be set up to produce results covering the period after the addition of test samples and left unattended for the duration of the experiment. This saves valuable time.[37]

iCELLigence real-time cell analysis system, in another study conducted to examine the cytotoxicity of drugs on cancer cell lines, it was emphasized that this system has a lower risk of contamination.[38]

MTT, XTT and iCELLigence tests are methods that offer different advantages in the in vitro assessment of drug toxicity. These differences are outlined in Table 1. MTT and XTT tests focus on cellular metabolism, providing fast and reliable results, but they cannot fully observe dynamic changes in cellular responses over time. iCELLigence, on the other hand, allows for real-time monitoring of cellular responses, providing more dynamic and comprehensive data. Each of these methods can help evaluate different aspects of drug toxicity and contribute to more comprehensive results throughout the drug development process.

The consumables used in the cell culture phase are common for all 3 methods. However, while the MTT and XTT reagents differ in the subsequent stages, there is no need for a separate reagent for the iCELLigence system. Similarly, while DMSO (dimethyl sulfoxide) is used as a solvent for MTT, DMSO and XTT solution are used for XTT, there is no need for a solvent in the iCELLigence system. Direct electrical response measurement without the need for a spectrophotometer is one of the most important advantages of iCELLigence. In addition to all these, the gold plate plate of the iCELLigence system and the 96 well plate that can be used in other methods are seen in Figure 5. In addition to all these, the iCELLigence method requires a main device that measures electrical impedance and a tablet system that will transfer the data it receives from there. This makes it a more expensive and complex method compared to other methods.

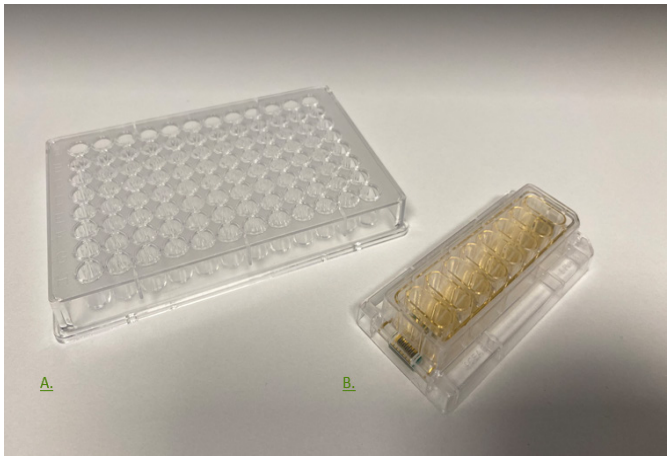

Figure 5. A) 96 well plate **B)** 8 well E-plate

MTT and XTT assays are widely used in cytotoxicity studies to screen new anticancer compounds due to their accuracy and relative simplicity. However, they cannot provide any information about the molecular mechanism of the drug's cytotoxic activity.

The fact that XTT is faster and less toxic than MTT makes it a preferred option in modern laboratories. On the other hand, the iCELLigence method is suitable for studies that require more sophisticated and advanced analyses. Although this

Table 1. Comparison of MTT, XTT and iCELLigence methods[24,28,34]

Method	Advantages	Limitations	Target
MTT assay	It is simple, economical and widely used. It gives fast results.	Measures only metabolic activity. Cannot observe all aspects of cell death.	Cellular metabolic activity
XTT assay	It provides more sensitive, effective at low cell densities and more stable results.	More expensive. Measures limited cellular mechanisms and only metabolic activity.	Cellular metabolic activity
ICELLigence system	It provides real-time monitoring and reliable results with fewer cells. It can perform in-depth analysis of cellular behavior.	It involves higher cost, technical difficulties, and more complex installation and operation.	Dynamic cellular behavior, real-time response monitoring

Table 2. Cellular response types and comparison by methods[39-41]

