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Measuring Urban Health in Türkiye: A Proposal for an Urban Health Index from a Capabilities Perspective

Türkiye'de Kentsel Sağlığın Ölçülmesi: Kapasite Yaklaşımı Perspektifinden Bir Kentsel Sağlık Endeksi Önerisi

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ABSTRACT

Aim: This study aims to develop an Urban health index (UHI) for Türkiye, grounded in the "capability to be healthy" framework.

Materials and Methods: Twenty-five routinely collected, publicly available provincial-level indicators were analyzed and categorized into four domains: economic, environmental conditions and services, and socio-cultural indicators. Using Microsoft Excel, the final UHI was calculated as a weighted average of 11 selected capability indicators. The under-five mortality rate (U5MR) was used as a proxy for population health. Geometric means, Spearman correlations, and Cronbach's alpha were calculated in SPSS version 31.0.

Results: The 11-indicator index demonstrated high internal consistency (Cronbach's alpha= 0.895). Strong negative correlations were observed between U5MR and indicators such as literacy (r= -0.881), poverty (r= -0.860), household overcrowding (r= -0.764), gender norms (r= -0.742), and civic engagement (r= -0.671).

Conclusion: The proposed UHI provides a novel, context-specific tool for assessing the structural and social determinants of health across Türkiye's provinces. It enables both cross-provincial comparisons and the monitoring of temporal changes in urban health, contributing to evidence-based urban policy and health equity efforts.

Keywords: Urban metrics, Urban health index, population health, capabilities approach, child mortality, ecological study

ÖZ

Amaç: Bu çalışma, "sağlıklı olma kapasitesi" çerçevesine dayalı olarak Türkiye için bir Kentsel sağlık indeksi (KSE) geliştirmeyi amaçlamaktadır.

Gereç ve Yöntem: Rutin olarak toplanan ve kamuya açık 25 il düzeyinde gösterge analiz edilerek dört alana ayrılmıştır: ekonomik, çevresel koşullar ve hizmetler ile sosyo-kültürel göstergeler. Microsoft Excel kullanılarak, nihai KSE 11 seçilmiş yetenek göstergesinin ağırlıklı ortalaması olarak hesaplanmıştır. Beş yaş altı ölüm oranı (U5MR), toplum sağlığının bir göstergesi olarak kullanılmıştır. Geometrik ortalama, Spearman korelasyon ve Cronbach alfa SPSS versiyon 31.0'da hesaplanmıştır.

Bulgular: On bir göstergeli endeks, yüksek iç tutarlılık göstermiştir (Cronbach alfa= 0,895). U5MR ile okuryazarlık (r= -0,881), yoksulluk (r= -0,860), hane halkı aşırı kalabalıklığı (r= -0,764), cinsiyet normları (r= -0,742) ve sivil katılım (r= -0,671) gibi göstergeler arasında güçlü negatif korelasyonlar gözlemlenmiştir.

Sonuç: Önerilen UHI, Türkiye'nin illerinde sağlığın yapısal ve sosyal belirleyicilerini değerlendirmek için yeni ve bağlama özgü bir araç sunmaktadır. İller arası karşılaştırmaların yanı sıra kentsel sağlıktaki zamansal değişikliklerin izlenmesini de olanaklı kılarak, kanıta dayalı kentsel politika ve sağlık eşitliği çabalarına katkıda bulunmaktadır.

Anahtar Kelimeler: Kentsel ölçütler, Kentsel sağlık endeksi, toplum sağlığı, kapasite yaklaşımı, beş yas altı ölüm oranı, ekolojik çalışma

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INTRODUCTION

Urbanization is one of the main trends affecting health. As the global population continues to urbanize, it is imperative to understand how divergent urban experiences shape health and well-being. Multiple aspects of urban living such as physical environment, social structures, and the access to health and social services, have profound implications for health[1]. Given the varying pace of urbanization and differences in city size, density, and social composition, "the relative importance of characteristics of the urban environment that may affect health may vary substantially in different cities and different parts of the world[1]." Türkiye has also experienced rapid urbanization over the past sixty years. According to recent data, over 75% of the population lives in urban areas[2]. This continued rural-to-urban migration has placed a significant strain on infrastructure and had major repercussions on public health.

In this article, we propose an Urban Health index (UHI) for Türkiye using routinely collected, publicly available data to comparatively examine and assess the health levels of all provinces in Türkiye. Using statistical data on health outcomes and health determinants, the proposed UHI seeks to identify and categorize numerous indicators related to provincial health outcomes and to analyze the extent to which determinants influence health in Türkiye.

UHIs are essential tools for understanding the impact of various factors on health in urban settings. In other words, these indexes provide baseline information on urban health allowing for the assessment of progress in health outcomes over time[3-5]. UHIs also enable inter-province comparisons and help identify associations between health determinants and impacts[3,4,6]. Lastly, UHIs facilitate the identification of health inequalities and the development of evidence-based policy recommendations on urban health[7]. Although UHIs are socially constructed, they are also "rational tools" for instigating policy change, balancing scientific robustness and political motivation[4]."

Although limited, several UHI indexes have been created for Türkiye. The UHI proposed in this article differs from other indicator sets because it adopts a "capability approach to being healthy" focusing on how social and physical conditions in provinces lead to positive or negative health outcomes rather than the individual choices. Thus, the focus is on the underlying determinants of health, defined as, "the factors and conditions which protect and promote the right to health beyond health services, goods and facilities[8]." The UHI outlined in this article is a tool for upholding the right to health in Türkiye. It accomplishes this by identifying areas for intervention to improve the conditions of health determinants. As the right to health is subject to "progressive realization," setting indicators and benchmarks is crucial to ensuring the enjoyment of this right[8]. In this article, we assess health from a capabilities perspective and propose a UHI to understand the extent to which the right to health is upheld in Türkiye.

Constructing an UHI from a Capability to Be Healthy Perspective

The capabilities perspective, originally developed by Amartya Sen and expanded by Nussbaum[9], focuses on the set of functions that a person possesses in order to achieve a meaningful and valuable life. This approach has also been applied to the concept of health.

In his book Health Justice, Sridhar Venkatapuram[8] argues that "every human being's moral entitlement to a capability to be healthy." He defines this capability as the ability to achieve or exercise a cluster of basic functions at a level that reflects human dignity[8]. Venkatapuram[8] views health as a "meta-capability" necessary for achieving the 10 basic capabilities identified by Nussbaum[9]. For Venkatapuram[8], this capability can be understood as "a human right to be healthy", which should be a central in discussions on social and global justice.

Living a long and healthy life requires more than just medical care and services. Essential elements for a healthy life include emotional nurturing, adequate nutrition, shelter, access to information, and protection from physical, psychological, and sexual abuse[8]. Defining health only as the prevention of disease and impairment, is an incomplete definition of health, as it represents only one aspect of health[8]. Health outcomes and capability to be healthy are shaped by social force including political, economic, legal, cultural and religious systems operating at local, national, and global levels. In other words, health is significantly shaped by our physical and social environments, and by the broader determinants of these environments[8]."

This article proposes a provincial-level UHI for Türkiye, using the capabilities approach. The objectives are twofold, 1) to identify and categorize the urban indicators that are related to health in Türkiye at the level of provinces and, 2) to analyze the extent to which these indicators influence urban health in Türkiye using the most up-to-date publicly available data. We believe this index will not only allow for comparisons across cities but will also provide a baseline for evaluating the impact future policies. It supports a shift toward the progressive realization of the right to health[10].

Adopting a capability-based health perspective allows us to distinguish between health as a functioning and health as a capability[11]. In our index, health as a functioning is representing by the outcome indicator, while health as a capability is assessed through social and physical determinants of health. The UHI examines the correlations between health outcomes and a set of capability (determinants of urban health) indicators.

It is important to note that the association between health outcomes and health capabilities may vary by context. For example, some associations that hold true in underdeveloped societies may not be true in developed societies. Patterns seen in low-income countries may not apply in high-income settings. Therefore, we selected indicators that reflect Türkiye's unique characteristics, to ensure the index is contextually appropriate. The UHI aims to offer insight into "possible and justifiable interventions" to improve the social and environmental conditions of health[8].

Although our index emphasizes the functioning and the capability aspects of health, it does not address the role of individual agency, that is the personal choices people make that influence their health[11]. Moreover, while access to healthcare services is a key component of the right to health, this article focuses instead on how the social and physical aspects of urban life affect health. For this reason, the analysis excludes data on access to healthcare services. Our perspective is that health outcomes are shaped by a range of social and environmental factors, and their interactions, outside of the healthcare system.

MATERIALS AND METHODS

This study aimed to develop a feasible UHI for Türkiye using routinely collected, publicly available data in order to comparatively examine and assess the health outcome of all the provinces in Türkiye. This study is a retrospective study that used secondary data to construct an UHI. Figure 1 summarizes the steps taken in the study.

Literature Review

A literature review was conducted in two stages. First, a comprehensive search of international online databases (Pubmed, Web of Science, Google Scholar, and Scielo, and ScienceDirect) and was carried out for the following terms: "urban health and methodology", "urban health indicators", "UHI," "healthy cities (or health city) and methodology", "healthy cities (or health city) and indicators", "healthy cities

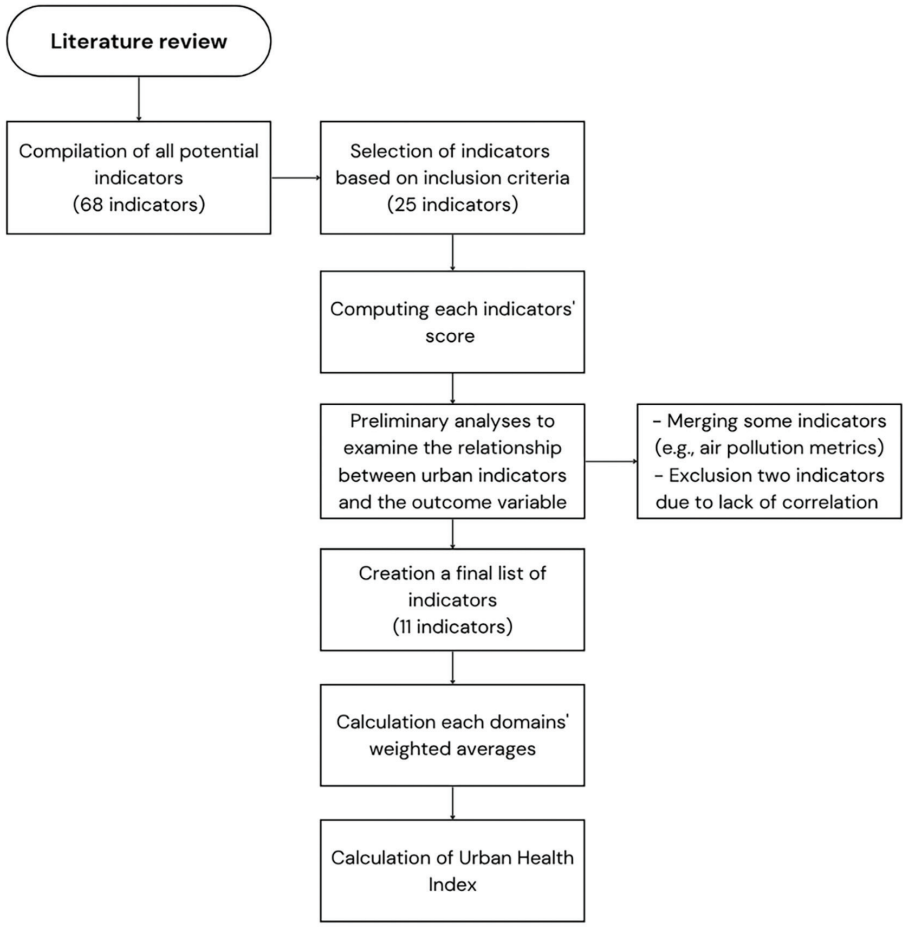


Figure 1. Summary of the study methodology

(or health city) and index." Then, the authors searched and reviewed all the studies related to urban health indicators conducted in Türkiye. A keyword search for "urban health and Türkiye" and "healthy cities and Türkiye" was conducted for both international databases and national (Ulakbim and Dergipark) databases. Finally, a non-systematic search of reports and proceedings on urban health and UHIs in Türkiye was conducted.

There is a vast literature on urban health indicators. According to a systematic review published in 2018, there are 145 UHIs composed of 8006 indicators from 26 countries[12]. Despite this vast literature, both the number and the quality of urban health indicator sets are limited in low and middle-income countries due to "a lack of quality data at the city level" and the limited capacities of some countries to analyze available data[7]. This holds true for the case of Türkiye as well.

Most literature on indicators focus on the development and validation of UHIs. However, there is a lack of research on the utilization UHIs in urban planning policy-making[4,7]. Constructing an UHI specifically for Türkiye is an important task because it calls attention to the particular characteristics of Turkish provinces and increases the visibility and acceptability of the impact of these characteristics on health. Furthermore, given limited number of UHIs tailored specifically for Türkiye, and the fact that many of these are incomplete and/or outdated, it can be argued that "the process of indicator development (in the case of Türkiye) is at least as important as achieving change as the eventual use of indicators[12]." This initiative marks a pivotal first step towards instigating policy change in urban health in Türkiye.

As stated above, many urban indicator sets have been created over time. Given the variability in the impact factors on urban health in different cities, the health assessment tools should also be adapted accordingly, ranging from environmental factors to broader socio-economic and socio-cultural factors. The World Health Organization (WHO) attempted to develop a tool to measure for the cities participating in the Health Cities project in the early 1990s. The WHO Healthy Cities Network developed a set of 32 indicators for the purpose of "changing the ways in which individuals, communities, private and voluntary organizations, local governments think about, understand, and make decisions about health[5]." The healthy cities indicators have been adopted with some revisions, to the case of Türkiye by the Turkish Healthy Cities Association as well.

In addition to the healthy city indicators, the WHO developed the Urban Health Equity Assessment and Response Tool (Urban HEART) which focuses on urban health inequalities[13]. In addition to the Urban HEART, the Euro-URHIS Project (European Urban Health indicators system) and the BRE Health Cities index are two international projects that created UHIs. These indexes focus on environmental indicators rather than health inequalities[14,15]. Although our UHI has been informed by all three indexes and entails the "lessons learned" from each of them, because our focus in this index has been to put forth a capability to be healthy perspective. For that reason, we had to create a novel index that included health as a functioning indicator combined with health as a capability indicator. This index incorporates both the social/cultural and the physical/environmental determinants of health.

In Türkiye, majority of research on UHIs has been conducted by municipalities rather than academic institutions. This research involves the creation of health profiles for cities and follow-up implementation projects. This led to a lack of comparative studies that could offer a holistic picture of urban health in Türkiye. Such studies are instrumental for developing actionable policy recommendations. The most comprehensive UHI project was undertaken by the Healthy Cities Movement Association in Türkiye (THCA).

In addition to the THCA healthy cities index, there are several studies have been conducted to develop composite index for measuring urban health in Türkiye. These studies focus on different aspects of urban health and its determinants. For instance, highlighting "the lack of a database for urban level (which) makes difficult to put forward the national relationship between urban and health", Kara[16] recommends to use 32 indicators under four categories. The fact that Kara's index includes indicators of health behavior at the individual level such as levels of alcohol consumption, drug use, smoking, physical exercise, fruit and vegetable consumption, rather than those related to the physical and social characteristics of cities, prompted us to create a novel UHI that paid more attention to the latter. In another study that focuses on quality of life in urban areas in Türkiye, Sari and Kindap[17] analyze the most repeated indicators in international indicator sets. The authors also refer to the lack of available data for measuring the quality of life in Turkish urban areas.

Selection Criteria of Indicators

A long list of all potential urban health indicators was created and were grouped following the domains borrowed from Pineo et al.[12]: environment, social, health, and economy. We revised Pineo's categories by splitting environment domain into two: environmental conditions and environmental health services. Whereas environmental conditions refer to the physical urban circumstances that are shaped by geographical location, previous urban planning etc., environmental health services refer to interventions that help ameliorate urban health.

Publicly available secondary data from Turkish Statistical Agency (TurkStat) and relevant Ministries (Ministry of Family, Labour and Social Services; Ministry of Forestry, Ministry of Health, Ministry of Justice, and Ministry of Education) were used as data sources to compose this index. In Türkiye, such data tend to be either available at the national, regional (NUTS I), subregion (NUTS II) or province-level (NUTS III).

We adopted the following criteria for selecting indicators: highly influenced by the built environment, demonstrates a link between the urban environment and health/well-being, globally relevant, and measurable by using public data[4]. The inclusion criteria for the indicators were as follows:

- Representing either the health as a functioning (health outcomes) or health as a capability (determinants of health) in provinces

- Having causal connection with health

- Available for all 81 provinces

- Publicly accessible

- Accurate, reliable

In addition to these criteria, we also assessed the validity and the reliability of the data collected. Another significant factor that contributes to creating a suitable measurement method is accessibility of data; utilizing routinely collected sources is considered an advantage[18].

As outlined in Figure 1, the third step, we included under-five mortality rate (U5MR) as a functioning indicator. This has been recommended by López Barreda et al.[19] as a suitable health indicator that reflects the population's achieved health status.

Finalizing the List of Indicators After Elimination

After searching for convenient indicators of urban health status, we considered the capability indicators that impact health as capability indicators under 4 separate yet interconnected domains: economy[4], environmental conditions[6] and environmental services[5], and social[8]. After exclusions, we analyze 25 capability indicators as seen Table 1 which provides a list of the indicators utilized in the study.

U5MR was defined as the probability of dying a child before reaching the age of five years per thousand live births. It reflects the access of children and communities to basic health interventions such as vaccination, medical treatment of infectious disease and adequate nutrition in place of poor living conditions. The data of U5MR (per 1000 live births) were taken for analysis as the average of the past five years, 2015-2019. However, the urban indicators were used converting score as explained the following part.

Constructing Urban Health Index

We adopted the calculation method of the human development index recommended by most UHI studies[20]. In brief, this method standardizes each indicator by using the following formula:

$I^S = [I - min^*(I)] / [max(I) - min^*(I)]$

In the formula, I is the value of an indicator for a given unit (actual value), max (I) is the maximum value of I the indicator over all units, min^* (I) is the minimum value of (I) over all units minus a small value or alternatively chosen[20].

The following indicators which negatively correlated with health outcomes were converted using formula 1 - I^S (4): poverty, the levels of air pollutants (SO_2 and PM_{10}), household size, classroom crowded, population growth rate, and child crime.

Standardization was carried out for indicators using data from all the 81 provinces in Türkiye. The similar indicators were aggregated using geometric averages for the weighed the domains. For this purpose, we aggregated the PM_{10} and SO4 as air pollution; household size and no. of students in classrooms as crowded in houses and schools; population served by drinking water treatment and wastewater treatment as environmental services; % of self-employed (entrepreneur) women, women employment in a secure job, and divorce rates as gender issues; and finally, no. of sport clubs and saloon for cinema and theatre as physical and social recreational opportunities. We excluded the social security coverage due to its low sensitivity to interprovincial differences and lack of correlation with U5MR. We also excluded the population density indicator was due to İstanbul's skewed density and lack of correlation.

The total UHI was calculated by geometric mean of all indicators presented in Figure 2.

Ethical Considerations

The data used in this study are anonymized, aggregate secondary data obtained from the Turkish Statistical Institute (TURKSTAT). Because the dataset is publicly accessible and contains no identifiable individual-level information, ethics committee approval was not required. Data collection and processing procedures at TURKSTAT comply fully with the European Statistics Code of Practice, ensuring statistical confidentiality, data protection, and scientific independence. All stages of the study were conducted in accordance with recognized standards of scientific integrity and publication ethics.