Response type	MTT assay	XTT assay	iCELLigence system
Metabolic activity (energy production)	High: MTT measures the metabolic activities of cells, specifically mitochondrial degradation.	High: XTT also measures metabolic activity, but is more sensitive and produces soluble formazan.	Medium-High: iCELLigence monitors cell behavior with electrical impedance measurements, an indirect indicator.
Cell proliferation	Medium: The MTT assay indirectly measures cell invasion, but is not specific.	Middle: Although XTT does not directly measure cell number, it monitors metabolic activity based on cell proliferation.	High: iCELLigence can monitor the spread and movement of cells in real time.
Apoptosis	Medium: MTT indirectly measures apoptosis but cannot recognize early stages.	High: The XTT assay can monitor metabolic changes due to apoptosis.	High: iCELLigence can monitor cell death and apoptosis processes based on electrical impedance changes.
Cell membrane integrity	Medium: Indirect monitoring for changes in cell membranes can be done with the MTT assay.	High: Soluble formazan formation in the XTT test provides information about the integrity of the cell membrane.	High: iCELLigence provides very precise, real-time data on cell membrane integrity.
Toxicity	Moderate: MTT indirectly measures toxicity, but early effects before cell membrane disruption cannot be detected.	High: XTT can more precisely measure the effects of toxic compounds on the cell.	High: iCELLigence detects toxic responses with real-time monitoring and can recognize effects faster.
Morphological changes	Low: The morphological status of cells is not directly observed in the MTT assay.	Low: XTT also does not focus on morphological changes, it monitors metabolic activity.	High: iCELLigence continuously monitors the physical morphology and behavior of cells.

method is ideal for monitoring biomarkers and observing the behaviour of cells in much more detail in real time, it is used in more limited areas due to high costs and the need for expertise.

If your experimental goal is to quickly assess the overall health and viability of cells, the XTT assay may provide more reliable and rapid results. Or if more in-depth biological analyses, complex tests such as apoptosis or biomarker monitoring are required, you can choose the iCELLigence method. Although the MTT assay is still economical and widely applicable, XTT or more advanced methods can be used in cases where biological factors other than cellular metabolism must be considered. Considering the advantages and disadvantages of each method according to your experimental goals will allow you to obtain more accurate and reliable results.

Most in vitro cytotoxicity tests focus on measuring cell necrosis. However, apoptosis, another important mechanism of cell death, is a process that needs to be evaluated with different methods. Inhibition of apoptosis is also an important parameter in terms of toxicology.

In addition, detailed studies on the changes in toxic effects on cells depending on dose and time provide important data by observing the reversibility of these effects as well as the effects on the cell cycle. Such studies provide valuable information on the mechanisms and types of toxicity, including necrosis, apoptosis and other cellular events. Comparisons according to the types of cellular responses are given in Table 2.[39-41]

RESULTS

As a result, in vitro cytotoxicity tests are required to determine cell death as a result of damage to basic cellular functions caused by a compound. These tests also provide the basis for understanding more complex parameters such as genotoxicity, mutation induction, and programmed cell death. Determining the dose at which 50% of the cells are affected (IC_{50}) allows comparison of the effects of a single compound in different systems or of several compounds in different systems. This review compares three different methods in many ways to help us understand the role of each method in the assessment of drug toxicity. It is possible to say that these data will be useful for decision makers and researchers, especially in drug development processes.

ETHICAL DECLARATIONS

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Review

MEDICINE PALLIATIVE CARE

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Comprehensive study on headache in otorhinolaryngology

Oğuzhan Oğuz[1], Zeynel Öztürk[2], Mustafa Yazır[3]

[1]*Department of Otolaryngology, Dr. Oğuzhan Oğuz Wellnose Clinic, İstanbul, Turkiye*
[2]*Department of Otolaryngology, Otolaryngology Clinic, Baypark Hospital, İstanbul, Turkiye*
[3]*Department of Otolaryngology, Yazır Private Otorhinolaryngology Clinic, İzmir, Turkiye*