Table 1. Indicator list, explanations and data sources

Domain/subcategories	Indicators of domains	Explanations	Data source and year
Health			
Child mortality	Under-five mortality rate (U5MR)	U5MR is defined as the probability of dying a child before reaching the age of 5 years per thousand live births. It reflects the access of children and communities to basic health interventions such as vaccination, medical treatment of infectious disease and adequate nutrition in place of poor living conditions.	TurkStat, 2015-2019
Economy			
Income	Gross domestic product (GDP) per capita ($)	GDP is the standard measure of the value added created through the production of goods and services in the provinces. The main limitation is being an average for the province but not showing the distribution of it to the people equally.	TurkStat, 2019
Employment	% of active employee with social security	Proportion of actively employed population under social insurance system among older than 15 years old. It reflects to employment level.	Social Security Institution, 2019
Poverty	% of population under poverty line according to universal health insurance	It is rate of under poverty according to Turkish universal health system who has income less than one of third of minimal wage besides not having any property. Although it likely to underestimate all poor people, it is included because of allowing to make estimation based on province.	Social Security Institution, 2019
Social security coverage	% of covered by social security (active, passive, dependents and under poverty line)	Proportion of population covered by social insurance system due to active, passive, dependent, and under poverty line. It reflects to shield under social right provided by social security system.	Social Security Institution, 2019
Environmental conditions			
City density	Population density (per km^2)	Population density per kilometer square.	TurkStat, 2019
Green spaces	m^2 of forest per 10.000 people	The indicator on m^2 per 10.000 people included to estimate green spaces, because the data on total green spaces in built environment including parks, recreation sites etc. is not available.	Ministry of Forestry, 2018
Air quality	Concentration of PM_{10} $\mu g/m^2$	It is included as an indicator because it is related to emissions of industrial pollution as well as other sources such as traffic, construction, etc.	Ministry of Environment and Urbanization, 2019
Air quality	Concentration of SO_2 $\mu g/m^2$	It is a major air pollutant primarily released by the combustion of fossil fuels.	Ministry of Environment and Urbanization, 2019
Housing/crowded	Household size	It reflects the crowding of houses which is the average number of household members. Crowding is directly associated infectious diseases and mental health problems.	TurkStat, 2019
Crowded	The number of students per classroom	It represents public primary schools. Density of classrooms is related to transmission of communicable diseases among schoolchildren.	Ministry of Education, 2017-18
Environment health services			
Infrastructure	Expenses for waste management (TL)	Expenses for current + investment to waste management system (per capita, TL). It reflects sustainability of waste management system. It is calculated per capita.	TurkStat, 2016
Infrastructure	Expenses for wastewater management (TL)	Expenses for current + investment to wastewater management system (per capita, TL). It reflects sustainability of wastewater management system. It is calculated per capita.	TurkStat, 2016 TurkStat, 20162018, and 2020
Infrastructure	Expenses for water supply management (TL)	Expenses for current + investment to water supply system (per capita, TL). It reflects sustainability of water in the provinces. It is calculated per capita.	TurkStat, 2016
Wastewater treatment	Proportion of population served wastewater treatment plants (%)	Wastewater treatment involves the biological processing of wastewater and sewage before it is discharged into the environment.	TurkStat, 2016 TurkStat, 2018 TurkStat, 2020

Table 1. Continued

Domain/subcategories	Indicators of domains	Explanations	Data source and year
Access to water	Proportion of population served by drinking water treatment plants (%)	Water treatment plants process surface water to make it safe for human consumption.	TurkStat, 2016
Waste management	Rate of population with solid waste collection (%)	It refers to the collection, transportation, processing, recycling, and disposal of waste materials in a way that minimizes their impact on human health and the environment.	TurkStat, 2016
Social-cultural indicators			
Population growth	Population growth rate between 2008 and 2018	It is used to explore to not only childbirths but also immigration. Migration is a reality in recent years including unregistered migrants, as well.	TurkStat, 2010-2019
Education	Literacy among older than 15 years old	Literacy is related to development besides is a component of socioeconomic status.	TurkStat, 2020
Gender equality	% of self-employed women	It is calculated using no. of self-employed women by divided to total no. of women (15 yrs+). Rate of self-employed women which reflects opportunities of women and women's entrepreneurship is an indicator for gender equality.	Social Security Institution, 2019
Women employment	% of women employees	This indicator represents the percentage of women employed under compulsory employer-based insurance. It reflects secure, formal employment, excluding flexible, part-time, or unpaid work arrangements such as family labor.	Social Security Institution, 2019
Conservativeness	Crude divorce rate (%)	Conservative social norms often discourage divorce and are closely associated with the continuation of marriages, even in cases of dissatisfaction. These patterns also reflect underlying gender dynamics.	TurkStat, 2019
Social recreation	No. of saloon for cinema and theatre per million population	It is calculated as total no. of cinema and theatre by divided to total population. It is related to social recreation opportunities.	TurkStat, 2019
Leisure/recreation	No. of sport club per 100.000 population	Although it is not reflecting whole population's physical activity, it is an approximate to sport activity in the provinces.	TurkStat, 2019
Child protection	No. of 5-14 years children crime per thousand	It is assumed that child crime is a social issue instead of security issue. Passport violations are not included because of misleading high rates in provinces of frontier.	TurkStat, 2017
Social networks	No. of society per 100.000 population	This represents the social networks and is related to social capital and civic engagement capacity of the population.	TurkStat, 2019

TurkStat: Turkish Statistical Agency

Statistical Analysis

After the data was prepared in Microsoft Excel® where each individual indicator was calculated and domain scores were constructed, the analyses were conducted using SPSS (IBM Statistical Package for Social Sciences), Version 31.0. In order to ensure internal consistency, Cronbach's alpha values were calculated for all the indicators (0.895) which is viewed as an acceptable point to measure reliability[21]. Then, Pearson's correlation coefficient was applied to explore the associations between U5MR as a functioning and health as a capability. Normality assumption of correlation was met with the normality criteria.

RESULTS

Table 2 and Figure 3 provides separate scores for each of the four domains as well as the total UHI score for Türkiye. Provinces' scores according to these four domains range broadly; the mean and median values indicate that distribution is not skewed except for the domain of environmental services which might stem from the variances in environmental services provided in provinces, especially with regards to investments.

According to our calculations, of the health as a functioning indicator, U5MR scores show significant correlations with UHIs of most domains (Table 3).

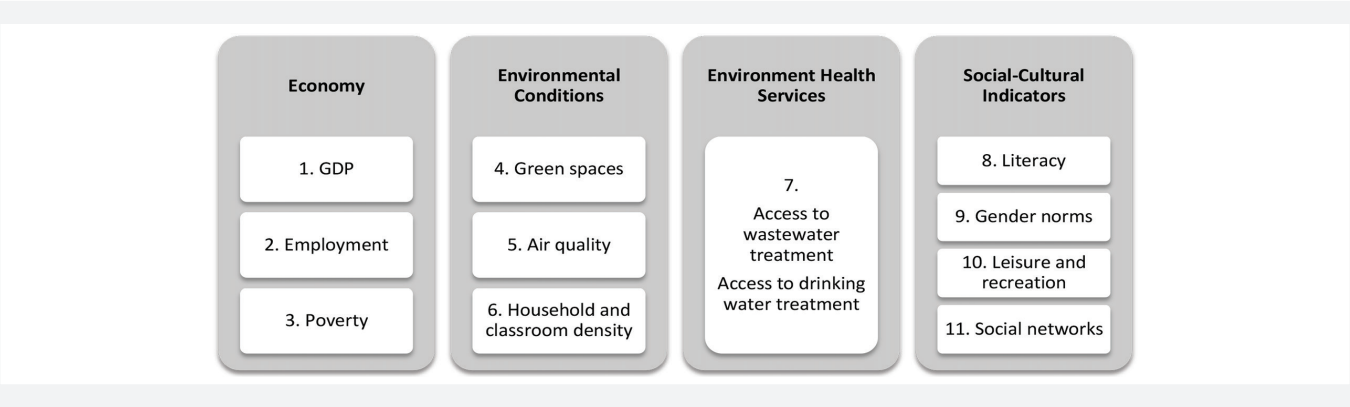


Figure 2. Indicators used to construct the Urban health index

GDP: Gross domestic product

Table 2. Descriptive statistics for indexes of urban health determinants by domain (no. provinces: 81)

	Domains				Total UHI score
	Economy	Environmental conditions	Environmental health services	Social-cultural indicators	
No. of provinces	81	81	81	81	81
Median	0.493	0.401	0.354	0.452	0.417
Mean	0.491	0.422	0.406	0.427	0.400
Standard deviation	0.210	0.194	0.326	0.175	0.169
Minimum	0.000	0.012	0.000	0.006	0.004
Maximum	0.972	0.938	1.000	0.714	0.694

UHI: Urban health index

Table 3. Correlations between under-five mortality rate (per 1000 live birth) as functioning indicator of and the scores of capability domains

Domains	Related issues	Indicators	Correlation coefficient
Economy	Income	Gross domestic product per capita ($)	-0.630**
	Employment	% of active worker with social security	-0.650**
	Poverty	% of population under poverty line (based on access to universal health) (converted)	-0.860**
		Economy domain	-0.752**
Environmental conditions	Green spaces	Meter square of forest per 10.000 people	-0.332**
	Air quality	Concentration of PM_{10} and SO_2 µg/m (converted)	-0.383*
	Housing/crowded	Household size and number of students per classroom (converted)	-0.764**
		Environmental conditions domain	-0.562**
Environmental health services	Wastewater and drinking water treatment	Proportion of population served wastewater treatment plants and drinking water treatment plants	-0.372**
		Environmental health services domain	-0.372**
Social-cultural indicators	Education	Literacy rate among older than 15 years old	-0.881**
	Gender norms	Proportion of self-employed women, women employees, and crude divorce rate	-0.742**
	Leisure and recreation	No. of sport club and cinema and theatre per population	-0.671**
	Social networks	No. of association per 100.000 population	-0.664**
	Total	Social facilities and opportunities	-0.871**
Total UHI score		**Weighted average of these indicators**	**-0.855****

*p<0.05, ** p<0.01, UHI: Urban health index

Among the domains, the scores of economy, environmental conditions, and social-cultural indicators show significantly moderate to strong correlations with U5MR, while the score of environmental services shows weak correlation to U5MR. As observed at Figure 3, the distribution of environmental health services score is wide which means that distributed more equally compared to the other UHI domains.

In the economy domain, while gross domestic product, employment and poverty indicators show moderate to strong correlations with U5MR, respectively coefficients -0.630, -0.650, and -0.860 (Table 2). The indicator of social security coverage is not correlated (r= 0.031, data not shown), therefore, was not included to the overall UHI score, as mentioned in the method.

Under the environmental condition domain, we found that population density (r= -0.082, data not shown), levels of PM_{10} in ambient air (r= -0.163) were not correlated with U5MR. The scores of green spaces, SO_2 as an air quality measure, and crowded in schools are observed moderate correlations while household size shows strong correlations with U5MR (r= -0.700). When we combine both air pollutants as one, air quality shows correlation with the child mortality (r= -0.383).

Under the environmental health services domain, we only used the wastewater treatment and drinking water treatment by excluding the other indicators which are the infrastructure expenses for waste management, wastewater management and water supply management, access to sewage and pipe system. The environmental health service score is weakly correlated with U5MR by the -0.372 correlation coefficient.

Lastly, our analysis shows that correlations between indicators in the social-cultral indicators domain and health as a functioning were generally robust. We found that literacy rate, gender norms, leisure and recreation indicators, number of societies per 100.000 are highly correlated with U5MR (ranging between 0.664 and 0.881).

Figures 4 and Figure 5 visualize the total UHI score and the domains' score across the provinces. The correlation of total UHI with U5MR presents a strong correlation as -0.855. As it can be seen from the map, there are inequalities between provinces in terms of UHI scores. Whereas provinces in Western parts of Türkiye tend to have higher UHI scores, provinces in Eastern and Southeastern Türkiye have lower UHI scores. Regarding the correlations of the domains, both economy and social-cultural indicators show strong correlation with U5MR (respectively -0.752 and -0.871) (Table 3, Figures 4,5), while environmental conditions present moderate correlation (r= -0.562) and environmental health services is weakly correlated (r= -0.372).

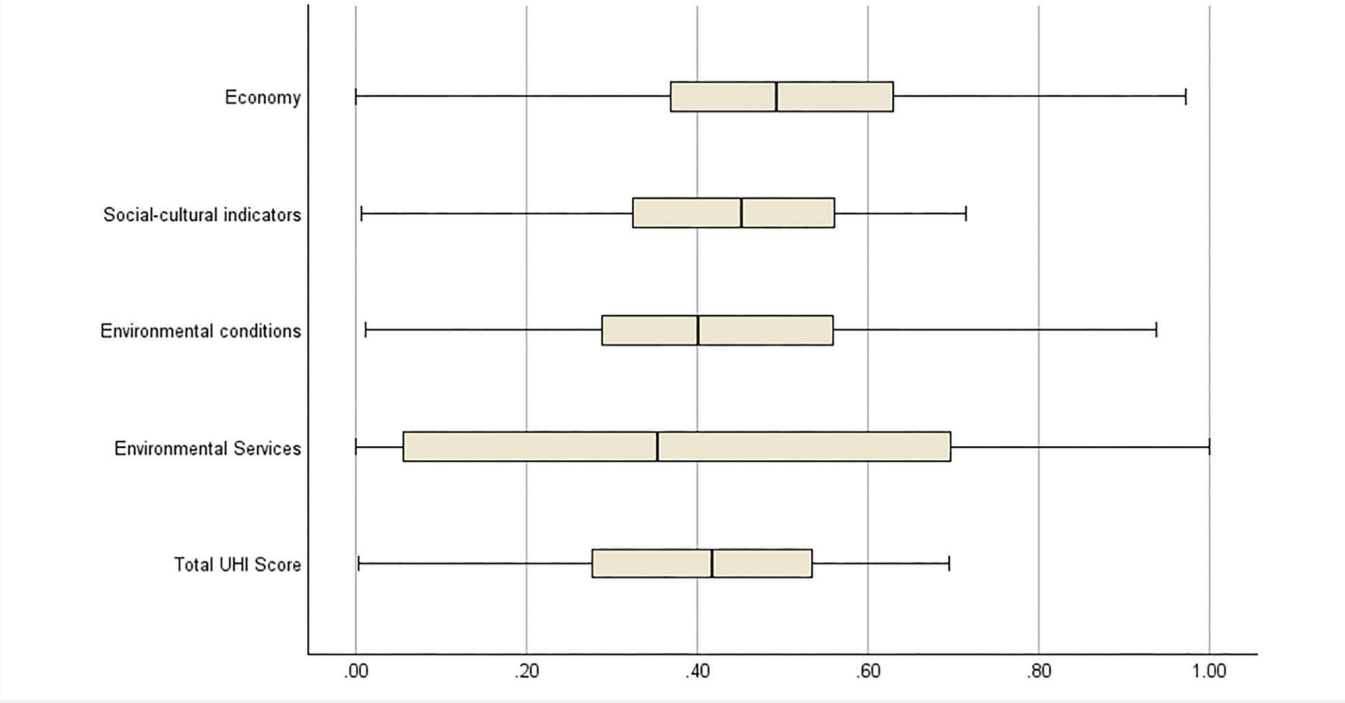


Figure 3. Box-plot presentation the total Urban health index and the domains (no. provinces: 81)

UHI: Urban health index

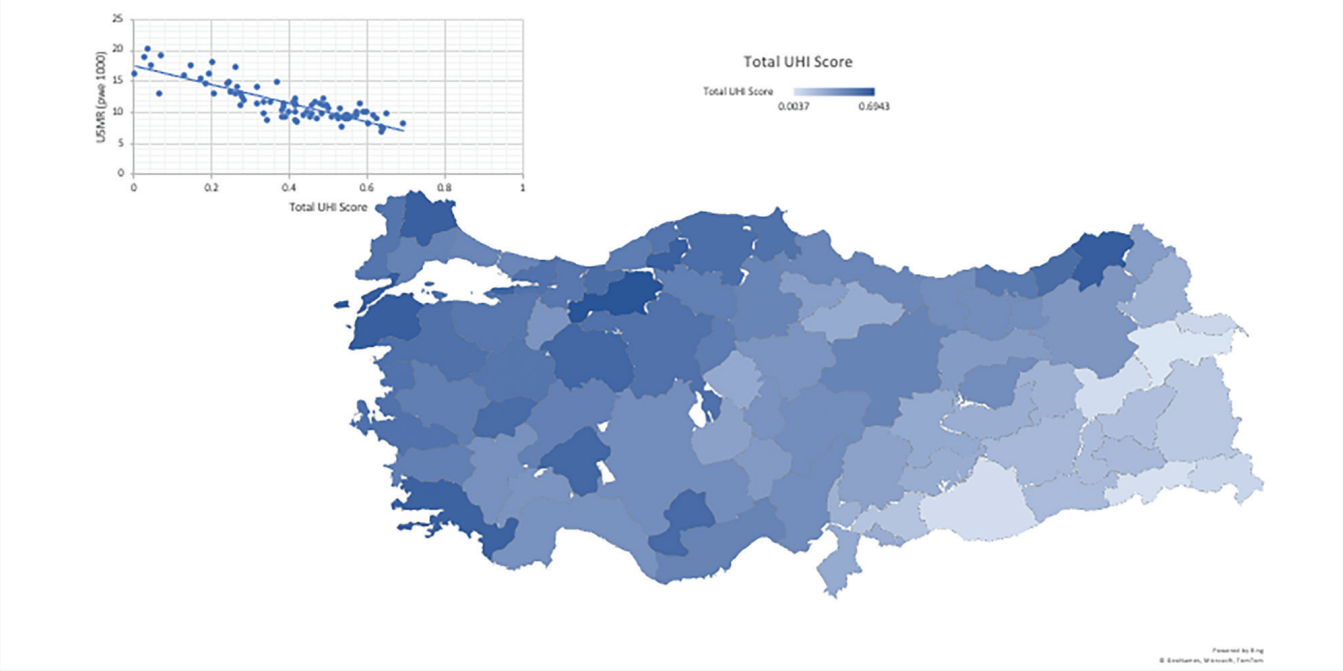


Figure 4. Map of the Urban health index's total score and its correlation with under-five mortality rate

UHI: Urban health index, U5MR: Under-five mortality rate

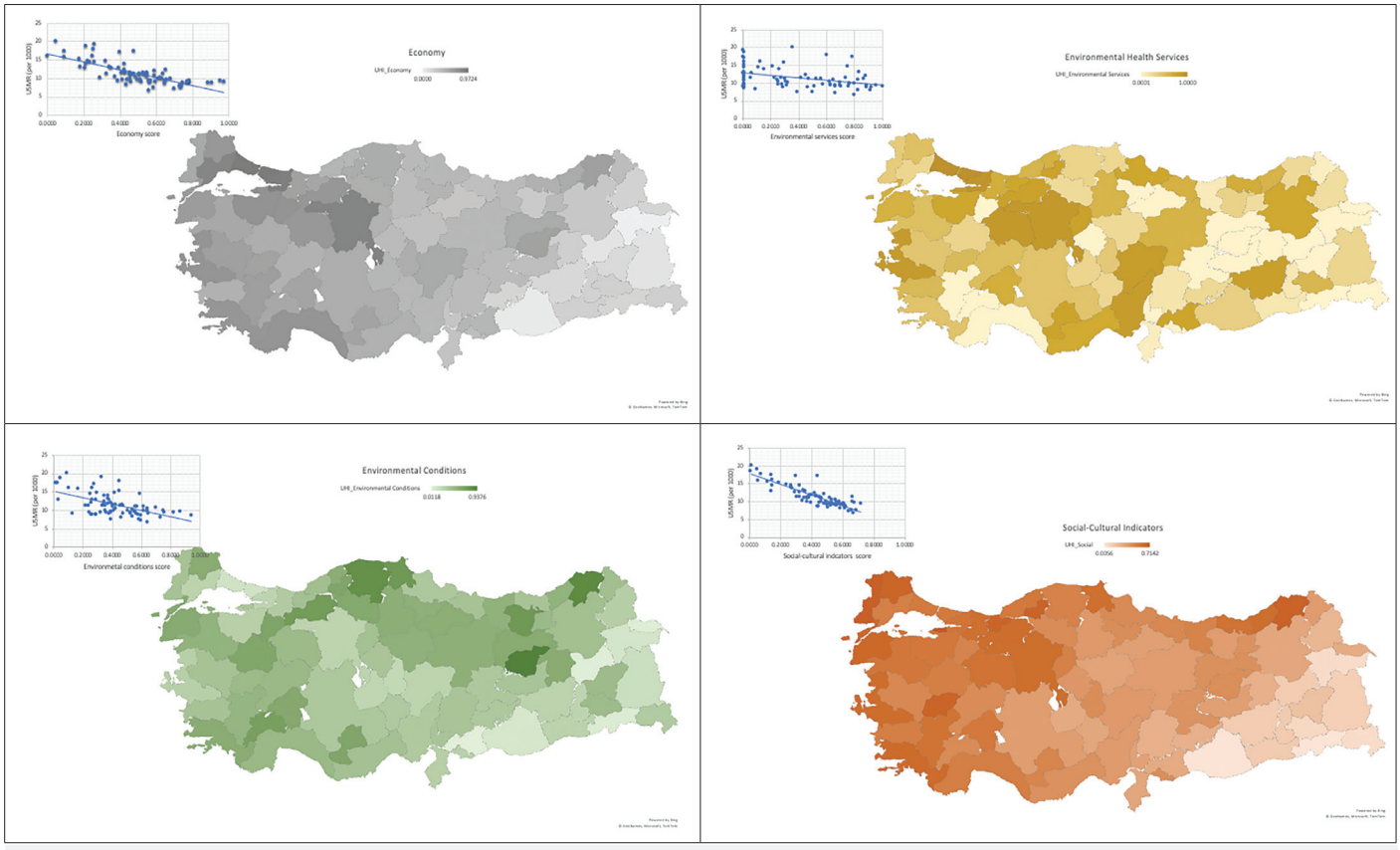


Figure 5. The maps of the scores of each domain and the correlations with under-five mortality rates

UHI: Urban health index, U5MR: Under-five mortality rate

DISCUSSION

In this study, we provide a list of indicators for assessing urban health at the province level as well as an UHI from a capability to be healthy perspective by taking into consideration the specific conditions and dynamics of Türkiye. This not only provides a tool for assessing urban characteristics associated with health but also allows us to compare and to follow up temporal change between the different provinces in Türkiye. Nevertheless, our index constitutes a first attempt at developing an accessible tool for monitoring urban health which can be utilized periodically in order to develop policies for improving the health and well-being of people in Türkiye. In this sense, our index not only allows us to make comparisons between different provinces in Türkiye but also serves a guideline for determining what kind of urban policies need to be implemented in order to ameliorate health conditions across Türkiye.

An understanding of health from a capabilities perspective is compatible with the vision laid out in 2030 Agenda for Sustainable Development in the sense that both approaches adopt a holistic view of urban health. Similar to the broader health-related sustainable development goals suggested by the United Nation, such as poverty eradication, equitable access to education, universal access to water and sanitation, full and decent employment; the indicators we suggest in our index also seek to provide a comprehensive approach to health. Such a conceptualization of urban health is crucial for upholding and achieving the right to health. We aimed to capture the relationship between several domains of health as a capability and different patterns of health. To this end, we used U5MR which is more related to urban health[13]. However, it is mostly a methodological study that the correlation with U5MR was used to check the quality of the final index.

Although there are studies that focus on various aspects of urban health or that attempt to create UHIs for Türkiye, our study differs from them in various ways. First of all, we employ an ecological model which seeks to integrate social and biological reasoning[22-25] rather than one-dimensional models that solely focus on individual health behavior or biological factors. Secondly, due to our focus on social and environmental determinants of urban health, we utilize data which can be summarized as anything that impacts the health of urban residents in Türkiye[1,26]. To this end, we excluded health indicators on access to healthcare and health services. Lastly, we attempted to link our discussion on urban health to "the right to health" by adapting the "capability to be healthy" perspective. This perspective allowed us to focus on determinants of health in urban settings, rather than access to healthcare and health services, and allowed us to "shift the focus away from disease outcome toward urban exposure, namely, the characteristics of the urban context that influence health and well-being[27]."

Although developing indexes using statistical analysis has been criticized as a rationalist approach, UHIs are nevertheless "one growing form of evidence that public health practitioners use to inform urban policy-makers[28]." They allow us to better understand the dynamics behind cities as indicators used in creating indexes reflect factors affecting community health.

Based on the results, literacy and poverty indicators showed the strongest correlations with U5MR across provinces. While other economic indicators such as employment and income also demonstrated notable associations with population health, their correlations were comparatively weaker. A substantial body of literature supports the link between socioeconomic status (SES) and health outcomes at both macro and individual levels[29-31]. Education and income are widely recognized as core components of SES and are frequently used in measuring health gradients[32,33]. These factors are closely intertwined, as higher educational attainment often leads to better income and improved living condition.

Poverty, however, encompasses more than just financial deprivation; it also reflects limited opportunities for employment, constrained capabilities, and reduced empowerment and security[34]. The poverty indicator used in this study, percentage of population under poverty line as defined by the Social Security Institution for universal health insurance eligibility captures only the financial dimension. Due to absence of province-level multidimensional poverty data, this measure served as a proxy for estimating the poverty. Notably, SSI data reported that 6.9% of the population was under the poverty line in 2019, while TurkStat estimated the figure at 14.4%[35]. This discrepancy suggests that out proxy likely underestimates the actual level of poverty, potentially inflating the strength of the observed correlation with the health indicator.

Although numerous environmental indicators are recommended for inclusion in the UHI frameworks, this study incorporated only air pollution, green space availability, and crowdedness in microenvironments (households and schools). Air quality is routinely monitored at the provincial level through measurement stations; however, these stations are not always representative of the broader geographic area[36]. Several province-level studies have reported significant correlations between air quality and premature mortality[36,37].

The green space indicator used in this study, measured by forest area size, does not include parks and smaller recreational areas within urban settlements due to data limitations. This is a notable shortcoming, as the built environment is closely associated with both physical and mental health through mechanisms such as stress reduction, reduced exposure to environmental pollutants, and improved access to spaces that support physical activity and social engagement[27]. Although indicators such as noise levels, drinking water quality, food

hygiene, and environmental pollution (e.g., heavy metals) are relevant to urban health, data on these factors are not publicly accessible. As a result, the environmental dimension of the UHI was limited to three available indicators. However, even these are subject to limitations in data quality.

As noted in the Methods section, we separated environmental health services from the broader environmental domain because they primarily reflect municipal-level interventions. Services such as water hygiene, wastewater management, and solid waste disposal are well-established public health measures that contribute significantly to improved population health outcomes. However, because coverage rates for waste management, sewage systems, and water supply networks are uniformly high across provinces, these indicators were excluded from the analysis due to their limited sensitivity in capturing regional differences[20]. However, the selected UHI indicators, the proportion of the population served by wastewater treatment and by drinking water treatment, show significant disparities across provinces, ranging from 0% to 99%. Though there is a weak correlation with the U5MR (r= -0.372), this wide variation may reflect structural and regional inequalities in infrastructure development, differences in municipal capacity, varying levels of urbanization, and uneven public investment across provinces[38]. Furthermore, broader spatial disparity analyses indicate that infrastructure and limited access to safe water and sanitation services persist in some regions, particularly those less urbanized or economically disadvantaged, due to historical neglect or geographic and environmental constraints[39].

As stated in many international publications, strict gender norms and gender inequalities persist in Türkiye[40]. Since TurkStat's Family Structure Survey includes valuable gender-related indicators-such as decision-making around marriage, perceptions of appropriate marriage age for men and women, and types of marriage (civil or religious), these indicators are not available at the provincial level[41]. A study analyzing the impact of gender inequality on population health across Organisation for Economic Co-operation and Development countries found that greater gender equality-measured through indicators such as education, political representation, and access to healthcare-s significantly associated with reductions in premature mortality, disability-adjusted life years, and years lived with disability, as well as improvements in overall life expectancy and health-adjusted life[42].

In developing the UHI, we initially planned to include indicators related to gender-based violence, such as number of women shelters per province. However, due to the lack of publicly available or complete data on women's shelters across all provinces, these indicators could not be incorporated into the final index. As alternatives, we included indicators that reflect gender norms and women's agency, specially the rate of women's self-employment as a proxy for women's entrepreneurship, and the crude divorce rate, both of which have been linked to shifting gender roles and autonomy in the literature[42-44].

Indicators reflecting gender norms demonstrated a strong correlation with the health outcome. These measures serve as proxies for women's economic participation, autonomy, and shifting social roles, all of which are recognized determinants of health. Research has shown that greater gender equity is associated with improved population health outcomes, including reductions in child mortality and increased life expectancy[9,42,45]. Enhanced participation of women in the workforce and public life is often linked to stronger investments in health, education, and well-being at both household and community levels[45].

In developing the UHI, we also considered indicators related to leisure and recreation opportunities, recognizing their important role in promoting physical activity, mental well-being, and stress reduction. Access to recreational and social spaces has been shown to contribute positively to both physical and psychological health outcomes by fostering active lifestyles, social connectedness, and healthier coping mechanisms[46,47]. These spaces serve as key components of supportive urban environments, particularly in mitigating the health effects of urban stressors and sedentary behaviors.

Finally, the indicator included in the UHI framework to represent social networks, measured by the number of civil associations per population, showed a strong correlation with U5MR. This indicator serves as a proxy for social capital and community level social support, which are widely recognized as important social determinants of health. High levels of social capital are associated with improved health outcomes through mechanisms such as increased access to information and resources, collective efficacy, and emotional support[48-50]. In particular, stronger civic engagement and trust within communities have been linked to lower child mortality and better overall population health[51].

A more recent study developed a health index of provinces in Türkiye using health statistics published by the Ministry of Health in 2016. The indexes divided into four categories: health infrastructure, health personnel, health services, and health status[52]. According to the study, majority of provinces in Eastern and Southeastern Türkiye rank demonstrate low performance across all indicators. Although this study differs from our UHI in that it focuses on the healthcare sector, it highlights disparities that exist at the regional and provincial level in Türkiye. As noted previously, we deliberately exclude the indicators on healthcare services because of our focus of the UHI. Although

the role of healthcare services in determining population health is important, research consistently shows about the healthcare accounts for only 10% to 20% of the overall determinants of population health[53].

Study Limitations

A major challenge in developing the UHI was limited access to regularly collected, publicly available data. As noted by other researchers working on UHIs in Türkiye[16,17,52] we found that reliable data is often unavailable. For example, we lacked access to provincial-level data on socially disadvantaged groups (e.g. children, women, LGBTI, and disabled people, water quality, food safety, gender equity (e.g. the number of women's shelter and their capacity), electricity usage by sector, and income inequality (e.g. Gini coefficient). Including these would have allowed for a more holistic and capability-based index.

Another limitation is the use of the U5MR as the outcome variable, which strongly reflects access to healthcare access. Life expectancy might have been broader indicator, but internal migration patterns such as retirees moving to coastal or rural provinces distort province-level reliability. Despite its limitations, U5MR was selected as the most feasible proxy for population health.

Finally, because this study uses province-level data, it cannot capture intra-province disparities or neighborhood-level health inequalities.

CONCLUSION

The development of a multidimensional UHI offers a valuable framework for assessing the social and structural determinants of health at the provincial level. The strong correlations observed between health outcomes and indicators such as such as literacy, employment, and gender norms, and civic engagement underscore the need to broaden health policy beyond the healthcare sector to address underlying social and cultural factors.

To support effective use of the UHI, national and local governments should ensure that province-level and disaggregated data, particularly on vulnerable populations-are publicly accessible. Improving data availability and transparency is essential for advancing health equity and informed decision-making.

The UHI provides actionable insights that can guide evidence-based, inclusive policymaking to promote urban health, resilience, and social justice across Türkiye's diverse provinces. Looking ahead, the collection and public dissemination of county-level data should be encouraged, as it would enable more granular assessments and further enhance the ability to address health disparities. Such efforts would make a meaningful contribution to improving population health and advancing health equity in Türkiye.

On the other hand, the component of the UHI highlight the key areas for enhancing population health through province-level policy. This study identifies potential local policy interventions, such as expanding leisure and recreation opportunities, promoting women empowerment, and improving built environment. Moreover, the findings underscore the importance of adopting a multidisciplinary, multidimensional, and participatory approach to urban health policymaking.

Ethics

Ethics Committee Approval: The data used in this study are anonymized, aggregate secondary data obtained from the Turkish Statistical Institute (TURKSTAT). Because the dataset is publicly accessible and contains no identifiable individual-level information, ethics committee approval was not required.

Informed Consent: This study is a retrospective study that used secondary data to construct an Urban health index.

Footnotes

Authorship Contributions

Concept: A.M., N.E., P.K., Design: A.M., N.E., Data Collection or Processing: N.E., Analysis or Interpretation: N.E., Writing: A.M., N.E., P.K.

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

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Anterolateral Ligament Injuries in Partial Anterior Cruciate Ligament Injuries

Kısmi Ön Çapraz Bağ Yaralanmalarında Anterolateral Bağ Yaralanması

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ABSTRACT

Aim: This study aimed to evaluate the relationship between the type of acute partial anterior cruciate ligament (ACL) injury and anterolateral ligament (ALL) injury.

Materials and Methods: One hundred and forty-eight patients aged 18-40 years with a clinical pre-diagnosis of ACL injury who underwent 3T knee magnetic resonance image between January 2016 and December 2020 were retrospectively analyzed. Acute ACL injury was defined as cases with a history of knee trauma within the last month. Demographic data, ALL visibility, classification of ALL components, presence and type of ACL and ALL injury, injury location, and accompanying lesions were recorded. Correlations between ACL/ALL injury characteristics and accompanying lesions were evaluated.

Results: There was a significant correlation between ACL injury type and both the presence and type of ALL injury (p=0.002 and p=0.013, respectively). There was also a significant correlation between ALL injury and lateral meniscus tear (p=0.024). The diameter of the ALL was significantly increased in injured patients compared to non-injured patients (p=0.001).

Conclusion: A high rate of ALL injury was observed in this series, which predominantly included partial ACL injury cases. Our results suggest that ALL injury should be considered in the management of partial ACL injuries to avoid potential complications.

Keywords: Anterolateral ligament, anterior cruciate ligament, partial lesions, sports injury

ÖZ

Amaç: Bu çalışmada amacımız, akut parsiyel ön çapraz bağ (ACL) yaralanması tipi ile anterolateral bağ (ALL) yaralanması arasındaki ilişkiyi ortaya koymaktır.

Gereç ve Yöntem: Ocak 2016 ile Aralık 2020 tarihleri arasında klinik olarak ACL yaralanması ön tanısı konulan ve diz manyetik rezonans görüntüleri 3T çekilen 18-40 yaşları arasındaki 148 hasta retrospektif olarak incelendi. Son bir ay içinde eş zamanlı diz travması öyküsü olan olgular arasında akut ACL yaralanması tespit edildi. Olguların demografik verileri, ALL'nin görünürlüğü, bileşenlerin sınıflandırılması, ACL ve ALL yaralanmasının varlığı, yaralanmanın yeri ve yaralanma tipi kaydedildi. ACL ve ALL yaralanma özellikleri ile eşlik eden lezyonlar arasındaki korelasyonlar değerlendirildi.

Bulgular: ACL yaralanma tipi ile ALL yaralanmasının varlığı ve tipi arasında anlamlı bir korelasyon vardı (sırasıyla p=0,002 ve p=0,013). ALL yaralanması varlığı ile lateral menisküs yırtığı arasında anlamlı bir korelasyon vardı (p=0,024). ALL çapı, yaralanması olan hastalarda yaralanması olmayanlara kıyasla artmıştı (p=0,001).

Sonuç: Çoğunlukla kısmi ACL yaralanması olan hastalardan oluşan vaka serimizde yüksek oranda ALL yaralanması tespit edildi. Bulgularımız, olası komplikasyonları önlemek için kısmi ACL yaralanması olan olguların tedavisinde ALL yaralanmasının varlığının dikkate alınması gerektiğini göstermektedir.

Anahtar Kelimeler: Anterolateral bağ, ön çapraz bağ, kısmi lezyonlar, spor yaralanması

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INTRODUCTION

Partial lesions comprise approximately 10% to 27% of isolated anterior cruciate ligament (ACL) lesions[1,2]. Conservative treatment is often recommended for stable partial lesions; however, success rates vary widely in young and active individuals. These lesions may progress to complete tears in 14% to 56% of cases and cause significant morbidity if not managed appropriately[3].

ACL injuries commonly occur via non-contact mechanisms involving valgus stress and internal rotation[4]. Similar mechanisms have been reported to cause injuries to the anterolateral ligament (ALL), first described by Segond in 1879[5]. Claes et al.[6] defined the ALL as a distinct ligament anterolateral to the knee and described its role as an important stabilizer against internal rotation, particularly between 30° and 90° of knee flexion. ALL injury has also been associated with a high-grade pivot-shift phenomenon in ACL-deficient knees[7].

Residual rotational instability may persist after ACL reconstruction[8,9]. It has been suggested that ALL reconstruction may help address persistent pivot-shift[10]. Despite extensive research on the ALL as a secondary restraint to the ACL, consensus on its anatomy and functional role remains lacking[11]. Moreover, most studies have focused on complete ACL tears, with limited data on ALL injury in partial ACL injuries.

This study aimed to investigate the relationship between acute partial ACL injury type and ALL injury in young, active individuals and to provide insights for future clinical and biomechanical studies.

MATERIALS AND METHODS

Our study was approved by the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (protocol number: 2021.46.02.09, date: 23.02.2021). Two hundred patients who were clinically pre-diagnosed with ACL injury and underwent knee magnetic resonance image (MRI) at our institution between January 2016 and December 2020 were retrospectively analyzed. Written informed consents of all cases were obtained. All images were created using an 8-channel knee coil on a 3T MRI system (Signa HDx; GE Healthcare, Chicago, IL, USA). Our standard knee imaging protocol included axial fat-suppressed proton density-weighted turbo spin-echo sequences [repetition time (TR): 2300 ms, echo time (TE): 35 ms], axial T2-weighted turbo spin-echo sequences (TR: 6700 ms, TE: 70 ms), sagittal fat-suppressed proton density-weighted turbo spin-echo sequences (TR: 3890 ms, TE: 35 ms), sagittal proton density-weighted sequences (TR: 3400 ms, TE: 35 ms), coronal fat-suppressed proton density-weighted turbo spin-echo sequences (TR: 4010 ms, TE: 37 ms), and coronal T1-weighted turbo spin-echo sequences (TR: 690 ms, TE: 9 ms). The section thicknesses were 3 mm for each sequence and the intersection gap was 0.3 mm. For all sequences the matrix size was 352×256 and the field of view was 16 cm.

The images were evaluated by a musculoskeletal radiologist and an orthopedic surgeon. One hundred and forty-eight patients between the ages of 18 and 40 years, who had acute ACL lesions (with a trauma history within the last month) were included in the study, whereas patients with no previous knee surgery, no arthrosis findings, no metal artifacts, or no inflammatory arthritis were excluded.

In the images on the picture archiving communication system, loss of integrity in any sequence or hyperintensity in T2 sequences was considered as ACL injury, and the injury was described as "partial" or "complete" according to the continuity of the fibers and clinical laxity[12]. The presence of the ALL was investigated in all sequences, the femoral origin was evaluated on the axial and coronal sequences while the meniscal attachment and tibial insertion were evaluated only on the coronal sequences[13]. The distance between the inferior aspect of the lateral meniscus and tibial insertion, and the thickness of the ALL at the subchondral bone level were measured. ALL injury was examined on the coronal T2 sequences. An increased intensity without disruption in ALL fiber continuity was examined. In the cases with disruption, the lesion was examined to check whether it was a partial or complete one (Figures 1,2)[6,13].

The demographic data of the cases, visibility of the ALL, classification of the components, presence of ACL and ALL injury, location of the injury, and injury type were recorded. Correlations between the ACL and ALL injury characteristics and accompanying lesions were evaluated. Interobserver reliability was also evaluated.

Statistical Analysis

Statistical analyses were performed using Number Cruncher Statistical system 2007 (Kaysville, UT, USA). Descriptive statistics (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used. Normality was tested with the Shapiro-Wilk test. The Mann-Whitney U test was used for quantitative data without normal distribution. Chi-square test was used for qualitative variables. Significance was set at $p<0.01$.

RESULTS

Results are summarized in Table 1. The majority (54.4%) of ACL lesions were partial and located proximally. The ALL could not be visualized in 10.8% of cases. Among cases where the ALL was visible, injury was detected in 81%, and the majority (81.3%) showed loss of continuity. Most ALL injuries (68.2%) occurred at the femoral origin. No bone avulsion was observed.

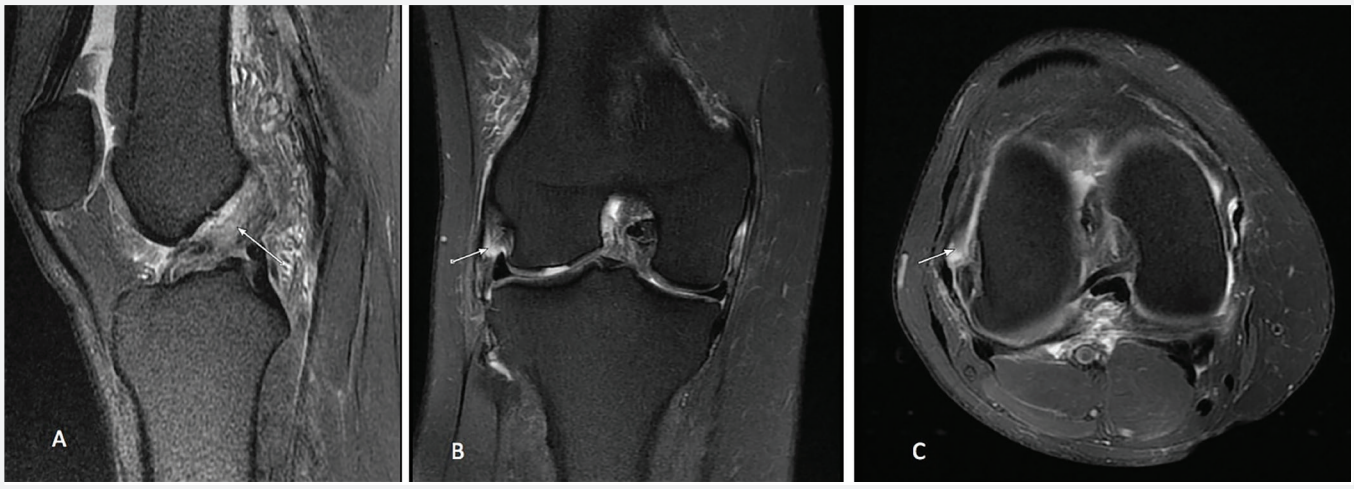


Figure 1. (A) Complete ACL injury on a sagittal T2 image. (B) Complete ALL injury in the femoral location on a coronal T2 image. (C) Complete ALL injury on the axial sequence

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament

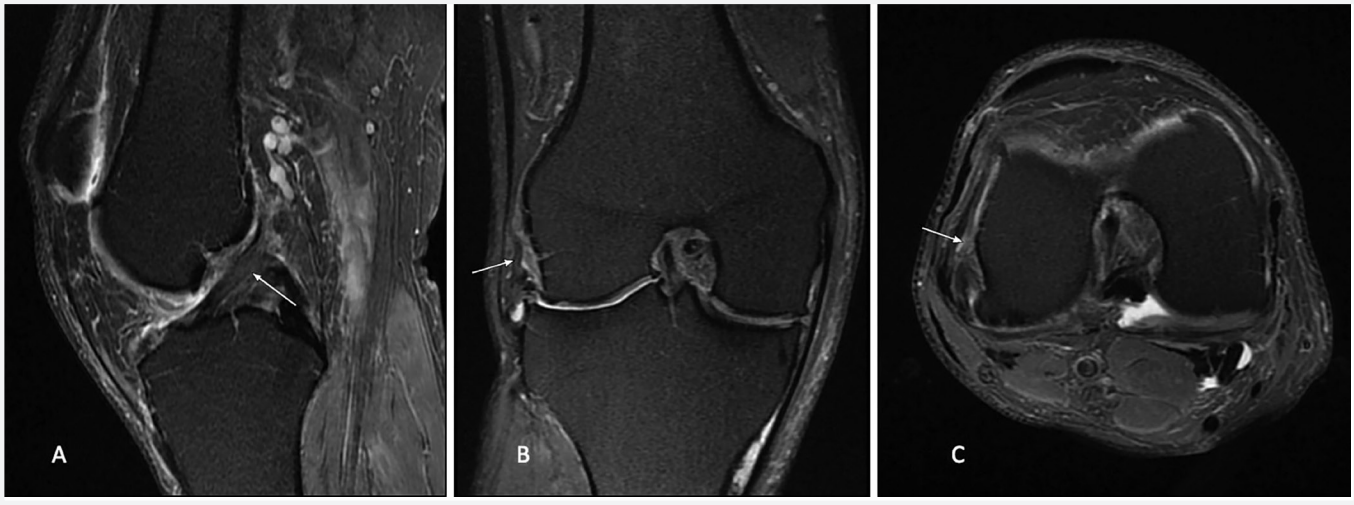


Figure 2. (A) Partial ACL injury on a sagittal T2 image. (B) Partial ALL injury in the femoral origin region on a coronal T2 image. (C) Partial ALL injury on an axial T2 image

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament

As shown in Table 2, there was a significant correlation between ACL injury type and presence of ALL injury (p=0.002). No significant correlation was found between ACL injury type and location (Table 3). A significant correlation existed between ACL injury type and both presence and type of ALL injury (p=0.002 and p=0.013, respectively) (Table 4). No correlation was observed between meniscal attachment morphology and ALL injury or lateral meniscus tear (Table 5). Medial meniscus tear was seen in only seven cases. Lateral meniscus tear occurred in 32 cases and was significantly associated with ALL injury (p=0.024). Medial kollateral ligament injury was detected in six cases and lateral collateral ligament (LCL) injury in 17 cases; no significant correlation was found with ALL injury or bone contusion (Table 6). ALL diameter was significantly increased in injured patients compared to non-injured patients (p=0.001) (Table 7). Interobserver reliability was excellent [confidence interval (CI): 0.98; 95% CI: 0.98-0.99].