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ABSTRACT

Otorhinolaryngologists are the first to receive numerous patients with headaches related to ear, nose, and throat (ENT) diseases. An otorhinolaryngologist frequently manages headaches unrelieved by a consultation practitioner with a general. Hence, it is natural that the etiology and treatments of headaches and ENT diseases have become the otorhinolaryngologist's concern. Approximately 47% of all US adults have experienced headaches or migraines in the past year. The most common type of headache is a tension-type headache. The second most common headache is reported as a primary headache, including migraine, tension-type headache, and cluster headache. According to the International classification of headache disorders, primary headaches, which are intrinsic and not caused by any other medical condition, and secondary headaches, which are symptomatic and arise from different illnesses or conditions, are related to otorhinolaryngology. Generally, primary headaches are more common than secondary ones. Migraines, tension-type headaches, and cluster headaches are common in this classification. Future research should primarily focus on the direction of the individually adapted treatment of the different headache entities with a special interest in comparing three arms per sub-entity: the placebo group as usual, the standard of treatment drug group, and a third arm with a substance of interest.

Keywords: Headache, otorhinolaryngology, sinusitis, rhinitis

INTRODUCTION

Headaches frequently accompany ear, nose, and throat (ENT) disorders. Otorhinolaryngologists are the first to receive numerous patients with headaches related to ENT diseases. An otorhinolaryngologist frequently manages headaches unrelieved by a consultation practitioner with a general. Hence, it is natural that the etiology and treatments of headaches and ENT diseases have become the otorhinolaryngologist's concern. Approximately 47% of all US adults have experienced headaches or migraines in the past year. The most common type of headache is a tension-type headache. The second most common headache is reported as a primary headache, including migraine, tension-type headache, and cluster headache. The type of headache may change with the progression of connected anatomical and physiological organs. Headaches may be connected directly to the organs of the head and neck or also to some factors of the cervical spine, such as the cervical prevertebral muscles, cervical dorsal, and spinal muscles, as well as a close relationship with the trigeminal afferents and posterior craniocervical afferents through the trigeminal-cervical and cervical-thalamic pathways. The close relationship with the trigeminal and spinal afferents is evidence of a continuous connection from the trigeminal region to the cervical spine, based on the theory that the main central mechanisms of migraine and tension-type headaches share the same pathways and that each has a different central sensitization in the trigeminal and cervical pathways. Because of the continuous relationship of the connected organs and the possible sharing aspect, if the pericranial pain inputs trigger those processes, it is crucial that the physician not only diagnose the etiology of the headache and treat it but also consider the other possible connected headaches or diseases.[1,2]

ANATOMY AND PHYSIOLOGY OF HEADACHE IN OTORHINOLARYNGOLOGY

The anatomical and physiological aspects of headache in connection with otorhinolaryngology include the relationships between head and neck structures, mastoiditis, petrositis, and diseases of the ear, nose, and paranasal sinuses. Knowledge of this topic is essential for diagnostic purposes. Headache is the most frequent cause of humans visiting neurologists and ENT clinics. It is a complicated and extensive issue, and it is hard to accurately diagnose the headache since it originated from several complex and intricate physiological networks. The relationship between local head and neck disorders and headaches has long been described. The pain of any part of the head is said to originate from the differentiation of the

Corresponding Author: Oğuzhan Oğuz, oguzhanoguz2023@outlook.com

innervation of the cranial dura mater referred to as the cervical periorbit.[3,4]

The intimate relationships between the pain neurons of the cranial dura, the delicate cervical joints, the muscular tissues that insert into the delicate junctions of the cervical spine, and, laterally, the sensory trigeminal nerves are said to be the source of headache regardless of the pathology of any structure. Meningeal afferents contain nerve trunks near the communicating innervations and the trigeminocervical system. The cranium and dura mater arteries are original with nociceptors and syringe afferents; hence, hypertension might cause headaches directly. In plasma and cerebrospinal fluid, certain substances are raised during the assaults. The MRI of the cranial migraineurs shows the linear significance of VOR along the prolonged trigeminal nerves and in their perivascular spaces; this finding is usually combined with the MRI findings of the sinus innervation of the dura. Such a finding is regarded as synaptic hyperactivity in the corticomedullary region.[1-4]