Table 1. Demographics and clinical examination and measurement results of the patients

Age (years)	31.8 (range: 20 to 40)
Gender (male: female)	100:48
Side (right: left)	72:76
Time between trauma and MRI (days)	11.43 (range: 1 to 25)
Distance to tibial attachment (mm)	4.26 (range: 4 to 10.4)
Diameter of the ALL (mm)	3.54 (range: 1.1 to 9)
Femoral origin	
Not visualized	16 (10.8%)
LE	116 (78.4%)
AD-LE	8 (5.4%)
PP-LE	8 (5.4%)
Tibial visualization	
Not visualized	16 (10.8%)
Visualized	132 (89.2%)
Meniscal attachment	
Not visualized	16 (10.8%)
Inferior	2 (1.4%)
Bipolar	52 (35.1%)
Complete	65 (43.9%)
Central	13 (8.8%)
ACL injury type	
Partial	80 (54.1%)
Complete	68 (45.9%)
ACL injury location	
Proximal	137 (92.6%)
Middle	2 (1.4%)
Distal	9 (6.1%)
ALL injury	
Not visualized	16 (10.8%)
Injured	107 (72.3%)
Non-injured	25 (16.9%)
ALL injury type (n=107)	
Increased intensity	20 (18.7%)
Partial	57 (53.3%)
Complete	30 (28.0%)
ALL injury location	
Femur	73 (68.2%)
Meniscus	5 (4.7%)
Tibia	13 (12.1%)
Complete	16 (15.0%)

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament, LE: Lateral epicondyle, AD: Anterodistal, PP: Posteroproximal, MRI: Magnetic resonance image

Table 2. Relationship between the ACL injury type and the presence of ALL injury

		Presence of ALL injury		p*
		Non-injured	Injured	
ACL injury type	**Partial**	20 (80%)	50 (46.7%)	0.002†
	Complete	5 (20%)	57 (53.3%)	

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament, *: Chi-square test, †: p<0.01

Table 3. Relationship between the ACL injury type and ALL injury location

		ACL injury type		p*
		Partial	Complete	
ALL injury location	**Proximal**	37 (52.9%)	36 (58.1%)	0.254
	Meniscal	3 (4.3%)	2 (3.2%)	
	Tibial	6 (8.6%)	7 (11.3%)	
	Complete	8 (11.4%)	8 (12.9%)	
	None	16 (22.9%)	9 (14.5%)	

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament, *: Chi-square test

Table 4. Relationship between the ACL injury type and ALL injury type

		ACL injury type		p*
		Partial	Complete	
ALL injury type	**Partial**	33 (47.1%)	24 (38.7%)	0.013†
	Complete	9 (12.9%)	21 (33.9%)	
	Increased intensity	12 (17.1%)	8 (12.9%)	
	None	16 (22.9%)	9 (14.5%)	

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament, *: Chi-square test, †: p<0.01

DISCUSSION

The most important finding of this study was the high rate of ALL injury accompanying partial ACL injuries.

There is still no consensus regarding the posttraumatic morphology of the ALL[6,14,15]. Following ACL injury, the ALL could not be visualized in 24% of cases in Claes et al.[6], 12.8% in Helito et al.[16] and 10.8% in our series. ALL injury rates were 32.6% in Helito et al.[16] and 78.8% in Claes et al.[6]. Our rate of 81% was notably high, possibly due to inclusion of increased signal intensity as injury and the predominance of partial ACL cases (Figure 3).

Lesion classification is as critical as radiographic anatomy when considering surgical reconstruction. Ferretti et al.[17] reported

Table 5. Relationship between meniscal attachment and the presence of ALL injury and lateral meniscus tear				
		Presence of ALL injury		p*
		Non-injured	Injured	
Meniscal attachment	Inferior	0 (0%)	2 (1.9%)	0.594
	Bipolar	8 (32%)	44 (41.1%)	
	Complete	13 (52%)	52 (48.6%)	
	Central	4 (16%)	9 (8.4%)	
		Lateral meniscus tear		p*
		Absent	Present	
Meniscal attachment	Inferior	2 (2%)	0 (0%)	0.390
	Bipolar	39 (39.8%)	13 (38.2%)	
	Complete	46 (47%)	19 (55.9%)	
	Central	11 (11.2%)	2 (5.9%)	

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)
ALL: Anterolateral ligament, *: Chi-square test

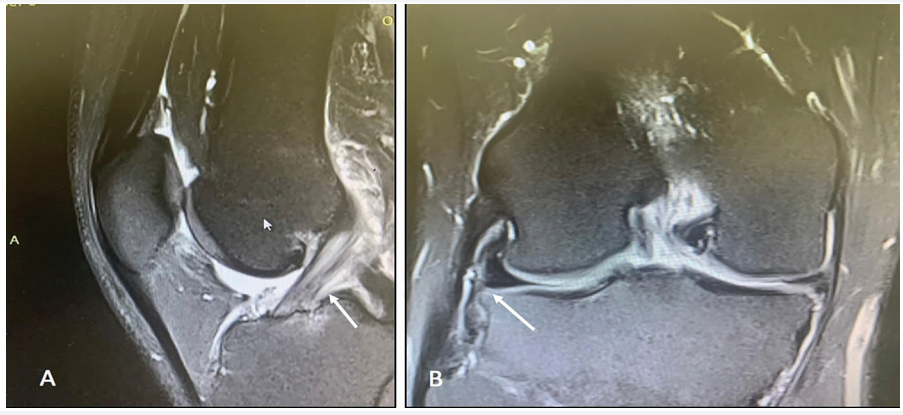


Figure 3. (A) Partial ACL injury on a sagittal image. (B) Increased intensity in the ALL on the coronal sequence
ACL: Anterior cruciate ligament, ALL: Anterolateral ligament

multi-level ALL ruptures in half of their surgical cases. In our MRI evaluation, 16% of cases showed ALL involvement at all three levels. Normal ALL thickness has been reported as 2.09 mm in men and 1.09 mm in women by Daggett et al.[18] and 1.75 mm by Kosy et al.[13] In our ACL injury cohort, ALL thickness was 3.54 mm overall and significantly higher in injured cases (4.03±1.11 mm vs. 3.3±0.59 mm). This increase, likely trauma-related, aligns with Ferretti et al.[15] observations of increased signal (79.4%) and thickness change (64.7%).

The most common site of ALL injury remains controversial. Some studies report tibial predominance,[6,11] while others note femoral predominance (72% in Helito et al.[16] 55.5% in Lee et al.[19]). In our series, 68.2% of injuries were at the femoral origin (Figure 4).

The trauma mechanism injuring the ACL can also affect adjacent structures. Lateral meniscus injury was seen in 22.9% of our cases, similar to 21.7% in Helito et al.[16] A significant correlation was found between ALL injury and lateral meniscus tear, suggesting a shared injury mechanism. Although not statistically significant, bone contusion of the lateral femoral condyle and LCL injury frequently co-occurred with ALL injury, consistent with the literature[20].

It is known that 14% to 56% of conservatively treated partial ACL injuries progress to complete tears within five years[1,21]. In patients under 30 years, Fayard et al.[3] reported progression in 39% of cases, while Fritschy et al.[22] reported 41.8%. Many studies on partial ACL progression have not assessed ALL injury[23]. ALL lesions have limited spontaneous healing potential, with 70% showing poor healing at one-year follow-up[19]. In our study, ALL

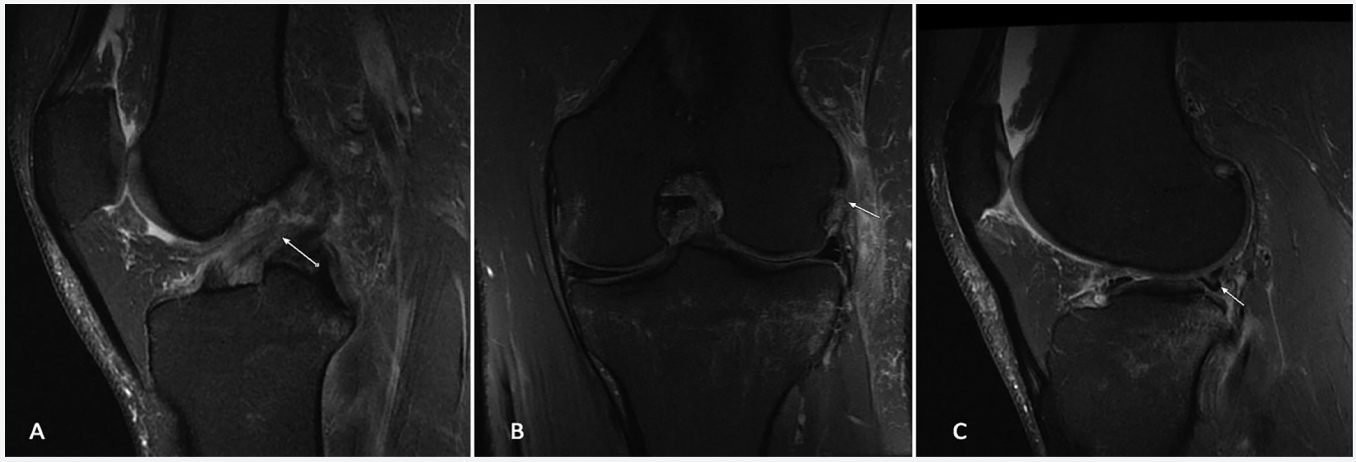


Figure 4. (A) Complete ACL rupture on a sagittal image. (B) Partial ALL injury and tibial bone contusion in the femoral origin region on a coronal T2 sequence. (C) Lateral meniscus injury

ACL: Anterior cruciate ligament, ALL: Anterolateral ligament

Table 6. Relationship between the presence of ALL injury and lateral meniscus tear, lateral collateral ligament injury and bone contusion

		Lateral meniscus tear		p*
		Absent	Present	
Presence of ALL injury	Non-injured	23 (23.5%)	2 (5.9%)	0.024
	Injured	75 (76.5%)	32 (94.1%)	
		LCL injury		
		Absent	Present	
	Non-injured	24 (21.1%)	1 (5.6%)	0.101
	Injured	90 (78.9%)	17 (94.4%)	
		Bone contusion		
		Absent	Present	
	Non-injured	24 (20.7%)	1 (6.3%)	0.167
	Injured	92 (79.3%)	15 (93.7%)	

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)

ALL: Anterolateral ligament, LCL: Lateral collateral ligament, *: Chi-square test

Table 7. Comparison of the ALL diameter with the presence of ALL injury

		n	Mean ± SD	Minimum-maxim (median)	p*
ALL diameter	Non-injured	25	3.3±0.59	1.6-4.1 (3.3)	**0.001**†
	Injured	107	4.03±1.11	1.2-9 (3.9)	

The data in parentheses indicate the incidence, while the data outside the parentheses indicate the number of cases (n)

ALL: Anterolateral ligament, SD: Standard deviation, *: Kruskal-Wallis test, †: p<0.01

injury was present in the majority of partial ACL cases (77% vs. 85% in complete ACL cases). Therefore, ALL injury should be considered in the progression of partial ACL tears and risk of re-rupture.

Study Limitations

This study has several limitations. It was based solely on radiological findings without clinical correlation. ALL injuries were not surgically verified. The sample size was relatively small, and clinical outcomes were not evaluated. However, the large proportion of partial ACL cases provides a unique perspective on

the relationship between ACL injury type and ALL injury. Further clinical studies on functional outcomes in isolated partial ACL lesions with ALL injury are warranted.

CONCLUSION

In conclusion, a high rate of ALL injury was detected in this series, which predominantly comprised partial ACL injury patients. Our results suggest that the presence of ALL injury should be considered in the management of young and active individuals with partial ACL injuries to avoid potential complications.

Ethics

Ethics Committee Approval: This study was approved by the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (protocol number: 2021.46.02.09, date: 23.02.2021).

Informed Consent: The study is a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.Ü.Ç., A.S., F.E., Concept: M.Ü.Ç., A.S., B.K.S., Design: M.Ü.Ç., A.S., F.E., B.K.S., Data Collection or Processing: M.Ü.Ç., A.S., F.E., B.K.S., Analysis or Interpretation: M.Ü.Ç., A.S., F.E., B.K.S., Literature Search: A.S., B.K.S., Writing: M.Ü.Ç., A.S., F.E., B.K.S.

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

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ORIGINAL ARTICLE

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Evaluation of Monocyte/HDL and Neutrophil/Lymphocyte Ratios in Patients with Hashimoto's Thyroiditis

Hashimoto Tiroiditi Hastalarında Monosit/HDL ve Nötrofil/Lenfosit Oranlarının Değerlendirilmesi

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ABSTRACT

Aim: Systemic inflammation related to Hashimoto's thyroiditis (HT) is thought to increase oxidative stress, endothelial dysfunction, and the risk of cardiovascular diseases. In this study, we aimed to indirectly evaluate this risk increase through easily measurable and cost-effective parameters such as the neutrophil/lymphocyte ratio (NLR) and monocyte/high-density lipoprotein ratio (MHR).

Materials and Methods: In this retrospective study, the laboratory data of patients aged between 18 and 80 who were diagnosed with hypothyroidism were reviewed. A total of 325 individuals who met the inclusion criteria were included in the study. Of these, 203 were diagnosed with HT and were divided into two subgroups: overt hypothyroidism (OH) and subclinical hypothyroidism (SH). These data were compared with those of 122 individuals in the healthy control group (CG).

Results: The mean age of the control and patient groups was similar. In the evaluation of inflammatory parameters, no significant differences were observed in C-reactive protein and sedimentation values between OH, SH, and the healthy CG. There were no statistically significant differences in terms of NLR and MHR between OH, SH, and CG (p: 0.507, p: 0.064, respectively). Additionally, no correlation was found between autoantibodies and NLR or MHR parameters in the OH, SH, and CG groups.

Conclusion: Parameters such as NLR and MHR could serve as practical tools in assessing oxidative stress, endothelial dysfunction, and cardiovascular disease risk in autoimmune and chronic inflammatory diseases like HT. However, further large-scale studies are needed to validate the clinical applicability of these parameters.

Keywords: Hashimoto's thyroiditis, neutrophil/lymphocyte ratio, monocyte/HDL ratio

ÖZ

Amaç: Hashimoto tiroiditi (HT) ile ilişkilendirilen sistemik enflamasyon artışının; oksidatif stres, endotel disfonksiyonu ve kardiyovasküler hastalık riskini artırdığı düşünülmektedir. Bu çalışmada, HT hastalarında risk artışını indirekt olarak değerlendirebilmek için, nötrofil/lenfosit oranı (NLR) ve monosit/yüksek yoğunluklu lipoprotein oranı (MHR) gibi kolay ve ucuz ölçülen parametreler incelenmiştir.

Gereç ve Yöntem: Bu retrospektif çalışmada HT tanısı almış 18-80 yaş aralığındaki hastaların laboratuvar verileri incelenmiştir. Çalışmaya alınma kriterlerine uyan toplam 325 kişi çalışmaya dahil edilmiştir. Dahil edilen bireylerin 203'ü HT hastası olup; aşikar hipotiroidi (AH) ve subklinik hipotiroidi (SH) olmak üzere iki alt gruba ayrılmıştır, bu veriler sağlıklı kontrol grubu (KG) olan 122 birey ile kıyaslanmıştır.

Bulgular: Kontrol ve hasta gruplarının yaş ortalaması benzerdi. Enflamasyon parametreleri açısından yapılan değerlendirmede AH, SH ve KG arasında C-reaktif protein ve sedim değerleri açısından benzerlik gözlemlenmiştir. AH, SH ve KG arasında NLR ve MHR parametreleri bakımından istatistiksel olarak anlamlı bir fark bulunmamıştır (sırasıyla p: 0,507, p: 0,064). Ayrıca, AH, SH ve KG de otoantikorlar ile NLR ve MHR parametreleri arasında herhangi bir ilişki saptanmamıştır.

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Sonuç: NLR ve MHR gibi parametreler, HT gibi diğer otoimmün ve kronik enflamatuvar hastalıklarda oksidatif stres artışı, endotel disfonksiyonu ve kardiyovasküler hastalık riskini belirlemede pratik birer parametre olabilir. Bununla birlikte, bu parametrelerin klinik kullanımıyla ilgili daha geniş ölçekli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Hashimato tiroiditi, nötrofil/lenfosit oranı, monosit/HDL oranı

INTRODUCTION

Hashimoto's thyroiditis (HT) is the most common chronic autoimmune disease of the thyroid gland, first described by Hakaru Hashimoto in 1912 as "struma lymphamotosa[1]." Patients with HT are often asymptomatic but may present with euthyroid, subclinical hypothyroidism (SH), overt hypothyroidism (OH), and rarely hyperthyroidism clinical manifestations[2]. If the diagnosis of SH is missed, the condition may progress to OH, leading to complications such as dyslipidemia, endothelial dysfunction, coronary artery disease, heart failure, and metabolic syndrome[3].

Elevated total cholesterol and low-density lipoprotein (LDL) levels are prone to oxidation and, under the effect of reactive oxygen species, accelerate lipid peroxidation. This process leads to increased oxidative stress, endothelial dysfunction, and increased peripheral resistance. The combination of arterial hypertension due to increased peripheral resistance and elevated cholesterol levels in thyroid hormone deficiency poses a significant risk for atherosclerotic cardiovascular disease (CVD) and cerebrovascular disease. Additionally, bradycardia and reduced ventricular contractility result in decreased cardiac output, predisposing to heart failure[4].

Recently, low-cost and easily measurable parameters such as neutrophil-to-lymphocyte ratio (NLR) and monocyte-to- high-density lipoprotein ratio (MHR) have gained popularity in assessing systemic inflammatory response[5]. Previous studies have indicated that NLR may serve as an independent predictor of CVD and mortality in acute heart failure[6]. Additionally, research suggests that NLR can be used as a marker of subclinical inflammation in non-cardiac conditions. For example, NLR has been reported as a helpful parameter in determining prognosis and guiding treatment decisions in cancer patients[7]. The association between NLR and rheumatologic diseases such as systemic lupus erythematosus and familial Mediterranean fever has also been investigated, revealing a significant correlation between NLR and disease prognosis[8]. Similarly, MHR has been found to be associated with various diseases including Parkinson's disease, hypertension, obstructive sleep apnea syndrome, ulcerative colitis, and vitiligo. In these conditions, increases in these parameters have been observed alongside elevated mortality[9].

In HT, the development of hypothyroidism alongside increased systemic inflammation constitutes a significant risk factor for endothelial dysfunction and atherosclerotic processes[10]. While previous studies have investigated the relationship between HT and NLR, no studies have, to our knowledge, examined the association between HT and MHR. In this study, we aimed to evaluate oxidative stress in patients with OH and SH due to HT by assessing NLR and MHR parameters.

MATERIALS AND METHODS

Data and Sources

This retrospective study reviewed data from patients diagnosed with hypertension based on ICD code screening via the hospital automation system, who visited the Internal Medicine Outpatient Clinics at Tekirdağ Namık Kemal University Hospital between January 1, 2020, and February 10, 2023. Approval for this study was obtained from the Ethics Committee of Tekirdağ Namık Kemal University, Faculty of Medicine, with the (protocol number: 2023.28.02.16, date: 28.02.2023). This article is derived from a thesis completed in 2024, registered under number 906166.

Patient Selection

Individuals under the age of 18 or over the age of 80, as well as those with CVD, pregnant women, individuals with active infections or chronic illnesses, those with autoimmune or rheumatologic diseases, and patients with immunosuppressive conditions who were using steroids were excluded from the study.

Laboratory Diagnostic Criteria

The diagnosis of HT was established based on anti-thyroid peroxidase (TPO) levels above 34 IU/mL and Anti-Tg levels above 115 IU/mL. Patients diagnosed with HT were divided into two groups according to their thyroid hormone levels: OH and SH. In the OH group, patients had a TSH level above 4.20 mIU/L, a free triiyodothyronine (T3) level below 2.0 pg/mL, and a free thyroxine (T4) level below 0.93 ng/dL. In the SH group, patients had a TSH level above 4.20 mIU/L, a free T3 level between 2.0-4.4 pg/mL, and a free T4 level between 0.93-1.7 ng/dL.

Distribution of Groups

OH group included 99 patients, while the SH group consisted of 104 patients. As the control group (CG), 122 healthy individuals with normal levels of anti-TPO, anti-Tg, TSH, free T4, and free T3 were included in the study. No euthyroid or hyperthyroid individuals were identified among the HT patients.

Laboratory Methods

Laboratory results for all participants, including TSH, T3, T4, C-reactive protein (CRP), sedimentation rate, anti-TPO, anti-Tg, NLR, MHR, cholesterol, triglycerides, HDL, and LDL, were recorded. NLR and MHR were calculated as the ratios of neutrophils to lymphocytes and monocytes to HDL, respectively. The obtained results were compared among the OH, SH, and CG.

Thyroid function tests and autoantibody measurements were performed using the electrochemiluminescence immunoassay method on Roche COBAS 8000 analyzers. HDL cholesterol, total cholesterol, and triglycerides were also analyzed on the same device using spectrophotometric biochemical reactions. LDL cholesterol values were calculated manually using the Friedewald formula (LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides /5).

Statistical Analysis

Statistical analyses were performed using SPSS for Mac, version 26. The normality of the distribution of variables was assessed using both visual methods (histograms and probability plots) and analytical tests (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics for normally distributed variables were presented as means and standard deviations. For normally distributed numerical data, comparisons between independent groups were made using one-way ANOVA with Bonferroni post-hoc analysis. For non-normally distributed data, the Mann-Whitney U test and Kruskal-Wallis test (with Mann-Whitney U post hoc analysis) were used. Spearman's correlation analysis was performed for non-normally distributed variables. Nominal variables were presented using cross-tabulations, and group comparisons were made using the chi-square test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 99 individuals with OH, 104 with SH, and 122 healthy individuals in the CG were included in the study. Among the participants, 67 (20.6%) were male and 258 (79.4%) were female. The mean age of the patients was 50.5±12.9 years in the OH group, 48.7±12.6 years in the SH group, and 47.1±14.7 years in the CG. No statistically significant difference in age was found between the groups (p: 0.175). When the patient and CG were evaluated in terms of gender, it was observed that female gender predominated in all groups, and this difference was statistically significant (p: 0.001) (Table 1).

Figures 1 and 2 present the distributions of NLR and MHR across the OH, SH, and CGs, respectively.

When the lipid profiles of the OH, SH, and CG groups were compared, total cholesterol and HDL levels were found to be significantly higher in both the OH and SH groups compared to the CG (p: 0.016, p: 0.011, respectively). However, no statistically significant difference was found between the OH and SH groups in terms of these parameters. When triglyceride and LDL cholesterol levels were evaluated, no significant difference was observed between the OH, SH, and CG groups, and their values remained at similar levels (p: 0.315, p: 0.234, respectively).

In the comparison of the patient groups with OH and SH and CG in terms of the NLR parameter, no statistically significant difference was found between the groups (p: 0.507). Similarly, the MHR parameter showed similar results across these three groups (p: 0.064). Anti-TPO and anti-Tg levels were found to be higher in the OH and SH groups than in the CG (p: 0.001 and p: 0.001, respectively). However, no statistically significant difference was found between the OH and SH groups in terms of these parameters. In the SH group with HT, a significant correlation was observed between NLR and MHR (Table 2), whereas no significant correlation was detected in the OH group (Table 3) (p: 0.018 and p: 0.719, respectively). Furthermore, when the relationships between antibodies and NLR and MHR were examined, no significant correlation was found between NLR and MHR in either patient group.

DISCUSSION

Certain parameters obtained from routine blood tests can provide valuable insights into disease prognosis and inflammatory processes[11]. This study aimed to evaluate the increased risk of CVD and cardiovascular and cerebrovascular disease which may result from enhanced oxidative stress and endothelial dysfunction associated with chronic inflammation in patients with HT, through the assessment of NLR and MHR parameters. By evaluating NLR and MHR together, the study sought to contribute to a better understanding of this pathophysiological process. When NLR and MHR parameters were compared among healthy control subjects and those in the OH and SH groups, no statistically significant differences were observed. However, a positive correlation between NLR and MHR (p: 0.018) was identified in the SH group, whereas no such correlation was found in the other groups. No significant correlation was found between thyroid autoantibodies and these parameters. Moreover, no significant correlation was observed between these parameters and other inflammatory markers such as erythrocyte sedimentation rate (ESR) and CRP. In the literature, parameters such as NLR and MHR have been reported to be associated with inflammatory diseases including cancer, lupus, psoriasis, and rheumatoid arthritis, and to be related to disease activity, inflammation severity, and prognosis. These findings indicate that inflammation plays an important role in various disease processes and suggest that these parameters may serve as potential biomarkers for

Table 1. Demographic and biochemical characteristics of the study participants

	Control group	Subclinical hypothyroidism	Overt hypothyroidism	p
Age (years)	47.1±14.7	48.7±12.6	50.5±12.9	0.175
Gender (female %)	74	92	92	0.001
TSH (mIU/L)	1.74±0.8	6.82±3.62	11.22±12.81	0.001
T3 (pg/mL)	3.10±0.48	2.84±0.47	2.49±0.51	0.001
T4 (ng/dL)	1.2±0.26	1.09±0.17	1.01±0.33	0.001
Anti-TPO (IU/dL)	11.04± 5.22	260.04±218.39	281.98±237.76	0.001
Anti-TG (IU/dL)	15.51±9.44	470.16±903.79	475.90±722.84	0.001
Cholesterol (mg/dL)	198.34± 46.61	214.2±45.17	212.17±44.28	0.016
HDL (mg/dL)	50.35±14.29	54.13±14.5	54.96±13.2	0.011
Triglyceride (mg/dL)	142.11±80.36	136.46±85.14	150.86±83.31	0.315
LDL (mg/dL)	122.11±60.36	116.08±44.93	135.46±43.31	0.234
Monocyte (10^3/uL)	0.48±0.18	0.45±0.15	0.48±0.21	0.274
MHR	10.53±5.17	8.95±4.25	9.34±5.83	0.064
Neutrophil (10^3/uL)	4.34±1.17	4.12±1.62	4.08±1.62	0.399
Lymphocyte (10^3/uL)	2.3±0.80	2.27±0.80	2.25±0.90	0.497
NLR	2.01±0.90	1.88±0.85	1.93±0.88	0.507
CRP (mg/L)	3.80±5.33	3.09±3.69	3.22±4.44	0.882
Sediment (mm/hour)	24.80±12.15	19.19±14.46	24.61±11.11	0.104

TSH: Thyroid stimulating hormone, T3: Triiyodothyronine, T4: Thyroxine, Anti-TPO: Antithyroid peroxidase, Anti-TG: Anti-thyroglobulin, HDL: High-density lipoprotein, MHR: Monocyte-high-density lipoprotein ratio, NLR: Neutrophil-lymphocyte ratio, CRP:C-reactive protein

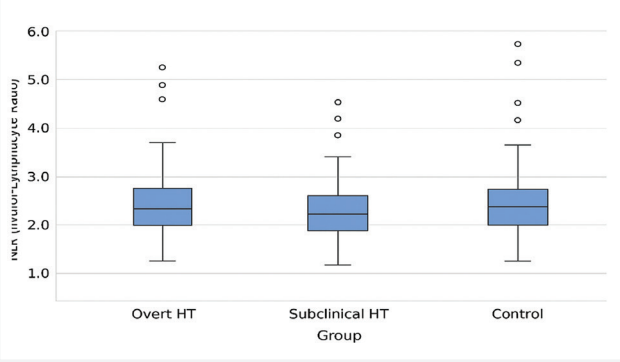


Figure 1. Representation of NLR between OH, SH and CG

NLR: Neutrophil-lymphocyte ratio, OH: Overt hypothyroidism, SH: Subclinical hypothyroidism, HT: Hashimoto's hyroiditis, CG: Control group

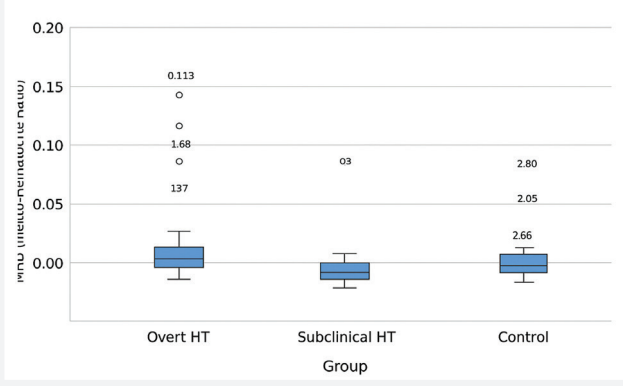


Figure 2: Representation of MHR between OH, SH and CG

MHR: Monocyte-high-density lipoprotein ratio, OH: Overt hypothyroidism, SH: Subclinical hypothyroidism, HT: Hashimoto's thyroiditis, CG: Control group

clinical assessment[7,8,12,13]. When previous studies examining the NLR parameter in HT patients are considered, Acay et al.[14] and Aksu et al.[15] reported, similar to our findings, that there was no statistically significant difference in NLR values between HT patients and CG (p: 0.23). Furthermore, consistent with our study, no significant relationship was found between autoantibodies and NLR1.

In contrast to our findings, Aktas et al.[16] reported that NLR levels were higher in patients with HT. The researchers suggested that NLR may serve as an indicator of inflammation, particularly in cases of complicated hypertension. Similarly to our study, Güneş et al.[17] divided HT patients into two groups as OH and SH.

Consistent with our findings, no statistically significant difference in NLR values was observed among the OH, SH, and CG. When reviewing previous studies, no research was found that specifically examined the relationship between MHR and disease status in patients with HT. Therefore, our study is among the first to investigate the association between the MHR parameter and HT. Examining studies that have explored the relationship between MHR and other diseases, Gembillo et al.[18] investigated the role of MHR in patients with resistant hypertension and found that MHR levels were higher in the resistant hypertension group. In addition, that study reported a positive correlation between MHR and CRP levels. In the study conducted by Demirbaş et al.[19] on patients with vitiligo, MHR values were found to be higher compared to the healthy CG, whereas the NLR parameter was found to be similar between the groups. Similarly, in the study by Yalçın et al.[20] on patients with ulcerative colitis, MHR, NLR, ESR, and CRP values were observed to be higher in the active disease group compared to the remission group.

The relationship between antibody titers and the degree of inflammation has gained importance as a current topic of research. In the literature, there are conflicting results regarding the association between antibody titers and the NLR and MHR parameters. For instance, in the studies by Aksu et al.[15] and Acay et al.[14] similar to our findings, no significant correlation was found between NLR and autoantibody levels. On the other hand, Keskin et al.[21] reported a positive correlation between autoantibodies and NLR; however, no relationship was identified between NLR and thyroid autoantibodies.

Table 2. Correlations of NLR and MHR in the SH group

	NLR		MHR	
	r	p	r	p
NLR	1	.	0.232	0.018
CRP	0.142	0.151	0.154	0.117
Sediment	0.03	0.763	0.177	0.072
Anti-TPO	0.102	0.301	-0.095	0.338
Anti-TG	0.082	0.407	0.065	0.514

SH: Subclinical hypothyroidism, MHR: Monocyte-high-density lipoprotein ratio, NLR: Neutrophil-lymphocyte ratio, CRP:C-reactive protein, Anti-TPO: Antithyroid peroxidase, Anti-TG: Anti-thyroglobulin

Table 3. Correlations of NLR and MHR in the OH group

		NLR		MHR
Parametre	r	p	r	p
NLR	1	.	0.037	0.719
CRP	0.081	0.426	0.161	0.112
Sediment	0.158	0.118	0.006	0.953
Anti-TPO	0.035	0.728	0.091	0.368
Anti-TG	-0.04	0.692	0.152	0.134

NLR: Neutrophil-lymphocyte ratio, MHR: Monocyte-high-density lipoprotein ratio, OH: Overt hypothyroidism, Anti-TPO: Antithyroid peroxidase, Anti-TG: Anti-thyroglobulin

The effects of hypothyroidism on serum cholesterol levels are well established. Previous studies have indicated that elevated TSH levels lead to an increase in serum cholesterol concentrations[22]. In our study, total cholesterol and HDL cholesterol levels were found to be significantly higher in both the OH and SH groups compared to the CG (p: 0.016 and p: 0.011, respectively). However, no statistically significant difference in these parameters was observed between the AH and SH groups. Regarding triglyceride and LDL levels, no statistically significant differences were detected among the AH, SH, and CG.

Study Limitations

The present study has several limitations. Since the data were retrospectively obtained from the hospital information system, it is possible that not all medications received by the patients were recorded in the database. This limitation may affect the completeness of the data and, consequently, the reliability of the results. To further evaluate inflammation in HT patients and to ensure the consistency and applicability of parameters such as NLR and MHR, prospective studies with larger patient populations are needed.

CONCLUSION

It is thought that in patients diagnosed with HT, concomitant hypothyroidism may lead to increased systemic inflammation, resulting in elevated endothelial dysfunction and oxidative stress. In this context, when NLR and MHR parameters were evaluated in hypertensive patients with OH and SH, these parameters were found to be similar among the groups. Although some studies have demonstrated an association between these parameters and atherosclerotic processes, further research and large-scale clinical studies are required to obtain more reliable and comprehensive results.

Ethics

Ethics Committee Approval: Approval for this study was obtained from the Ethics Committee of Tekirdağ Namık Kemal University, Faculty of Medicine, with the (protocol number: 2023.28.02.16, date: 28.02.2023).

Informed Consent: This is retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.Y., A.Ç., Concept: B.B., Design: B,B., Data Collection or Processing: D.Y., Analysis or Interpretation: D.Y., B,B., Literature Search: D.Y., Writing: D.Y.

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

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ORIGINAL ARTICLE

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Anatomy in the Clinical Years: A Document-based Comparative Mapping of National and International Curricula

Klinik Yıllarda Anatomi: Ulusal ve Uluslararası Müfredatların Belge Temelli Karşılaştırmalı Haritalanması

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ABSTRACT

Aim: This study aimed to systematically map and compare the existence, content, and pedagogical characteristics of clinical-year anatomy education in Türkiye and internationally, and to interpret the findings within the frameworks of vertical integration, cognitive integration, and experiential learning.

Materials and Methods: A document-based descriptive and comparative design was used. Official curricula, course catalogs, and publicly accessible syllabi of 54 medical schools (30 national, 24 international) were analyzed according to the curricular analysis and reporting in document analysis framework. Data were extracted on course title, phase (pre-clinical vs. clinical), instructional materials, and thematic content. Frequencies and thematic patterns were reported through inductive coding.

Results: Anatomy was universally present in pre-clinical years (100%), whereas clinical-year anatomy was identified in only 25% (6/24) of international schools and in none of the Turkish schools. Among 12 identified courses, 91.7% were in year 4, focusing mainly on regional/clinical (75%) and gross anatomy (66.7%). Cadaver dissection remained the dominant instructional material (58.3%), while radiological and microanatomy content was scarce.

Conclusion: Anatomy education shows strong early-phase intensity but a late-phase gap. The absence of structured anatomy sessions in the clinical years of Turkish curricula and the limited global implementation of vertical integration indicate a critical pedagogical deficiency. Re-establishing longitudinal anatomy threads through cadaver-based, radiology-integrated, and small-group approaches is essential to strengthen clinical reasoning, procedural safety, and professional readiness.

Keywords: Anatomy education, clinical years, vertical integration, learning

ÖZ

Amaç: Bu çalışma, Türkiye'de ve uluslararası düzeyde klinik yıl anatomi eğitiminin varlığını, içeriğini ve pedagojik özelliklerini sistematik olarak haritalandırmak ve bulguları dikey entegrasyon, bilişsel entegrasyon ve deneyimsel öğrenme çerçevelerinde yorumlamak amacıyla yapılmıştır.

Gereç ve Yöntem: Bu araştırmada belge temelli tanımlayıcı ve karşılaştırmalı bir tasarım kullanılmıştır. Elli dört tıp fakültesinin (30 ulusal, 24 uluslararası) resmi öğretim planları, ders katalogları ve çevrim içi erişilebilir müfredat dokümanları belge analizinde müfredat incelemesi ve raporlanması çerçevesine göre analiz edilmiştir. Ders adı, dönem (preklinik/klinik), öğretim materyalleri ve tematik içerik verileri çıkarılmış; frekanslar ve tematik örüntüler indüktif kodlama yoluyla raporlanmıştır.

Bulgular: Anatomi eğitimi preklinik yıllarda tüm kurumlarda (%100) yer alırken, klinik yıl anatomisi yalnızca uluslararası kurumların %25'inde (6/24) saptanmış; Türk kurumlarında ise hiçbirinde tanımlanmamıştır. Belirlenen 12 dersin %91,7'si 4. sınıfta yer almakta olup, içerikler ağırlıklı olarak bölgesel/klinik anatomi (%75) ve makro anatomi (%66,7) temalarına odaklanmaktadır. Kadavra diseksiyonu en yaygın öğretim materyalidir (%58,3); radyolojik ve mikroanatomi içerikleri oldukça sınırlıdır.

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Sonuç: Anatomi eğitimi erken dönemde yoğun, ileri klinik dönemde ise belirgin bir boşluk göstermektedir. Türkiye'de klinik yıllarda yapılandırılmış anatomi oturumlarının bulunmaması ve uluslararası düzeyde dikey entegrasyonun sınırlı biçimde uygulanması önemli bir pedagojik eksikliğe işaret etmektedir. Anatomi eğitiminin kadavra temelli, radyolojiyle entegre ve küçük grup odaklı yaklaşımlarla klinik yıllara uzanan süreğen bir müfredat bileşeni hâline getirilmesi, klinik akıl yürütme, girişimsel güvenlik ve mesleki hazırlığı güçlendirmek açısından gereklidir.

Anahtar Kelimeler: Anatomi eğitimi, klinik yıllar, dikey entegrasyon, öğrenme

INTRODUCTION

Anatomy provides the cognitive framework for clinical reasoning, procedural safety, and professional identity formation; however, over the past forty years, teaching hours, opportunities for cadaver dissection, and the number of teaching staff have declined in many countries, raising concerns that this situation has fallen below the threshold for patient safety[1,2]. The disruption of access to laboratories during the coronavirus disease 2019 pandemic, coupled with the rapid proliferation of remote and hybrid approaches, has made this vulnerability more apparent; dissection and face-to-face laboratory-based learning have quickly given way to online and visually-focused solutions[3,4]. The disruption of laboratory access during the pandemic limited opportunities for applied anatomy, particularly during clinical years, and this situation created a permanent learning gap that could not be fully recovered after the pandemic. However, national and regional surveys show that the time allocated to teaching anatomy in modern medical curricula, as well as the methods used, vary significantly across countries and institutions[5,6]. In contemporary medical education, the principle of vertical integration aims to integrate basic sciences with clinical sciences throughout the curriculum, embed fundamental content in a clinical context from an early stage, and maintain this relationship throughout the clinical years[7]. This approach requires design decisions at the session level, enabling the learner to establish cognitive integration between basic and clinical concepts in their minds, not just to organize the timeline[8]. The spiral curriculum concept supports this vertical continuity by suggesting that the same themes be revisited at increasing levels of complexity and that prior learning be reinforced through application and decision-making in clinical years[9]. Experiential learning, on the other hand, operates through the "experience–reflection–conceptualization-application" cycle using real or realistic tasks in a clinical context; anatomy laboratories and clinical anatomy sessions are among the most direct tools for this cycle[10].

In recent years, integrating clinical anatomy directly into clinical year rotations has yielded findings suggesting that it can enhance students' ability to recall information, build confidence, and improve contextual reasoning skills. Workshops and near-peer teaching models conducted during surgical rotations have facilitated upper-level students' transfer of anatomical knowledge acquired during the preclinical period to clinical scenarios; meaningful improvements in self-efficacy, orientation in the operating room, and confidence in structure identification have been reported[11-13]. The acceptability and perceived effectiveness of year-specific, focused anatomy workshops and vertical integration-based courses are high; it is recommended that this model be widely adopted to accompany internships[14,15]. This trend is consistent with findings that cadaver-based simulations enhance knowledge, skills, and confidence in the post-graduation stage and points to continuity between pre- and post-graduation[16].

In the Turkish context, the National Standards for Pre-Graduation Medical Education and the National Core Education Program explicitly define horizontal and vertical integration as a normative expectation; many faculties' self-assessment reports state that integration with basic sciences continues during the clinical period[17,18]. However, there is no systematic, comparative, and document-based mapping study at the national level regarding how anatomy content (scope, duration, pedagogical methods, assessment approach) is positioned among institutions during the clinical years. Internationally, there is also a heterogeneous picture, with strong anatomy teaching in the pre-clinical period that becomes increasingly sparse in the clinical period[5,6]. This gap is critical both for providing an evidence-based perspective on the local implementation of the principle of vertical integration and for developing policy and program recommendations for clinical anatomy designs that are consistent with the principles of cognitive integration and spiral curriculum[8,9].

The lack of a systematic national comparison of the position of the clinical year anatomy in the curriculum creates a significant decision-making gap for both policymakers and curriculum developers. This gap makes it difficult to compare how institutions plan clinical-year integration and prevents the development of an evidence-based perspective on where students' clinical competencies need support. Therefore, objectively documenting national trends in clinical year anatomy is critical to assessing the sustainability of vertical integration. This study aims to systematically compare the existence, content, teaching methods, and assessment approaches of clinical-year anatomy by examining the official curricula and publicly available documents of medical schools in Türkiye and international examples; to interpret the findings within the frameworks of vertical integration, cognitive integration, and experiential learning; and to discuss the alignment of the evidence obtained with national standards and global trends.

In this way, we aim to provide evidence-based, applicable, and contextual recommendations for the sustainable enhancement of anatomy education in clinical years[7,8,10].

MATERIALS AND METHODS

Study Design

This study is designed as a descriptive and comparative, document-based study to compare the anatomy education curricula of top-ranked medical schools in Türkiye and internationally. The systematic review of curriculum documents was chosen because it is conducive to revealing institutional curriculum structures and evidence of vertical integration within the context[19]. Further, the curricular analysis and reporting in document analysis (CARDA) framework has been adopted as a methodological framework that provides documentation review-specific reporting standards in healthcare professional education[20]. Elements of the CARDA framework were applied at multiple stages of the analysis. Curriculum structure and resource specification guided the identification and mapping of course characteristics, whereas content mapping and evidence integration informed the inductive thematic coding. The reporting transparency component of CARDA was used to structure the presentation of results and ensure traceability.

Setting and Sample

The international sample was created based on institutions listed in the QS World University Rankings by Subject 2024: Medicine[21]. The national sample was selected from the universe of "universities with medical faculties" in the URAP 2024-2025 Türkiye Ranking[22]. The inclusion criterion was that the curriculum/course catalog information on the official website of the relevant institutions be publicly accessible. In contrast, the exclusion criterion was the absence of a medical faculty or open access to the curriculum. During the selection process, two institutions without a medical faculty and four institutions without open access to the curriculum were excluded; 24 international and 30 national medical faculties were included in the final sample.

Data Collection and Coding

The curriculum pages, course catalogs, syllabi, and/or academic handbooks sections of each institution were systematically scanned. The search was conducted independently by three anatomists, all experts in the field, and any potential discrepancies were resolved through a consensus meeting. Any discrepancies between coders were discussed and resolved through consensus meetings among the three anatomists; inter-rater agreement was not formally quantified. During the scan, the keywords "anatomy," "dissection," and "foundation" were used in page-internal and site-internal searches; for each course, the variables class year (preclinical vs. clinical), course title, educational materials (e.g. cadaver dissection, laboratory materials, microscope, digital/visual resources, video content, specialty equipment), and integration approach were recorded on a standardized data extraction sheet. These keywords were selected to maintain consistency across institutions and because they are the most frequently used terms to denote anatomy-related content in course catalogs. Although broader terms might reveal additional activities, we prioritized a standardized and reproducible search strategy.

In this study, the distinction between the preclinical and clinical periods was made based on both the institutions' own curriculum classifications and the educational stages defined in the literature. In foreign universities, the definitions of "preclinical" and "clinical" specified in the course catalogs were accepted directly in line with the institution's own classification. This approach is consistent with the literature, indicating that the boundary between basic sciences and clinical sciences varies structurally across different education systems[23-25]. However, in Turkish medical schools, the distinction between pre-clinical and clinical is generally structured to coincide with the start of clinical internships. Therefore, in Turkish institutions, the distinction is based on the start of the clinical clerkship period; in international institutions, the institution's official classification serves as the basis.

Statistical Analysis

Document analysis was conducted in accordance with the CARDA checklist, covering the dimensions of curriculum structure, content mapping, integration of evidence, resource specification, and reporting transparency[20]. Within the scope of quantitative description, frequencies (n) and percentages (%) were reported. Inductive thematic analysis was applied to the content of the courses included in the clinical years[26]. During thematic coding, content themes (e.g. gross anatomy, radiological anatomy, regional anatomy, clinical anatomy, microanatomy, embryology) and materials/integration themes were considered together; a lesson may contain more than one material/theme. Since the study was conducted on publicly available documents, it does not involve human/animal subjects and does not require ethics committee approval.