CLASSIFICATION OF HEADACHES RELEVANT TO OTORHINOLARYNGOLOGY

According to the International classification of headache disorders, primary headaches, which are intrinsic and not caused by any other medical condition, and secondary headaches, or those that are symptomatic and arise from different illnesses or conditions, are related to otorhinolaryngology. Generally, primary headaches are more common than secondary ones. Migraines, tension-type headaches, and cluster headaches are common in this classification. Moreover, headaches that may coincide with otorhinolaryngology diseases, such as vestibular migraines and those that trigger Meniere syndrome, vestibular schwannoma, and aural fullness, are also included in this classification. In contrast, secondary headaches are rare in the field of otorhinolaryngology. Examples include headaches stemming from cervical pathology, cervicogenic headaches, and intracranial vascular headaches resulting from acute head and neck diseases in the otorhinolaryngology field. The distinction between primary and secondary headaches is crucial for patients presenting with otorhinolaryngological signs and symptoms. Migraines, tension-type headaches, and cluster headaches are the primary clinical entities in the headaches category. A thorough examination of patients with primary headaches is valuable for making a definitive diagnosis by an otorhinolaryngologist. The headaches that may coincide with otorhinolaryngology diseases interest both neurologists and otorhinolaryngologists. However, secondary headaches, such as intracranial vascular headaches caused by acute head and neck diseases, should not be overlooked in the treatment of patients exhibiting cluster-like headaches.[2-4]

Primary Headaches

Primary headaches do not arise from a head injury, sinus or otologic inflammation, cervical abnormalities, or other facial or cranial structures. The International headache society proposed a more specific classification. They are subdivided into four headache types: migraine, tension-type headache, trigeminal autonomic cephalalgias, and other primary headache disorders. The three most common primary headaches in clinics are migraines, tension-type headaches, and cluster headaches.[5]

Epidemiological data in non-selected populations suggest that migraines are more prevalent in women than in men. Conversely, both men and women are equally affected by tension-type headaches, with a prevalence of 45% across their lifespan. In comparison, cluster headaches are more common in men than in women and have an incidence ranging from 45 to 69 per 100.000 people. Concerning their clinical presentation, tension-type headaches and migraines follow an episodic or chronic pattern. Episodic tension-type headaches occur for less than 15 days per month, while chronic tension-type headaches last for 15 days or more per month. Migraines can similarly be classified as episodic or chronic disorders based on headache frequency. Lastly, the underlying pathophysiology of these headaches may be neurogenetic in origin and is usually triggered by one or a combination of triggers, including stress, psychiatric diseases, physical exertion, head or neck pain, and upper respiratory tract infection.[5-7]

Genetics can predispose individuals to develop these headaches, but not all people with these genes will experience headaches. The first gene related to migraines was identified in 1996. When mutated, this gene was found to be associated with typical migraines and other related issues, such as hemiplegic migraines, inherited episodic ataxias, and other progressive neurodegenerative diseases. These genetic mutations could explain the clinical heterogeneity of disorders seen within families and the variability in attack frequency, severity, and duration. While a genetic basis is suspected, the triggers for migraines and tension-type headaches remain somewhat controversial. Headache triggers are mainly related to lifestyle or environmental factors. For example, lifestyle factors such as excessive caffeine intake, insufficient sleep, and bad posture can exacerbate headache frequency or intensity. Food triggers can vary, but alcoholic beverages and nitrate-rich foods are believed to be common migraine triggers. Lifestyle factors that may aggravate headaches include stress, physical activity, and the Valsalva maneuver. Daily headache diaries are proven to help identify headache triggers. Consequently, triggers vary among individuals according to their respective lifestyles and environments. Therefore, both medical and behavioral approaches are needed for consultation and explanation. Importantly, not all patients are aware of the triggers that exacerbate headache frequency or intensity, so patients are often advised to visit their headache specialist regularly.[3,4]