RESULTS

Institutional Coverage and Selection Flow

The study included 24 international medical schools from the QS-Medicine 2024 ranking that met the selection criteria, and 30 national medical schools from the URAP 2024 ranking. In the international group, two institutions without a medical school and four institutions without open access to the curriculum were excluded. A total of 54 medical schools were analyzed.

The reasons for exclusion and the number of institutions are summarized in Table 1.

Distribution of Anatomy Education Across Phases

Anatomy education is available in both groups during the pre-clinical period (years 1-2). At least one introductory anatomy course is offered in 24/24 (100%) institutions in the international group and 30/30 (100%) institutions in the national group. This indicates that anatomy education is a mandatory curriculum component in both systems during the pre-clinical period (Table 2). In the clinical period (years 3-6), anatomy education was identified in 6 international institutions (25%); a total of 12 courses were included in the analysis. 1 course is at year 3 (8.3%), 11 courses are at year 4 (91.7%); there is no independent anatomy education at the year 5-6 levels. In the national group, no clinical anatomy course defined as independent or modular was identified in the clinical period. Taken together, these findings indicate that while anatomy is universally present in the pre-clinical years in both settings, only the international sample retains explicitly defined anatomy courses in the clinical phase.

Characteristics of Clinical-year Anatomy Courses

A total of 12 clinical-year anatomy courses have been identified. Most are offered in year 4. Institutions offering clinical-year anatomy education include: University of California, San Francisco (UCSF), University of British Columbia (UBC), Imperial College London, and University of Michigan. At UCL, the Advanced Anatomy course is conducted with cadaver and dissection plans, while at UCSF, three modules based on regional dissection (Head & Neck, Musculoskeletal, Thorax-Abdomen-Pelvis) have been defined. UBC offers courses in Human Anatomy, Microscopic Anatomy, Neuroanatomy, and Directed Studies in Anatomy. At the University of Michigan, Anatomy in Surgery and Head and Neck Teaching Elective courses are defined as pre-clinical preparation modules, while the Residency Preparatory Course is defined as a pre-specialty anatomy review. Imperial College London has defined a clinical anatomy module, but detailed information is not available.

The most frequently used teaching material in clinical year courses was cadaver dissection (7/12; 58.3%). Laboratory materials (3D models etc.) ranked second (3/12; 25%). Microscope, video content, and specialty equipment were each used in one course (1/12; 8.3%). Material information was not included in four courses. In cases where more than one material was used, cadavers and radiological sources were used together (Figure 1).

A summary of course types, instructional materials, content themes, and year levels across the identified clinical-year anatomy courses is presented in Table 3.

Thematic analysis identified six main content themes for the 12 courses in the clinical period: regional/clinical anatomy (n=9; 75%), gross anatomy (n=8; 66.7%), microanatomy (n=2; 16.7%), embryology (n=1; 8.3%), radiological anatomy (n=1; 8.3%), and unspecified (n=1; 8.3%). The distribution and proportional visualization of the themes are presented in Figure 2. In contrast, no comparable, explicitly structured clinical-year anatomy modules were identified in the national sample, highlighting a divergence in how anatomy is represented during the clinical phase across the two contexts.

As these observations are based on a limited number of clinical-year courses (n=12), the findings should be interpreted with appropriate caution.

DISCUSSION

This study shows that anatomy is universal in the pre-clinical period; however, when moving to the clinical-year phase, anatomy remains a module/elective in only 25% (6/24) of the

Table 1. Selection and inclusion of medical schools (QS 2024-URAP 2024)

Group	Initial number of institutions	Exclusion criteria	Final included (n)
International (QS 2024)	30	2 without medical schools; 4 lacking open-access curricula	**24**
National (URAP 2024)	30	—	**30**
Total	**60**	**6 excluded**	**54 included**

PRISMA-style summary of included and excluded institutions. Six schools were excluded (two without medical programs, four lacking open-access curricula)

Table 2. Distribution of anatomy education across pre-clinical and clinical phases

Group	Pre-clinical anatomy (years 1-2)	Clinical anatomy (years 3-6)	Number of clinical-year courses	Year-wise distribution
International	24/24 (100%)	6/24 (25%)	12	Year 3: 1 (8.3%) Year 4: 11 (91.7%) Year 5-6: 0
National	30/30 (100%)	0/30 (0%)	—	—

Presence of anatomy courses by training phase and academic year among international and national medical schools

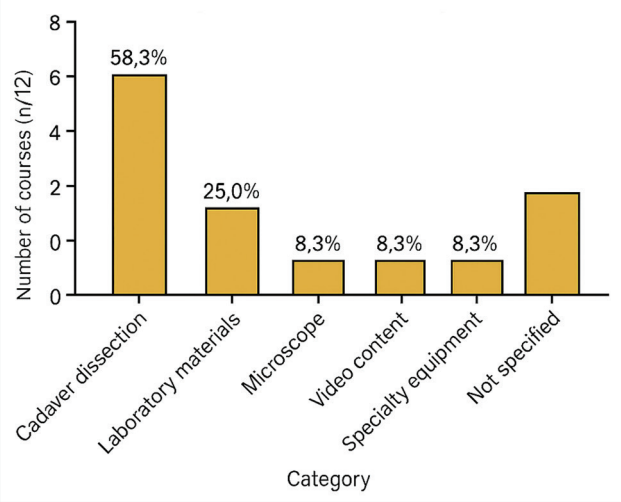


Figure 1. Distribution of instructional materials used in clinical-year anatomy courses (n=12)

Proportion (%) and frequency (n) of instructional materials reported across 12 clinical-year anatomy courses. Multiple materials may be used within a single course; therefore, totals exceed 12.

Cadaver dissection (7/12; 58.3%) was the most frequently used material, followed by laboratory materials (3/12; 25.0%). Microscope, video content, and specialty equipment were each reported in one course (8.3%), while four courses did not specify any materials

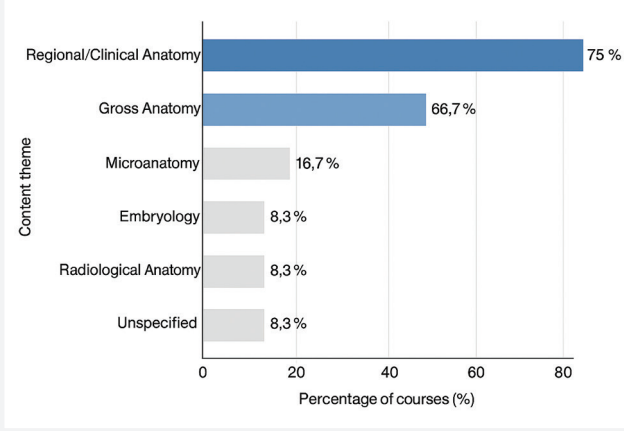


Figure 2. Thematic distribution of clinical-year anatomy courses (n=12)

Proportion (%) and frequency (n) of content themes identified through thematic analysis. regional/clinical and gross anatomy themes were the most frequent, followed by microanatomy, embryology, and radiological anatomy

During the preparation of this article, the authors used OpenAI's ChatGPT-5 tool to create figures. The figures were prepared with artificial intelligence support to enhance visual presentation and clarity; they were manually reviewed, edited, and approved by the authors for scientific accuracy and consistency with the study data. The authors assume full responsibility for all visual materials included in the article

Table 3. Summary of course types, instructional materials, content themes, and year levels identified across 12 clinical-year anatomy courses

Variable	Categories	Frequency (n=12)	Percentage
Course type	Elective	7	58.3%
	Module (regional dissection-based)	3	25.0%
	Preparation module (review/pre-surgical)	2	16.7%
Teaching materials	Cadaver dissection	7	58.3%
	Laboratory materials/3D models	3	25.0%
	Microscope	1	8.3%
	Digital/video-assisted materials	1	8.3%
Content themes	Regional/clinical anatomy	9	75.0%
	Gross anatomy	8	66.7%
	Microanatomy	2	16.7%
	Embryology	1	8.3%
	Radiologic anatomy	1	8.3%
Year level	Year 3	1	8.3%
	Year 4	11	91.7%

This table provides a descriptive overview of the instructional characteristics identified across all 12 clinical-year anatomy courses in the international sample. "Elective" refers to student-selected, non-mandatory anatomy courses offered during clinical training. "Module (regional dissection-based)" denotes structured, region-specific anatomy sessions conducted through cadaveric dissection, typically organized as part of a series (e.g., head and neck, musculoskeletal, thorax/abdomen/pelvis). "Preparation module" includes pre-surgical or residency preparation courses designed to reinforce anatomical knowledge prior to clinical specialization. Instructional materials were coded based on primary modalities explicitly stated in curriculum documents. Content themes reflect dominant anatomical domains emphasized within each course. Year level indicates the academic stage at which courses are offered, with the majority occurring in year 4 and a small number in year 3.

international sample, and in Türkiye, none of the institutions examined had a clearly defined clinical-year anatomy. Of the 12 courses offered, 91.7% were concentrated in year 4; no courses were reported in years 5/6. Content centers on regional/clinical anatomy and gross anatomy, while radiological anatomy and embryology are extremely rare; at the methodological level, cadaver dissection remains the dominant modality. This picture indicates that, despite claims of vertical integration, anatomy has failed to become a systematic longitudinal thread in the clinical phase. Over the past 20-30 years, global reports have shown a decrease in anatomy hours and a decline in the intensity of dissection/laboratory work, while integration has been designed more in the early stages. Our findings confirm this long-term trend as a gap that becomes apparent in the clinical phase[27].

In our study, clinical-year anatomy content was identified in only 25% of international institutions; the majority of these offered it as an elective in year 4. In Türkiye, however, no independent anatomy course or laboratory-based review has been defined for the clinical phase. These findings are consistent with international observations that anatomy teaching is concentrated in the early years and extends only to a limited extent into the clinical phase. Indeed, in a national survey of 39 medical schools conducted by Smith et al.[6] in the United Kingdom-Ireland region, it was reported that gross anatomy education is primarily delivered in years 1-2, with only 37% of institutions extending it beyond two years. This data indicates that the time allocated to anatomy in the clinical phase is limited and that integration is confined to the pre-clinical stage. Therefore, when our document-based analysis is evaluated alongside Smith et al.[6] empirical findings, it reveals that anatomy education follows an "early intensity - late gap" pattern at the national-international level. This situation shows that, although the principle of vertical integration is theoretically accepted, it cannot be practically transferred to the clinical phase. This situation demonstrates that, despite theoretical acceptance of the principle of vertical integration, it has not been adequately translated into practice in the clinical setting. This "early intensity-late gap" pattern suggests that it may be beneficial to consciously support clinical-year curricula with longitudinal anatomy touchpoints; thus, fundamental anatomy knowledge can be reinforced during clinical practice periods, when students use anatomical reasoning most intensively. In practice, this approach could be implemented by incorporating short anatomy refresher sessions into clinical rotations, utilizing radiology-integrated sessions, or applying targeted anatomy reviews prior to surgery.

The regional variation observed across institutions may stem from several structural and contextual factors. Differences in faculty staffing, curriculum governance, resource availability, and institutional priorities shape how and when anatomy is integrated into clinical training. In some settings, the dominance of discipline-based curricula limits opportunities for longitudinal anatomy reinforcement, whereas schools with stronger infrastructure or surgical teaching traditions more readily maintain clinical anatomy exposure. Cultural expectations regarding the role of dissection and hands-on anatomy also contribute to cross-regional variation. These findings align with Barut et al.[28], who demonstrated that regional and institutional contexts substantially influence approaches to anatomy learning, underscoring the need for context-sensitive and longitudinally structured educational designs.

It has been emphasized that vertical integration is a design that requires establishing cognitive bridges at the session level, not just on the timeline[8]. In contrast, cognitive integration is achieved by establishing causal links between basic and clinical concepts in the learner's mind. In light of our findings, these bridges may not be sustainable at the institutional level due to the scarcity of defined anatomy threads in clinical years.

In the Turkish context, the absence of clearly defined clinical-year anatomy courses may also reflect structural differences in how internships are organized and how clinical teaching responsibilities are distributed across departments. Clinical rotations often prioritize service-based learning, which may limit opportunities for structured anatomy reinforcement unless intentionally embedded. Moreover, institutional variability in faculty capacity, access to laboratory resources, and the presence of dedicated clinical anatomy units contributes to inconsistent integration practices across medical schools. Recent evidence further indicates that well-designed and structured anatomy modules can significantly enhance learner engagement and perceived educational value[29]. These findings underscore the importance of context-sensitive and deliberately structured approaches to sustain anatomy learning during the clinical years.

During the pandemic, access to cadaver dissection sessions was temporarily suspended, and many institutions turned to digital and hybrid materials.

However, when laboratories reopened after the pandemic, it was reported that these digital alternatives became permanent, and dissection-based hours did not return to their previous levels[3,30,31]. Therefore, the lack of a laboratory-based anatomy comeback in the clinical phase may be consistent with this global restructuring trend. On the other hand, radiology-integrated small group sessions, which are evidence-based bridging modalities, have been shown to improve anatomy performance, and point-of-care ultrasound (POCUS) integration has been shown to enhance anatomy comprehension and short-term learning outcomes[32]. In our study, radiological anatomy was identified in only 1/12 sessions, and no systematic

evidence was found for POCUS integration, suggesting an evidence-practice gap. Our findings reveal that anatomy is not structured into the curricula of national and international medical schools, particularly during the clinical years. In contrast, a recent literature analysis shows that systematic clinical anatomy teaching practices can increase student success and interest[33].

The relegation of anatomy sessions to the background during clinical years may indicate not only a local curriculum preference but also a systemic deficiency in instructional design and resource allocation.

While Walker et al.[33] reports the positive effects of systematically presented clinical anatomy sessions, our findings show that such practices are absent or very limited in Türkiye and in the institutions included in the study.

This situation suggests that pedagogical frameworks such as vertical integration, cognitive integration, and experiential learning are not being effectively implemented.

This deficiency can be considered risky, especially in the context of clinical internships, interventional practices, and patient care preparation, as the literature emphasizes the relationship between anatomical knowledge and clinical decision-making and practice safety[34].

Therefore, it is critically important for medical education programs to reposition the "representation of anatomy in the clinic" in their core curricula in order to enhance the clinical preparedness capacity of the programs and reinforce the safe practice skills of graduates.

Study Limitations

The strongest aspect of this study is its use of a multi-centered and official document-based analysis design. Curriculum data were systematically examined using the CARDA framework, ensuring standardized, transparent, and traceable data extraction and reporting processes. Using the same methodological framework for both national and international examples enhanced the comparability of findings. However, the study has some limitations. First, hidden or informal curriculum elements were outside the scope of this research. Pre-internship briefings, brief in-service clinical reminders, or case-linked anatomy presentations are often not listed in official course catalogs. They may therefore not have been counted toward the clinical-year anatomy course. Second, the heterogeneity of course titles and classifications across institutions may have led to misclassification; clinically relevant anatomy sessions such as radiology-integrated or surgical anatomy workshops may not have been captured if they were not explicitly labeled under "anatomy," creating a potential risk of false negatives. In addition, elective anatomy courses are often listed inconsistently or embedded within broader modules on institutional websites, which may result in certain anatomy offerings remaining partially or completely invisible during document extraction. Third, as the study was based solely on publicly available documents, student or graduate outcomes (e.g. objective structured clinical examination performance, error rates, clinical safety indicators) could not be evaluated. Additionally, unavailable or incompletely reported documents represent another limitation. Since not all institutions share their curriculum data publicly and in an up-to-date manner, access to some sources may have been unavailable, or content may not have been presented with sufficient clarity. The lack of direct communication with institutions and the absence of verification constitute a structural limitation of this research, inherent to the nature of document analysis. Differences in how institutions define the boundary between pre-clinical and clinical phases may influence cross-country comparability; therefore, findings should be interpreted with this structural heterogeneity in mind. As this study relied solely on publicly available curriculum documents, some interpretations necessarily reflect document-based inferences rather than direct observation, and should therefore be understood within the limits of this methodological approach.

CONCLUSION

The findings of this study show that anatomy education in clinical years remains weak in terms of continuity and content at the international level, while structured anatomy sessions are almost non-existent in the clinical phase in Türkiye. When comparing examples from Türkiye and the rest of the world, it is clear that the principles of vertical integration and cognitive integration are not sufficiently implemented at the clinical year level. The placement of anatomy sessions primarily as electives in year 4 and their absence in years 5/6 falls short of providing the depth expected at times when anatomy is critical for clinical practice and procedural safety. In this context, it is recommended that medical school programs carry a longitudinal anatomy thread into the clinical years, incorporating both laboratory-based (e.g. cadaver/prosection) sessions and radiology-integrated, POCUS-assisted, and small group approaches. Such a restructuring could transform anatomy education from being merely an early-stage requirement into a strong foundation for clinical decision-making, safe procedures, and professional skills ready for post-graduation practice.

Ethics

Ethics Committee Approval: This study was conducted using publicly available institutional documents and did not involve human or animal participants; therefore, ethics committee approval was not required.

Informed Consent: The study was conducted using publicly available documents, it does not involve human or animal subjects.

Footnotes

Presentation: This study was presented as an oral communication at the 12th Anatomy Winter Days congress (abstract no: 9491796).

Authorship Contributions

Concept: F.T.K., Design: F.T.K., M.Y., Data Collection or Processing: F.T.K., B.K., F.O., M.Y., Analysis or Interpretation: F.T.K., B.K., F.O., M.Y., Literature Search: F.T.K., B.K., F.O., M.Y., Writing: F.T.K.

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

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ORIGINAL ARTICLE

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Do Inflammatory Indices Predict Neoadjuvant Chemotherapy Response in Hormone Receptor-positive, HER2-negative Breast Cancer?

Enflamatuvar İndeksler Hormon Reseptörü Pozitif HER2-negatif Meme Kanserinde Neoadjuvan Kemoterapi Yanıtını Predikte Eder Mi?

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ABSTRACT

Aim: The aim of this study was to evaluate clinical, pathological, and inflammatory markers that can predict pathological complete response (pCR) after neoadjuvant chemotherapy (NACT) in patients with hormone receptor-positive (HR+)/human epidermal growth factor receptor 2 (HER2) HER2- breast cancer.

Materials and Methods: A retrospective analysis was conducted on 109 patients aged 18-80 years with HR+/HER2- breast cancer who underwent NACT at University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital between January 1, 2020, and May 1, 2025. All patients received standard anthracycline-taxane-based chemotherapy. Pre-treatment blood tests were used to calculate the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII). pCR was defined as the absence of residual invasive tumor in the breast and axillary lymph nodes. Factors affecting pCR were evaluated using logistic regression analysis.

Results: pCR was achieved in 21.1% of patients. Most of the pCR group was under 50 years of age and had the luminal B subtype. ROC analysis showed that NLR, PLR, and SII were not significant predictors of pCR ($p>0.05$). In univariate analysis, luminal B subtype, low estrogen receptor (ER) levels, high Ki-67, and high tumor grade were significantly associated with pCR. In multivariate analysis, only low ER levels and high Ki-67 remained independent predictors.

Conclusion: Inflammatory markers are insufficient to predict pCR in HR+/HER2- breast cancer. Low ER levels and high Ki-67 are associated with a better response to NACT. The findings highlight the importance of biomarkers in personalizing treatment response.

Keywords: Breast cancer, neoadjuvant chemotherapy, pathological complete response, inflammatory markers, Ki-67, estrogen receptor

ÖZ

Amaç: Bu çalışmanın amacı, hormon reseptörü pozitif (HR+)/insan epidermal büyüme faktörü reseptörü 2 (HER2) HER2- meme kanseri olan hastalarda neoadjuvan kemoterapi (NACT) sonrası patolojik tam yanıtı (pCR) öngörebilen klinik, patolojik ve enflamatuvar belirteçleri değerlendirmektir.

Gereç ve Yöntem: 1 Ocak 2020 ile 1 Mayıs 2025 tarihleri arasında Sağlık Bilimleri Üniversitesi, Balıkesir Atatürk Şehir Hastanesi'nde NACT uygulanan 18-80 yaş arası HR+/HER2- meme kanseri olan 109 hasta üzerinde retrospektif bir analiz yürütülmüştür. Tüm hastalar standart antrasiklin-taksan bazlı kemoterapi almıştır. Tedavi öncesi kan testleri kullanılarak nötrofil-lenfosit oranı (NLR), trombosit-lenfosit oranı (PLR) ve sistemik immün-enflamasyon indeksi (SII) hesaplanmıştır. pCR, meme ve aksiller lenf düğümlerinde rezidüel invaziv tümör bulunmaması olarak tanımlanmıştır. pCR'yi etkileyen faktörler lojistik regresyon analizi kullanılarak değerlendirilmiştir.

Bulgular: Hastaların %21,1'inde pCR elde edildi. pCR grubunun çoğu 50 yaşın altındaydı ve luminal B alt tipine sahipti. ROC analizi, NLR, PLR ve SII'nin pCR'nin anlamlı öngörücüleri olmadığını gösterdi ($p>0,05$). Tek değişkenli analizde, luminal B alt tipi, düşük östrojen reseptörü (ER) seviyeleri,

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yüksek Ki-67 ve yüksek tümör derecesi pCR ile anlamlı şekilde ilişkiliydi. Çok değişkenli analizde, yalnızca düşük ER seviyeleri ve yüksek Ki-67 bağımsız öngörücüler olarak kaldı.

Sonuç: Enflamatuvar belirteçler, HR+/HER2- meme kanserinde pCR'yi öngörmede yetersizdir. Düşük ER seviyeleri ve yüksek Ki-67, NACT daha iyi yanıtla ilişkilidir. Bulgular, tedavi yanıtının kişiselleştirilmesinde biyobelirteçlerin önemini vurgulamaktadır.

Anahtar Kelimeler: Meme kanseri, neoadjuvan kemoterapi, patolojik tam yanıt, enflamatuvar belirteçler, Ki-67, östrojen reseptörü

INTRODUCTION

Breast cancer (BC) is the most common type of cancer found in women[1]. More than 90% of BCs are confined to the locoregional area at the time of diagnosis, allowing for curative treatment[2]. One such approach, neoadjuvant therapy, involves administering systemic therapy before primary surgery and has made significant advances in recent years[3]. This strategy aims to reduce tumor size, enabling less invasive surgical procedures and improving patient outcomes[3]. Despite this, approximately 20% of patients experience recurrence after initial treatment[2]. Prediction of patients at risk of recurrence is primarily based on clinicopathological risk factors such as receptor status, tumor size, and nodal status[2]. Studies have shown that patients who achieve a pathological complete response (pCR) after neoadjuvant therapy have better long-term outcomes[4].