Secondary Headaches

Secondary headaches occur due to underlying disorders or other medical conditions known as "organic" headaches. Their identification is of utmost importance for accurate assessment to prevent possible severe pathologies, and without their ruling, a primary headache diagnosis cannot be confirmed. Sinusitis, other inflammatory pathologies, and neck disorders can present headaches as a leading symptom. Headache can also arise from central mediators involving the medulla and the upper spinal cord. Head trauma can either directly provoke intracranial pathology or lead to a diffuse axonal

injury. Clinical evaluation is mandatory for the diagnostic work-up. Still, in most of these cases, particularly intracranial pathologies, imaging is a key factor in confirming the diagnosis, preventing further mishaps, and planning the necessary intervention. The diagnostic assessment is, therefore, divided based on its etiology and pathophysiology. If headaches are severe or comport other neurological signs, a computed tomography (CT) scan or magnetic resonance imaging (MRI) should also be performed in the emergency setting or later. Depending on the organic condition, inflammation, infectious processes, space-occupying lesions, lesions affecting the fifth cranial nerve, and pulmonary infections can give rise to secondary headaches. The underlying organic disorder could be present in sinus pathology, intracranial pathology, cervical pain from nerve root compression, fibromuscular pain, and temporal arteritis. The pulsating character of the pain can indicate a vascular abnormality, leading to a secondary headache. Patients should be questioned about the localization of the pain, as posterior headache may be much more specific. Management and therapy are based on a treatment that must manage headache and the related primary condition responsible for the secondary headache. The guidelines are lacking in the literature, and no evidence-based recommended treatments are present. Medical societies must work to provide well-designed studies and agreed-upon pain management guidelines. Finally, it must be known that managing headaches is imperative to managing the secondary disorder. If a secondary condition involving the nervous system or brain is present, only when the secondary headache is treated can the clinical symptoms pointing to the diagnosis be obscured.[7,8]

EPIDEMIOLOGY OF HEADACHES IN OTORHINOLARYNGOLOGY

Headaches are one of the significant complaints presented to an otorhinolaryngologist and an allergist, neurologist, or an ENT specialist in their daily clinical practice. Aim: The objective of this review article was to have an in-depth discussion on the various aspects of headaches in ENT practice. It has been specialized into five sections, enunciating a broad coverage of the current scenario of headaches in the field of otorhinolaryngology. As ENT gets overlooked, numerous undiagnosed and untreated headaches flowing from the nose, sinuses, or pharynx result in chronic headaches that could have been cured had an early intervention been made.[8,9]

This article discusses the global perspective, advanced diagnosis, and management of primary headaches attributed to temporomandibular disorders. The international scenario illustrates that headaches can affect high scores worldwide, with some experiencing infrequent attacks while others have frequent headache attacks that require prophylaxis. Aim: This article is a narrative collation of their global prevalence as headaches are exclusively managed in the upper airway. Many risk factors are associated with headaches, exacerbating many comorbid conditions affecting health and negatively impacting a person's quality of life. One of the otorhinolaryngological frequent outpatient department visits is headache, and these patients are a set of demanding clients with multiple health problems. If all comorbid conditions are managed exquisitely, the headache will remain, yet the routine still is not considered in an otorhinolaryngologist's practice. In this narrative review, the focus is on headaches rather than the rhinogenic type. All the above minutiae have made us consolidate an exclusive focus on this topic for future guidelines. The statistical version of this data and spot verification would make things clear.[9,10]

It is indispensable to underline the epidemiological aspects of headaches in the crucial ENT structures of rhinological and otologic domains. Prevalence: The prevalence of headaches is a common health concern in otorhinolaryngological practice, affecting more than one-third of the general population of a geographic area. Overall, based on the data analysis from more than thirty studies, the prevalence is estimated at a 38% rate. Globally, the prevalence and frequency of headaches in high-income groups are much higher than those in low-income groups. It is further mentioned that biographical background, ethnicity, and socioeconomic and demographic groups in earlier studies have an essential influence on the increased prevalence of headache distribution. Gender: The prevalence rate is approximately 5-20% higher in females. Age: The occurrence of headaches in middle age is higher than in young and old populations. This trend has also been documented in various countries. Apart from these, there are no more findings on the general epidemiology of headaches in ENT. The aforementioned statistical inputs will help one to understand the burden of nuisance in a detailed patient encounter, intervention, and management of improved therapeutic outcomes. The geographic distribution shall help intensify the strategies to mitigate the burden of this nuisance in different continents. The younger populations will help expand the academic ecosystems in these countries regarding head and neck diseases, including headaches. In summary, it will help one understand the importance of managing headaches in the ENT fraternity field, which will also refine individual dedicated practice patterns.[9,10]

CLINICAL PRESENTATION AND DIAGNOSTIC EVALUATION OF HEADACHES IN OTORHINOLARYNGOLOGY

This section aims to guide a comprehensive study on headaches, including the up-to-date scientific literature and subjective knowledge through the experience of well-known experts. Headaches are frequently encountered complaints in otorhinolaryngology, as any structural abnormality, infection, or disease involving the ear, upper respiratory system, pharynx, or neck may result in headache. Clinicians should thoroughly question headache features, including onset, periodicity, time of the day or night, duration, localization, rapidity and degree of progression, character, quality, associated symptoms, medical and surgical history, and medications. At which crossroads they are diagnosed, and if a diagnosis of 'primary headache' has already been confirmed, they will be referred for reassurance, symptomatic therapy, or prophylactic treatment. Physical examination is critical in patients with a history and suspicion of a headache related to otorhinolaryngological structures or functions. Clinical and especially otorhinolaryngological examinations may reveal signs of primary and secondary headaches,

facilitating the differential diagnosis. Questionnaires and visual analog scales to evaluate headache pain, degree of disability, and quality of life are widely used in diagnosing primary and secondary headaches and monitoring the effects of headache treatments. A physical examination is crucial because almost all localizations of the primary and secondary headaches fall within or close to the structures examined by an otorhinolaryngologist. Neuroimaging is usually carried out with a CT scan and/or MRI in patients with headaches with signs and symptoms associated with neurological findings or a progressive, severe, or peculiar headache rather than in all patients with headaches. Patients should be categorized into organic, unspecified etiology, or non-organic/reassurance recommended by considering their clinical and corresponding imaging findings. Thus, it is essential to use clinical data, imaging findings, and experience in determining a differential diagnosis by considering the patient's primary or secondary headache disorder according to the localization and anatomic structures achieved. Patients with headaches are subject to a multidisciplinary approach in the form of functional neurology or pain service with an integrated headache clinic, both in primary and tertiary care. A comprehensive preoperative history and physical examination for optimizing patient selection and preventing unexpected headaches is necessary in patients undergoing otorhinolaryngologic surgery. Central and peripheral mechanisms are considered adequate in the genesis of headaches. Regardless of the headache's location and degree of severity, its diagnostic strategy is made by considering all clinical diagnostic findings.[11]

History Taking and Physical Examination

A standard brain exam can identify the majority of secondary etiologies in patients with a nonspecific headache. History-taking remains a valuable tool for diagnosing headaches. A well-conducted history identifies the characteristics of the headaches and applies to the various entities of headaches. In practice, a structured consultation can be organized around a set of questions focused on three goals: to rule out secondary headaches, to define the headache to provide etiological orientation and to understand the impact of this headache on the patient's quality of life.[10]