Breast tumors behave differently depending on the biological properties of their originating cells[5]. The most commonly used clinical markers for tumor biology classification are estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)[5]. The commonly accepted molecular subsets include luminal A [ER+/PR+/HER2-, low proliferation]; luminal B (ER+, low PR, HER2-, elevated proliferation); HER2+) (ER+ and ER- comprise unique HER2 subsets); and basal-like (commonly ER-/PR-/HER2-, triple-negative BC)[6]. In general, hormone receptor negative (HR-) (ER and PR negative) or HER2+ tumors are sensitive to chemotherapy and respond well to neoadjuvant chemotherapy (NACT)[4]. However, the indication for NACT in HR-positive (HR+)/HER2- tumors is controversial[7]. Although NACT is a treatment option for early-stage HR+/HER2- BC, pathological response rates are generally lower in this subtype[8]. pCR rates range from 0% to 18%, while breast-conserving surgery (BCS) can be achieved in up to 60% of tumors[7].

BC treatment is heterogeneous, and understanding who benefits most from specific treatments is vital[9]. Molecular methods such as prosigna gene testing on biopsy samples can influence neoadjuvant treatment decision-making in early-stage HR+/HER2- BC[10]. BC gene mutation status can help personalize treatment decisions[9]. Unfortunately, there are currently no completely reliable predictors[11]. The medical need for reliable biomarkers to predict response to NACT has not been met[12].

Persistent subclinical inflammation is closely associated with cancer development and progression, and neutrophils, platelets, and lymphocytes are key mediators of chronic inflammation[13]. Systemic inflammatory markers, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), have been identified as potential prognostic and predictive indicators in various malignancies, including BC[14]. Studies in the literature support the hypothesis that NLR, PLR, and SII may serve as predictive biomarkers for pCR in BC patients receiving NACT[15]. However, there are still inconsistent research results regarding the value of peripheral blood inflammatory markers in assessing the efficacy of neoadjuvant therapy in BC[16].

The aim of this retrospective study was to evaluate factors, including inflammatory markers, that may predict pCR after NACT in HR+/HER2- BC patients.

MATERIALS AND METHODS

Selection and Description of Cases

In our study, we retrospectively analyzed the data of HR+/HER2- BC patients aged 18-80 who underwent NACT at University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital between January 1, 2020, and May 1, 2025. Patients who received standard chemotherapy regimens (4 cycles of 600 mg/m^2 cyclophosphamide + 60 mg/m^2 doxorubicin every 2 weeks, followed by either 4 cycles of 175 mg/m^2 paclitaxel every 2 weeks or 12 cycles of 80 mg/m^2 paclitaxel weekly) were included in the study. Patients with clinical node positivity before treatment were included in the study. Patients with metastatic initial status, male gender, a second primary cancer other than BC, and those who received chemotherapy regimens other than those considered standard were excluded from the study. Patients with diseases that could affect blood results (rheumatological disease, chronic renal failure, liver cirrhosis, infection) and those taking medications (steroids, immunosuppressive therapy, chemotherapy) were excluded from the study.

The protocol for this retrospective study was approved Ethics Committee of University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital (decision number: 2025/07/67, date: 24.07.2025). Since this study was a retrospective archive search, informed consent was not obtained from the patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Technical Information

Immunohistochemistry (IHC) for ER, PR, and HER2, and double-probe fluorescence in situ hybridization (FISH) for HER2 were evaluated according to the 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines using Food and Drug Administration-approved tests[17,18]. ER and PR positivity was defined as at least 1% nuclear staining in tumor cells[17,18]. HER2 IHC was scored as positive (3+), equivocal (2+ or 1+ to 2+), or negative (0 or 1+) according to the 2018 ASCO/CAP guidelines[17,19]. All HER2 IHC equivocal (2+ or 1+ to 2+) cases underwent reflex HER2 dual-probe FISH testing (HER2 IQFISH pharmDx; DAKO). A positive HER2 FISH result was defined as a HER2/CEP17 ratio ≥2.0 or a mean HER2 copy number ≥6.0 signals per cell[17,19] . In our study and the 2021 St. Gallen update, ER-positive cancers are sometimes classified as "luminal A-like" (lower grade, lower Ki-67, strong ER/PR expression) or "luminal B-like" (higher grade, higher Ki-67, lower ER/PR expression levels)[20].

pCR was defined as no residual invasive carcinoma (ypT0/is ypN0) in the breast and axillary lymph nodes after surgical resection[17]. Patients with tumor and nodal pathologic stages of all other stages, including T1mi and N1mi, were considered the non-pCR group. Microinvasive BC was defined as an invasive component not exceeding 1 mm, most often in the setting of ductal carcinoma in situ[21]. Foci measuring ≤2 mm in the lymph node were also considered micrometastases[22]. Clinical and pathological tumor staging was performed based on the 8th edition of the International Union on Cancer TNM classification.

In our study, NLR, PLR, and SII were calculated according to the following equations: NLR = neutrophil count/lymphocyte count; PLR = platelet count/lymphocyte count; SII = (neutrophil count × platelet count)/lymphocyte count[23]. Laboratory examinations, including routine blood tests, for which data were recorded, were selected from examinations performed within the last month before the start of NACT.

Neoadjuvant treatment decisions were made in accordance with international guidelines and based on tumor biology, clinical stage, and patient-specific characteristics. All cases were evaluated in a multidisciplinary tumor board including medical oncologists, breast surgeons, radiologists, and pathologists, and treatment plans were finalized by consensus. Clinical staging was performed using the 8th edition of the American Joint Committee on Cancer staging system[24]. Diagnostic evaluation included physical examination, breast and axillary ultrasonography, and mammography, with breast MRI performed when indicated. To exclude distant metastasis, additional imaging such as chest computed tomography (CT), abdominal ultrasonography/CT, and bone scintigraphy or positron emission tomography-CT was used according to guideline-based indications.

Statistical Analysis

Statistical analyses and data processing were performed using the SPSS Statistics software, version 24 (SPSS Inc., Chicago, IL) and the R programming language (R Core Team, 2024). The ideal cutoff value for the logarithmic formula in distinguishing pCR from non-pCR, as well as specificity and sensitivity values, were determined using ROC analysis. Factors predicting pCR were calculated using univariate and multivariate analyses and binary logistic regression. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported, and a p value of <0.05 was considered statistically significant.

RESULTS

A total of 109 patients were included in the study. pCR was achieved in 23 patients (21.1%). Among patients who achieved pCR, 14 patients (60.9%) were under 50 years of age. The majority of this group had the luminal B subtype (20 patients, 87.0%). The Ki-67 proliferation index was above 20% in 20 patients (87.0%), and 9 patients (39.1%) had grade 3 tumors. Histologically, ductal type carcinoma was observed in 21 patients (91.3%). PR levels were low (<20%) in 8 patients (34.8%) (Table 1).

ROC curve analysis was conducted to evaluate the performance of inflammatory markers (NLR, PLR, and SII) in predicting pCR. The area under the curve for PLR was 0.451 (95% CI: 0.310-0.591; p=0.469), for NLR 0.595 (95% CI: 0.465-0.726; p=0.162), and for SII 0.583 (95% CI: 0.460-0.706; p=0.223). These results indicated that NLR, PLR, and SII do not exhibit adequate diagnostic value for predicting pCR; therefore, an optimal cut-off value could not be established (Table 2, Figure 1).

Logistic regression analysis was performed to identify predictors of pCR in HR+/HER2- BC. In univariate analysis, the luminal B subtype demonstrated approximately a five-fold higher odds of achieving pCR compared with luminal A (OR: 5.03; 95% CI: 1.39-18.22; p=0.014). A significant negative association was found between ER level and pCR, indicating that higher ER levels reduced the likelihood of achieving pCR (OR: 0.97; 95% CI: 0.96-0.99; p=0.004). Additionally, patients with a high Ki-67 index had significantly increased odds of achieving pCR (OR: 1.05; 95% CI: 1.01-1.09; p=0.009). Higher tumor grade also increased the likelihood of pCR (OR: 3.04; 95% CI: 1.11-8.32; p=0.030) (Table 3).

In multivariate analysis, only ER level (OR: 0.97; 95% CI: 0.96-0.99; p=0.009) and Ki-67 (OR: 1.05; 95% CI: 1.01-1.09; p=0.024) remained independent predictors of pCR. Molecular subtype, tumor grade, histological type, menopausal status, body mass index, inflammatory indices (NLR, PLR, SII), and clinical T stage were not significant in the multivariate model (Table 3). As all VIF values were <5, no multicollinearity was detected in the regression model (Table 3).

Table 1. Clinical and pathological characteristics of patients who achieved and did not achieve a complete pathological response after neoadjuvant chemotherapy

	Number of patients (n=109)	%	Non-PCR (n=86)	Non-PCR %	PCR (n=23)	PCR %
Age						
<50	47	43.1	33	38.4	14	60.9
≥50	62	56.9	53	61.6	9	39.1
Molecular subtype						
Luminal A	40	36.6	37	43.0	3	13.0
Luminal B*	69	63.3	49	57.0	20	87.0
Histology						
Ductal	89	81.7	68	79.1	21	91.3
Others	20	18.3	18	20.9	2	8.7
PR						
<20	32	29.4	24	27.9	8	34.8
≥20	77	70.6	62	72.1	15	65.2
Ki-67						
<20	40	36.6	37	43.0	3	13.0
≥20	69	63.3	49	57.0	20	87.0
Grade						
Grade 1-2	85	78.0	71	82.6	14	60.9
Grade 3	24	22.0	15	17.4	9	39.1
Clinical T stage						
T1	30	27.5	24	27.9	6	26.1
T2-T3	79	72.5	62	72.1	17	73.9
Menopause status						
Pre-peri menopausal	41	37.6	30	34.9	11	47.8
Postmenopausal	68	62.4	56	65.1	12	52.2
BMI						
<25	41	37.6	38	44.2	13	56.5
≥25	68	62.4	48	55.8	10	43.5
NLR (median)						
<3.3	53	48.6	45	52.3	8	34.8
≥3.3	56	51.4	41	47.7	15	65.2
PLR (median)						
<374	54	49.5	40	46.5	14	60.9
≥374	55	50.5	46	53.5	9	39.1
SII (median)						
<936	54	49.5	44	51.2	10	43.5
≥936	55	50.5	42	48.8	13	56.5

*:Ki-67 was determined as 20% for luminal A-B distinction. Cut-offs derived from literature, not ROC in this cohort

PCR: Pathological complete response, PR: Progesterone receptor, BMI: Body mass index NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index

Table 2. ROC-curve analysis to determine cut-off for factors predicting pathological complete response

Test result variable (s)	Area under the curve	Area under the curve at 95% confidence interval		
		Lower limit	Upper limit	p
PLR	0.451	0.310	0.591	0.469
NLR	0.595	0.465	0.726	0.162
SII	0.583	0.460	0.706	0.223

ROC: Receiver operating characteristic, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic immune-inflammation index

Table 3. Univariate and multivariate logistic regression analysis of clinical and pathological predictors of pathological complete response after neoadjuvant chemotherapy in HR-positive/HER2-negative breast cancer patients

Variable	Category	Univariate analysis		Multivariate analysis		
		OR (95% CI)	p	OR (95% CI)	p	VIF
Age	<50/≥50	0.40 (0.16-1.03)	0.057	-		
Molecular subtype	Luminal A/luminal B	5.03 (1.39-18.22)	**0.014**	2.37 (0.48-11.80)	0.293	1.058
Histology	Ductal/others	0.36 (0.08-1.68)	0.193			
ER (%)	Continuous	0.97 (0.96-0.99)	**0.004**	0.97 (0.96-0.99)	**0.009**	**1.051**
PR (%)	Continuous	1.00 (0.98-1.01)	0.606	-		
Ki-67 (%)	Continuous	1.05 (1.01-1.09)	**0.009**	1.05 (1.01-1.09)	**0.024**	**1.106**
Grade	1-2/3	3.04 (1.11-8.32)	**0.030**	1.66 (0.54-5.16)	0.378	1.069
Clinical T stage	T1-2 /T3	1.10 (0.39-3.11)	0.862	-		
Menopause status	Pre/peri/post	0.61 (0.24-1.54)	0.295	-		
BMI	<25/≥25	0.58 (0.23-1.48)	0.258	-		
NLR	Continuous	1.00 (0.91-1.09)	0.959	-		

HR: Hormone receptor, HER2: Human epidermal growth factor receptor 2, OR: Odds ratio, CI: Confidence interval, ER: Estrogen receptor, PR: Progesterone receptor, BMI: Body mass index, NLR: Neutrophil-to-lymphocyte ratio,

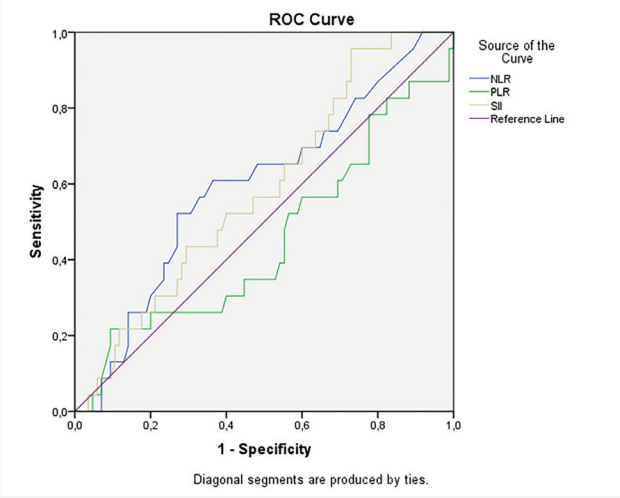


Figure 1. ROC-curve analysis for cutoff predicting complete response for NLR, PLR, and SII

NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, SII: Systemic inflammation index

DISCUSSION

This study investigated the factors predicting pCR after NACT in patients with HR+ BC. Univariate analysis revealed increased pCR in patients with luminal B, low ER, high Ki-67, and high-grade tumors. Multivariate analysis identified ER level and Ki-67 as independent predictors of pCR. NLR, PLR, and SII were found to have insufficient diagnostic value in predicting pCR.

pCR rates after NACT are lower in HR+/HER2- BC compared to other subtypes (7-16% in HR+/HER2- BC; 26-43% in HR+/HER2+ BC; 39-55% in triple-negative BC; and 46-90.5% in HER2+ BC)[25].

The 21.1% pCR achieved in our study may be attributable to the selection of patients who were relatively more suitable for NACT, with 63.3% having luminal B disease, 77.1% having Ki-67 ≥14, and 72.5% having cT2-cT3. The importance of pCR lies in the fact that large clinical trials of NACT have demonstrated improved disease-free survival (DFS) and overall survival (OS) rates in patients with pCR compared to those with residual disease[26]. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-18, patients achieving pCR had a DFS and OS of 75% and 85%, respectively, at a median follow-up of 9 years, compared to 58% and 73% in patients with residual disease[26].

It has been reported that luminal tumors with high Ki-67 expression respond well to chemotherapy, likely reflecting their high proliferative activity[27]. In a study by Aktas et al.[28] the Ki-67 level was identified as a guide for personalized treatment. Our study results are consistent with the literature in this regard. However, it should be noted that the European Society for Medical Oncology states that there is no consensus on a definitive cut-off value for Ki-67, but that values less than 10% are considered low and values greater than 30% are considered high[29]. Although stage, menopausal status, age, Ki-67, grade, HER2 expression, HR status, and even multiple gene expression analyses are used to assess whether neoadjuvant therapies will benefit a patient, identifying patients who will respond to NACT remains a challenging decision[30].

The recommendation that NACT can be used instead of adjuvant chemotherapy in HR+/HER2- BC is based on data from the NSABP B-18 study, which showed no difference in DFS or OS between adjuvant and neoadjuvant treatment in patients with stage II-III BC[31]. Factors guiding the decision-making process for adjuvant systemic therapy (e.g., lymph node status, tumor

grade, and comorbidities) can also be used to select patients with HR+/HER2- BC for whom NACT is appropriate, although it should be recognized that some factors, such as lymph node status, may be better assessed following definitive surgery[31]. The benefit of NACT in luminal subtype BC include performing BCS; however, whether neoadjuvant therapy improves prognosis in luminal subtype BC is controversial[32].

Yang et al.[13] reported that pretreatment NLR, PLR, and SII predicted pCR in BC patients undergoing NACT. Cullinane et al.[33] reported in their meta-analysis that NLR was a predictor of pCR in BC patients. A study by Kaytaz Tekyol et al.[34] demonstrated that low PLR levels were associated with higher chemotherapy sensitivity in NACT-treated BC, independent of molecular subtypes, but no association was found between NLR levels and pCR. A study by Eryilmaz et al.[35] also found no association between pCR and pretreatment NLR values. In the study by Hu et al.[36] lower pretreatment PLR was associated with higher pCR rates after NACT, but no such association was demonstrated with NLR. In the study by Wang et al.[37] higher pretreatment NLR and PLR were associated with a higher probability of achieving pCR, but pretreatment SII was not associated with a higher pCR.

In our study, no association was found between NLR, PLR, and SII and pCR. In the literature, different studies use different cutoff values for inflammatory markers, and the lack of a standard cutoff value may explain the discrepancies in the results of the studies[34]. Furthermore, many studies evaluated BC patients of all molecular subtypes together. Our study evaluated only HR+/HER2- patients to obtain more reliable results.

Study Limitations

Limitations of the study include the small number of patients conducted at a single center and its retrospective design. In addition, due to its retrospective design, it is possible that some conditions such as diseases and medication use that could affect blood values could not be evaluated due to incomplete records and could affect the results.

CONCLUSION

Predicting patients who will respond well to NACT in BC treatment is important. Inflammatory indices are inexpensive, reproducible, and easily accessible parameters that can be calculated with routine blood count analysis. However, given the conflicting results in the literature, negative studies including ours, and the multitude of factors that can influence blood parameters, their suitability for routine use in predicting chemotherapy response is questionable. Prospective clinical studies of sufficient size are needed to determine whether inflammatory indices can be a significant prognostic indicator of pCR.

Ethics

Ethics Committee Approval: The study received approval from the Ethics Committee of University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital (decision number: 2025/07/67, date: 24.07.2025).

Informed Consent: This study is a retrospective cross-sectional analysis of medical records of patients experiencing neoadjuvant chemotherapy at University of Health Sciences Türkiye, Balıkesir Atatürk City Hospital from January 2020 to May 2025.

Footnotes

Authorship Contributions

Concept: S.S., Design: S.S, Data Collection or Processing: S.S., Y.İ., Analysis or Interpretation: S.S., Z.E., Literature Search: S.S., Writing: S.S., Y.İ.

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

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ORIGINAL ARTICLE

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Impact of COVID-19 on Anemia, Polypharmacy and Medication Use: A Retrospective Analysis of Pre- and Post-infection Health Outcomes

COVID-19'un Anemi, Polifarmasi ve İlaç Kullanımı Üzerindeki Etkisi: Enfeksiyon Öncesi ve Sonrası Sağlık Sonuçlarının Retrospektif Analizi

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ABSTRACT

Aim: This study aimed to investigate the impact of coronavirus disease 2019 (COVID-19) infection on anemia, polypharmacy, inappropriate medication use, and the prescription of antidepressants, antipsychotics, and analgesics. Specifically, it compared medication use and associated health outcomes before and after COVID-19 infection.

Materials and Methods: A retrospective analysis was conducted using data from the national health registry system, including 354 patients who had COVID-19 between March 2020 and December 2022. Data on comorbidities, medication use, and hemoglobin levels were collected for six months before and after COVID-19 diagnosis.

Results: The study revealed significant increases in anemia, polypharmacy, antidepressant use, antipsychotic use, and analgesic use post-COVID-19 infection ($p<0.05$). Subgroup analyses showed significant increases in analgesic use, including paracetamol, non-steroidal anti-inflammatory drugs, opioids, and gabapentinoids (gabapentin and pregabalin). Multivariate logistic regression indicated that antipsychotic use after COVID-19 infection independently increased the likelihood of anemia (odds ratio: 2.99, 95%, confidence interval: 1.08-8.29, $p<0.05$).

Conclusion: The study found significant increases in anemia, polypharmacy, and the use of antidepressants, antipsychotics, and analgesics following COVID-19 infection. These findings emphasize the need for careful monitoring of medication use and anemia in post-COVID-19 patients and highlight the importance of appropriate prescription practices to mitigate long-term healthcare risks.

Keywords: Anemia, polypharmacy, antidepressants, COVID-19 pandemic

ÖZ

Amaç: Bu çalışma, 2019 koronavirüs hastalığı (COVID-19) enfeksiyonunun anemi, polifarmasi (çoklu ilaç kullanımı), uygunsuz ilaç kullanımı ve antidepresan, antipsikotik ile analjezik reçetelenmesi üzerindeki etkilerini araştırmayı amaçlamıştır. Özellikle, COVID-19 enfeksiyonu öncesi ve sonrası dönemdeki ilaç kullanımı ve ilişkili sağlık sonuçları karşılaştırılmıştır.

Gereç ve Yöntem: Mart 2020 ile Aralık 2022 tarihleri arasında COVID-19 geçirmiş 354 hastayı içeren ulusal sağlık kayıt sistemi verileri kullanılarak retrospektif bir analiz gerçekleştirilmiştir. COVID-19 tanısından önceki ve sonraki altı aylık dönemler için hastaların ek hastalıkları, ilaç kullanımları ve hemoglobin düzeyleri toplanmıştır.

Bulgular: Çalışmada, COVID-19 sonrası dönemde anemi, polifarmasi, antidepresan, antipsikotik ve analjezik kullanımında anlamlı artışlar tespit edilmiştir ($p<0,05$). Alt grup analizlerinde; parasetamol, nonsteroid antienflamatuvar ilaçlar, opioidler ve gabapentinoidler (gabapentin ve pregabalin) dahil olmak üzere analjezik kullanımında da anlamlı artışlar görülmüştür. Çok değişkenli lojistik regresyon analizine göre, COVID-19

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sonrası antipsikotik kullanımı, bağımsız olarak anemi riskini artıran bir faktör olarak bulunmuştur (olasılık oranı: 2,99, %95, güven aralığı: 1,08-8,29, p<0,05).