Checklist: headache, previous treatments, type of family history. The second stage of the consultation consists of searching for elements in the history that focus on the potentially determining or triggering elements. Triggers from the ENT field should be explored, and interesting questions include infection risk factors, chronic otitis, and ENT procedures. It is then essential to focus on neurological symptoms associated with headaches. In the presence of one or more elements suggesting secondary etiology, additional evaluations are often necessary, but not always; for example, the absence of a modified neurological examination may not be present in all forms of tumors, and the presence of a significant disorder does not exclude migraines or exacerbations.[9,10]

The physical examination has several objectives: to carry out a neurological examination, to identify changes in ENT and craniofacial structures, and to identify extracranial mechanisms likely to produce headaches. A complete examination of craniofacial structures must, therefore, be carried out. An initial interview must guide clinical maneuvers to determine if symptoms, typically epistaxis, hearing loss, vision loss, or facial discomfort, interfere with basic functions identified in the history. It is essential to exercise caution with specific patients or during a physical examination of the craniofacial region to ensure normal conduct and see if, under tension, signs of possible discomfort appear, as well as to ignore changes that are considered secondary. As with history taking, the physical examination may be routine but must not contain signals that suggest an abnormality. This double approach makes it possible, in part, to account for the diagnostic accuracy of headaches. They are complementary; the absence of abnormal elements in the history or the physical examination is likewise expected to be observed in those with the primary headache as in patients suffering from a chronic secondary etiology. History and physical examination must be carried out, depending on how methodically they are used to solicit all pertinent elements. Every question not asked represents an element of information, although not what could be used to confirm or rule out an abnormal process. Furthermore, the answers must be summarized at regular intervals to draw up lines of management. In conclusion, comprehensive history and physical examination are critical in guided management decisions.[11,12]

Imaging Studies

The role of imaging is to investigate the etiology, identify pathology, and explore possible causes of headaches resistant to medical management. Such imaging may be necessary for patients with warning signs and early morning headaches, where elevated intracranial pressure resulting from hydrocephalus needs to be ruled out. In all cases of new-onset headache accompanied by focal or generalized seizures, any neurological deficits or chronic progressive headaches should be considered. Patients with a history of recent significant trauma and those with potential post-trauma effects should also be evaluated. Imaging studies have diagnostic applications for identifying the etiology of headaches, including temporal bone inflammatory conditions and intracranial issues masses. There is no gold standard investigation for imaging of headache diagnosis; however, the most commonly used modality is MRI with and without intravenous gadolinium administration. MRI is the imaging modality used most frequently for anatomical investigation and physiological evaluation of the brain. CT investigates soft tissue and bone in the initial diagnosis or when MRI is contraindicated. Different studies have shown the superiority of these modalities in diagnosing various anatomical and physiological etiologies of headaches without any statistical evidence of the superiority of these imaging modalities. Head and neck digital subtraction angiography is reserved for vascular causes of headache in patients in whom other imaging modalities have failed to yield a diagnosis. Multi-detector-row CT angiography and MR imaging of the arterial and venous systems of the head and neck in various countries are currently performed with preference to MR due to its lower-risk applications. The radiation effects should be considered when CTA is performed in adult patients, as radiation exposure may have a more significant impact on brain tissue in younger patients because

of prolonged exposure periods and increased susceptibility to genetic mutations.[12,13] Unique protocols should be considered when ordering imaging, including:

- Indications for imaging.
- Appropriate timing and sequences for the relevant modality.
- Safety measures to prevent radiation exposure and adverse reactions in sensitive patients.

The balance between the yield of the diseases diagnosed and the safety measures to be undertaken has resulted in the following indications and safety checklist. Imaging must balance searching for yield and reducing the chance of complications, including a checklist comprising contraindications to be taken when ordering an imaging study in a headache patient. It should be noted that imaging findings such as temporal bone fistula, contaminated sinus, and sphenoid disease must correlate clinically. Rates of sinusitis detection at radiographic evaluation after clinical clearance vary from 14% to 53% of patients. This indicates that paranasal sinus abnormalities from radiographic studies contrast with the absence of clinical evidence of sinusitis, as demonstrated in children with headaches.

TREATMENT STRATEGIES FOR HEADACHES IN OTORHINOLARYNGOLOGY

Although the available treatment options for headaches within otorhinolaryngology have broadened due to the recent identification of several new noncerebral targets and the development of new treatments that utilize antibodies or neuromodulation techniques, invasive neuromodulation disputes the broad implementation of these endpoints. Monoclonal antibodies have shown modest effects in both trials and daily clinical practice, while there is still a lot of missing data. In addition, neuromodulation of pre-fixed stimulation sites has successfully treated several headache-inducing syndromes. Still, there is a lack of investigation into the modulation of electrically active stimulation sites. Future research into headache interventions should concentrate on patient-specific engineering, ranging from genetic profiles used to match drugs to the individual's migrainous burden to in vivo models that study novel molecules that might impart protection against headaches induced by compressive nerve injuries. It is conceivable that exploring the efficacy of interventions targeting nerves outside the cranium, either preventative or abortive, may also be relevant to the otorhinolaryngologist's practice. The mentioned drugs are tested in ongoing clinical trials.[14,15]

No genetic profiling studies regarding headache treatment in otorhinolaryngology have been published, but research in headaches is continuing. Pain's DNA profiling is also relevant for the ENT field. They are anticipated to come to the fore within the following years. The consensus concluded that a multidisciplinary approach offers an added advantage. More knowledge and education concerning headaches are required to help healthcare professionals disseminate newly found abilities and understandings. Using cumulative insights to drive future plans and identify unmet needs. Healthcare professionals used terms such as 'facets in understanding the headache' and 'moving with the times' to describe a forward-looking viewpoint. Recognition of additional techniques offers a new therapeutic perspective angle. There is a call for intensive research in TRH to develop and assess the feasibility and accuracy of the investigation. The evaluation of the practice of laboratory diagnostics presented in this paper also highlights the need for such research. Ongoing studies and consortium research in headaches will shed more light on this topic shortly. Ongoing research projects were discussed, including pharmacogenomics in primary headache disorders and using a new investigation technique in a patient with chronic headache.[14,15]

CONCLUSION

A comprehensive overview of the different entities of headaches in otorhinolaryngology and the modern knowledge on these topics was presented. Special emphasis was placed on the importance of correct diagnosis in order to agree on an interdisciplinary management strategy between specialists. Although different lines of the new treatment era have been addressed recently, such as monoclonal antibodies, neuromodulators, and special nerve stimulation techniques, focus was placed on evolving treatment modalities to close gaps in diagnostics and tailor individually adapted treatments. Particular attention was given to current research within otorhinolaryngology, such as exhale ventilatory inhibition in AVR and neurophysiological imaging in superior canal dehiscence.[4,6,14]

There is overwhelming modern evidence of the high interconnectivity of the pain processing network, not only between different sectors of the CNS but also in the potential interaction along the primary-afferent signaling transmitted along the periphery. This knowledge stresses the need to expand the education to specialists from other medical fields involved in caring for patients with headaches in otorhinolaryngology. Integrating this knowledge into an interdisciplinary treatment concept might reflect the individual constellation of possible interacting headache comorbidities and optimize treatment. The willingness to collaborate in multidisciplinary headache management could interlink neurologists with several substances of interest in headache patients from the field of otorhinolaryngology. However, this assumption requires validation in future trials. Future research should primarily focus on the direction of the individually adapted treatment of the different headache entities with a special interest in comparing three arms per sub-entity: the placebo group as usual, the standard of treatment drug group, and a third arm with a substance of interest.

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All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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