Sonuç: Bu çalışma, COVID-19 enfeksiyonu sonrası dönemde anemi, polifarmasi ve antidepresan, antipsikotik ile analjezik kullanımında anlamlı artışlar olduğunu ortaya koymuştur. Bu bulgular, COVID-19 sonrası hastalarda ilaç kullanımının ve aneminin dikkatle izlenmesi gerektiğini vurgulamakta ve uzun vadeli sağlık risklerini azaltmak için uygun reçeteleme uygulamalarının önemine dikkat çekmektedir.

Anahtar Kelimeler: Anemi, polifarmasi, antidepresanlar, COVID-19 pandemisi

INTRODUCTION

The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused one of the largest public health crises worldwide to date. Although the effects of the pandemic are considered to have passed, the economic burden caused by the disease is still impacting governments. After the coronavirus disease 2019 (COVID-19) infection, particularly the increased consumption of certain drug groups has led to an increase in inappropriate drug use. This has led to the development of the Turkish Inappropriate Medication Use in the Elderly (TIME) guideline, which aims to help healthcare professionals write optimal prescriptions and reduce inappropriate medication use in older adults[1].

Antidepressants are commonly used not only for depression but also for medical conditions such as post-traumatic stress disorder, panic disorder, and obsessive-compulsive disorder. Additionally, they are used for conditions like chronic pain and insomnia[2]. After the COVID-19 infection, it can be said that the use of antidepressants may increase due to the rising issues and economic problems for governments.

So far, the results of case series published on anemia in COVID-19 are generally contradictory. In some, it was reported that the hemoglobin (HGB) concentrations of patients who survived and those who died due to SARS-CoV-2 infection were similar, while no significant difference was found between patients followed in the intensive care unit (ICU) and those outside the ICU. Other studies, however, have reported lower HGB levels in patients with severe disease[3].

When more than five medications are taken in a day, this is called polypharmacy. The use of such a number of medications increases the likelihood of drug interactions, some of which can lead to more significant health issues. The portion of the used medications whose adverse effects outweigh their expected benefits is referred to as potentially inappropriate medication (PIM)[4]. In Türkiye, the Turkish Geriatrics Academy has developed the TIME criteria in these indications.

In our study, data from the national health registry system were examined for patients who had experienced a COVID-19 infection, comparing the period before the infection with the period after the infection. The aim was to investigate the relationship between anemia, polypharmacy, inappropriate medication use, and the usage rates of certain medications such as antidepressants, antipsychotics, and analgesics. We hypothesized that COVID-19 infection may increase the prevalence of anemia, polypharmacy, and the use of certain high-risk medications.

MATERIALS AND METHODS

Design and Sample

This retrospective study included 620 patients who were admitted to the University Hospital for different diseases and health problems between March 1, 2020 and December 12, 2022 during the COVID-19 pandemic. Of these 620 patients, data on comorbidities and visits to healthcare facilities were available for analysis for 354 patients. This study included only geriatric patients aged 65 years and older with PCR-confirmed COVID-19. The flow diagram of the patients included in the study is shown in Figure 1.

Ethical Considerations

All procedures for studies involving human participants were performed in accordance with the 1964 Declaration of Helsinki and its subsequent revisions. The study was approved by the Clinical Research Ethics Committee of Gaziantep University (approval number: 2022/10, date: 07.02.2022).

Data Collection

Data for all patients, including comorbidities, prescriptions, and the number of medications they should be taking for chronic diseases, were obtained by scanning the national health registry system, where health records are stored for at least 8 years. Anemia was defined according to the World Health Organization (WHO) HGB thresholds, and comorbidities were identified based on documented ICD-10 diagnostic codes within the national health registry system. Anemia was defined as HGB <13 g/dL in men and <12 g/dL in non-pregnant women according to WHO criteria[5]. The study period was divided into two parts: 6 months before and 6 months after the diagnosis of COVID-19 for each patient. COVID-19 diagnosis for each patient was confirmed using the polymerase chain reaction (PCR) method, and the recovery period was determined through subsequent PCR tests. Polypharmacy is defined as the simultaneous use of five or more medications. Polypharmacy was recorded if at least

three prescriptions for five or more medications were regularly prescribed within 4 weeks or less from the date of COVID-19 diagnosis. The patients' inappropriate medication use status was determined using the TIME guideline.

Statistical Analysis

The statistical analysis of the study was performed using IBM SPSS for Windows 24.0 statistical software. Parametric tests were applied to data showing normal distribution, while non-parametric tests were used for data not showing normal distribution. The normality of the data was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Descriptive statistical methods, including frequency, mean, standard deviation, median, minimum, and maximum values, were used to compare quantitative data. The significance of the difference between the means of two normally distributed groups was determined by the paired samples t-test, while the Wilcoxon signed-rank test was applied for data not showing normal distribution. McNemar's test was used to evaluate the relationship between categorical variables, and Pearson's correlation coefficient was calculated to assess the relationship between numerical variables. Multivariate binary logistic regression analysis was performed to determine the independent factors related to the parameters. In the multivariate binary logistic regression analysis, all variables listed in Table 4 were included in the model. Sex, age, length of hospital stays, diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, polypharmacy status (before and after COVID-19), total number of medications (before and after COVID-19), antipsychotic use after infection, and analgesic use before infection were entered as independent variables. Potential confounders, including age, sex, and major chronic comorbidities, were adjusted for in the final model to reduce bias and to ensure the robustness of the estimated associations. Results with a p-value less than 0.05 were considered statistically significant.

RESULTS

In our retrospective study, 620 geriatric patients with a COVID-19 infection history from the national health system records were included. Due to missing data, 266 participants were excluded, leaving 354 patients (182 women and 172 men) for analysis. A total of 354 geriatric patients (182 women, 172 men) were included in the final analysis (Figure 1). Descriptive data and comparisons of age, days of hospitalization during active COVID-19 infection, medications used for comorbidities, and HGB levels in both genders are shown in Table 1. It was found that men had a longer hospital stay due to COVID-19 infection ($p<0.05$). In our study, a comparison was made between the six months before and after the diagnosis of COVID-19 regarding anemia, pharmaceutical status, and the prescription of various drugs (Table 2). It was determined that anemia, polypharmacy ($p<0.001$), antidepressant use ($p<0.05$), antipsychotic use, and analgesic use significantly increased ($p<0.01$). In subgroup analyses, it was found that the use of analgesics such as paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and gabapentinoid (GABA) analogues significantly increased ($p<0.01$). Table 3 summarizes the comparison of polypharmacy, antidepressant and analgesic prescriptions, and anemia in the six months

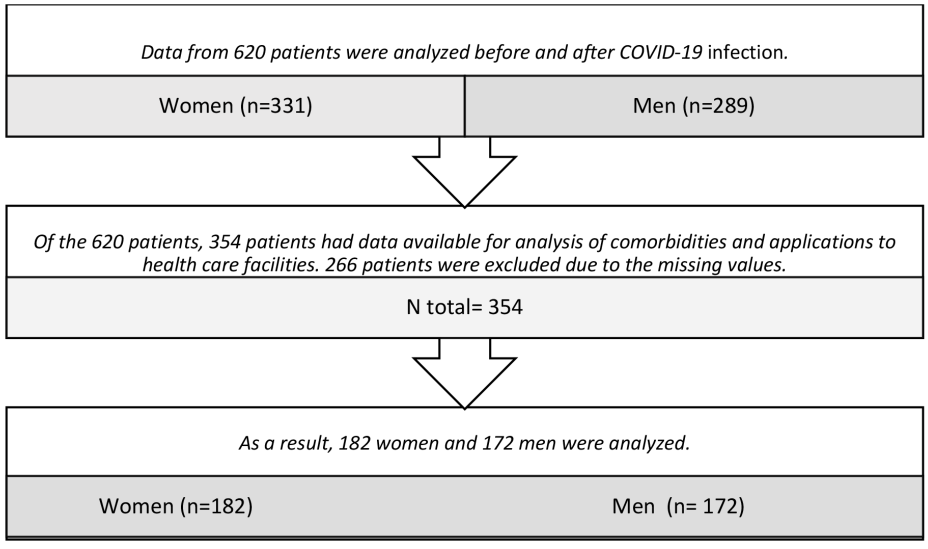


Figure 1. Flow diagram of the patients included in the study

COVID-19: Coronavirus disease 2019

Table 1. Descriptive data and comparisons of patients' age, days hospitalized during active COVID-19 disease, medications for co-morbidities and hemoglobin levels in both sexes

Variables	Total		Gender (n=354)				p
			Women (n=182)		Men (n=172)		
	Mean (median)	SD (min-max)	Mean (median)	SD (min-max)	Mean (median)	SD (min-max)	
Age$^{\Phi}$	73.06 (72)	(65-96)	73.82 (72)	(65-96)	72.25 (71)	(65-91)	0.021*
Duration of hospital stay $^{0\Phi}$	7.9 (7)	(0-37)	7.18 (7)	(0-24)	8.66 (7)	(0-37)	0.037*
Regular drugs before$^{1\Phi}$	5.01 (5)	(0-13)	5.03 (5)	(0-13)	4.98 (6)	(0-15)	0.882
Regular drugs after$^{2\Phi}$	6.49 (6)	(0-15)	6.48 (5)	(0-13)	6.51 (6)	(0-14)	0.994
HGB before$^{3\Psi}$	13.43	±1.64	12.935	±1.42	13.96	±1.69	<0,001*
HGB after$^{4\Phi}$	13.02 (13.18)	(8.1-17.3)	12.7 (12.9)	(8.1-16.9)	13.2 (13.6)	(8.1-17.3)	0.006*

$^{\Phi}$Mann-Whitney U test was performed for non-parametric data. Mean values were also given for non-parametric data.

$^{\Psi}$Student's t-test used for parametric data. Mean and standard deviation values were used instead of median and minimum maximum values for parametric data.

*Significant at 0.05 level.

^{0}: Length of the index COVID-19 hospital admission only.

^{1}: Number of drugs regularly prescribed to patients for their chronic diseases in the six months before COVID-19.

^{2}: Number of drugs regularly prescribed to patients for their chronic diseases in the six months after COVID-19.

^{3}: Hemoglobin values of the patients in the six months before COVID-19.

^{4}: Hemoglobin values of the patients in the six months after COVID-19.

COVID-19: Coronavirus disease 2019, HGB: Hemoglobin, SD: Standard deviation

before and after the COVID-19 diagnosis, by gender. In the female group, the use of polypharmacy and analgesic subgroups including paracetamol, NSAIDs, opioids, and GABA analogues significantly increased ($p<0.05$). In the male group, it was found that anemia, polypharmacy, antidepressant use, and analgesic subgroups such as paracetamol, NSAIDs, and opioids increased significantly ($p<0.05$). The multivariate logistic regression analysis of independent variables for anemia in the six months before and after the COVID-19 diagnosis is shown in Table 4. Accordingly, antipsychotic drug use after COVID-19 infection was found to independently and positively increase the presence of anemia [odds ratio (OR) 2.99, 95% confidence interval (CI): 1.08-8.29, $p<0.05$].

DISCUSSION

In our study, we determined that the rates of anemia, polypharmacy, antidepressant use, antipsychotic use, and analgesic use significantly increased in the period following the COVID-19 infection. Additionally, through regression analysis, we found that the use of antipsychotic drugs after COVID-19 infection independently increased the occurrence of anemia in these patients.

The relationship between COVID-19 and anemia is complex. Both anemia and COVID-19 lead to immune system dysfunction, making patients more susceptible to autoimmune complications that can effectively transform into one another[6,7]. Inflammation causes characteristic changes in iron homeostasis. This results in increased iron uptake and accumulation in macrophages, along with a decrease in iron absorption from the intestines[8]. This situation leads to a decrease in circulating iron levels. Additionally, it results in a reduction of the metal levels necessary for erythropoiesis. As a result, anemia of inflammation occurs due to cytokines that inhibit erythropoiesis, shortened erythrocyte lifespan, and decreased biological activity of erythropoietin[9,10]. In our study, anemia increased among male patients following COVID-19 infection, whereas no significant change was observed in females. Only a limited number of studies have evaluated HGB levels in COVID-19 patients by sex. Therefore, our findings regarding the post-infection increase in anemia among male patients provide a novel contribution to the literature. Notably, the increase in anemia was statistically significant only in men.

In a previous study, the identification of anemia as an independent risk factor for the prognosis of COVID-19 patients[11] highlights the importance of the disease's impact on hematological parameters.

Table 2. Medication use and anemia before and after COVID-19

Variables	Period				
	Before COVID-19		After COVID-19		p
	n	%	n	%	
Anemia	91	25.7	118	33.3	**<0.001***
Polypharmacy	189	53.4	265	74.9	**<0.001***
Potentially inappropriate medication use (TIME criteria)	113	31.9	122	34.5	0.396
Antidepressant use	78	22.0	98	27.7	**0.021**
Escitalopram	36	10.2	46	13.0	0.175
Sertraline	10	2.8	12	3.4	0.791
Duloxetine	16	4.5	22	6.2	0.238
Mirtazapine	6	1.7	10	2.8	0.344
Amitriptyline	5	1.4	9	2.5	0.289
Fluoxetine	4	1.1	1	0.3	0.375
Paroxetine	3	0.8	3	0.8	1.000
Trazodone	3	0.8	6	1.7	1.000
Venlafaxine	2	0.6	3	0.8	1.000
Benzodiazepine use	15	4.2	17	4.8	0.832
Medazepam	11	3.1	14	4.0	0.629
Alprazolam	3	0.8	3	0.8	1.000
Antipsychotic	8	2.3	20	5.6	**0.008***
Quetiapine	4	1.1	9	2.5	0.180
Haloperidol	1	0.3	2	0.6	1.000
Olanzapine	1	0.3	4	1.1	0.250
Risperidone	1	0.3	3	0.8	0.625
Aripiprazole	1	0.3	1	0.3	1.000
Analgesic use	327	92.4	336	94.9	**0.004***
Paracetamol	255	72.0	290	81.9	**<0.001***
NSAIDs	272	76.8	291	82.2	**<0.001**
Opioid	38	10.7	58	16.4	**<0.001***
GABA	43	12.1	65	18.4	**<0.001***

*Significant at 0.05 level, The McNemar test was performed for independent categorical data.

TIME: Turkish Inappropriate Medication Use in the Elderly, NSAIDs: Non-steroidal anti-inflammatory drugs, GABA: Gabapentinoids (gabapentin or pregabalin), COVID-19: Coronavirus disease 2019

In elderly patients, the presence of multiple comorbidities is common, leading to the use of numerous medications. Additionally, the physiological functions of older patients decline, and pharmacodynamic and pharmacokinetic changes increase the risk of drug interactions[12]. Drug-drug interactions can exacerbate these undesirable side effects. Polypharmacy ranks third among the causes of hospitalization in elderly patients and is also the leading cause of hospital-associated diseases[13].

In a cross-sectional study conducted by Chen et al.[13] on COVID-19 patients hospitalized with a diagnosis of COVID-19, high prevalence rates of polypharmacy (77.67%), PIMs (66.99%), and drug-drug interactions (61.65%) were identified. Additionally, diuretics, followed by benzodiazepines and benzodiazepine receptor agonist hypnotics, insulin, antipsychotics, and rivaroxaban or dabigatran were found to be the most commonly used PIMs. In our study, we also found that the proportion of polypharmacy patients increased after COVID-19 infection. It is well known that polypharmacy and inappropriate medication use lead to negative health outcomes for patients. In this study, no significant difference was observed in the rates of inappropriate medication use before and after COVID-19 (31.9% and 34.5%, respectively; p=0.396). This finding differs from the high PIMs rate (66.9%) reported by Chen et al.[13] This discrepancy may be due to differences in study populations, variations in healthcare

Table 3. Comparison of polypharmacy, antidepressant and analgesic prescriptions and anemia in both sexes in the six months before and after diagnosis of COVID-19 disease

Gender	Variables	Period				
		Before COVID-19		After COVID-19		p
		n	%	n	%	
Women	**Anemia**	43	23.6	48	26.4	0.511
	Polypharmacy	96	52.7	134	73.6	**<0.001***
	Antidepressant use	50	27.5	58	31.9	0.256
	Analgesic use	170	93.4	175	96.2	0.063
	Paracetamol	137	75.3	149	81.9	**0.017***
	NSAIDs	146	80.2	156	85.7	**0.013***
	Opioid	17	9.3	30	16.5	**<0.001***
	GABA	26	14.3	43	23.6	**<0.001***
Men	Anemia	48	27.9	70	40.7	**<0.001***
	Polypharmacy	93	54.1	131	76.2	**<0.001***
	Antidepressant use	28	16.3	40	23.3	**0.045***
	Analgesic use	157	91.4	161	93.6	0.125
	Paracetamol	118	68.6	141	82.0	**<0.001***
	NSAIDs	126	73.3	135	78.5	**0.049***
	Opioid	21	12.2	28	16.3	**0.016***
	GABA	17	9.9	22	12.8	0.063

*Significant at 0.05 level

The McNemar test was performed for independent categorical data.

NSAIDs: Non-steroidal anti-inflammatory drugs, GABA: Gabapentinoids (gabapentin or pregabalin), COVID-19: Coronavirus disease 2019

system practices, and the use of different criteria for assessing inappropriate medication use.

Worldwide, significant research is being conducted to assess changes in the rates of psychological disorders and psychiatric diagnoses associated with the COVID-19 pandemic. These studies are being carried out despite the decreasing trend in the number of SARS-CoV-2 infections and COVID-19 cases, driven by concerns about the potential long-term mental health impacts of the pandemic[14,15]. According to national data obtained from the United Kingdom, it has been shown that overall mental health scores have decreased since the start of the pandemic, and the proportion of individuals reporting symptoms of depression has nearly doubled compared to pre-pandemic levels[16].

Data from Europe and the United States provide reliable evidence showing an increase in antidepressant prescription and pharmacy requests during the pandemic period compared to the pre-pandemic period[16]. In our study, the rates of antidepressant use before and after the COVID-19 period were compared, and in line with the literature, it was determined that the usage increased in the post-pandemic period.

Under the shadow of the COVID-19 pandemic, a second crisis has emerged, resulting in a significant increase in psychiatric disorders. Along with this rise, the usage rates of antipsychotic medications have inevitably increased[17].

Long-term use of antipsychotic medications has been clearly associated with an increased risk of death in older adults. Antipsychotics, which cause numerous negative medical effects, have been reported to lead to serious respiratory side effects and are also linked to potential immune function disorders[18].

A large study conducted with adults from different age groups demonstrated a bidirectional interaction between psychiatric problems and COVID-19. The study found that survivors of COVID-19 are at an increased risk of psychiatric issues, including dementia[18]. Along with this information, it is of great importance to assess the survivors of COVID-19 for depression and psychiatric conditions in the long term and to ensure regular follow-up evaluations.

Hematologic side effects related to psychotropic drugs are very rare, but it should not be forgotten that they can be life-threatening. The hematologic side effects caused by psychotropic drugs have been explained through different pathophysiological mechanisms. These include bone marrow suppression, destruction of immune-related cells, the formation of active or toxic metabolites, and direct bone toxicity[19,20]. In this context, our study provides a noteworthy

Table 4. Multivariate binary logistic regression analysis of the independent variables for anemia, both prior to and following a six-month period subsequent to a diagnosis of COVID-19

Variables	B	SE	OR (95% CI)	p-value
Sex	0.714	0.264	2.04 (1.22-3.42)	**0.007***
Age	0.057	0.020	1.06 (1.02-1.10)	**0.005***
Hospital stay[1]	0.040	0.019	1.04 (1.003-1.080)	0.033
DM[2]	0.379	0.298	1.46 (0.81-2.62)	0.204
CKD[3]	1.980	0.613	7.24 (2.18-24.08)	**0.001***
COPD[4]	0.473	0.330	1.61 (0.84-3.07)	0.152
Polypharmacy, before[5]	0.702	0.445	2.02 (0.84-4.83)	0.114
Drugs, before[6]	-0.121	0.128	0.89 (0.69-1.14)	0.343
Polypharmacy, after[7]	-0.474	0.486	0.62 (0.24-1.61)	0.329
Drugs, after[8]	0.212	0.117	1.24 (0.98-1.56)	0.069
Antipsychotics, after[9]	1.098	0.519	2.99 (1.08-8.29)	**0.035***
Analgesics, before[10]	-0.553	0.481	0.58 (0.22-1.48)	0.250

*p<0.05 according to multivariate binary logistic regression analysis. B: Beta, CI: Confidence interval, OR: Odds ratio, COVID-19: Coronavirus disease 2019, SE: Standard error, CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, DM: Diabetes mellitus, Constant p value =0.22

[1]Length of the index COVID-19 hospital admission only.

[2]Diabetes mellitus

[3]Chronic kidney disease

[4]Chronic obstructive pulmonary disease

[5]Polypharmacy status of the patients before the COVID-19 diagnosis within the last 6 months.

[6]Number of drugs regularly prescribed to patients for their chronic diseases in the six months before COVID-19.

[7]Polypharmacy status of the patients after the COVID-19 diagnosis within the first 6 months.

[8]Number of drugs regularly prescribed to patients for their chronic diseases in the six months after COVID-19.

[9]The number of prescriptions for antipsychotic drugs given to patients within the first six months after their diagnosis of the COVID-19 disease.

[10]The number of prescriptions for analgesic drugs given to patients within the last six months before their diagnosis of the COVID-19 disease

contribution to the literature by demonstrating that post-COVID-19 antipsychotic use is independently associated with anemia, even after adjusting for age, sex, and major comorbid conditions. This independent association suggests that the hematologic vulnerability observed in some patients after COVID-19 infection may be exacerbated by antipsychotic exposure, possibly through one or more of the mechanisms described above. Highlighting the need for closer monitoring of hematologic parameters in patients who require antipsychotic treatment following COVID-19 infection.

One of the known but rare side effects in patients undergoing antipsychotic treatment is anemia. This condition is defined as a decrease in the total number of erythrocytes or the HGB content in red blood cells. The presence of anemia is associated with a reduced capacity of cells to carry oxygen[21,22]. In our study, we found a significant increase in the rate of anemia in patients following a COVID-19 infection. According to the multivariate binary logistic regression analysis results of independent variables related to anemia in the six-month periods before and after the COVID-19 diagnosis, we determined that antipsychotic use after COVID-19 infection independently and positively affected anemia (OR: 2.99, 95% confidence interval CI: 1.08-8.29, p<0.05). We believe this finding contributes to the literature due to the limited number of studies on this topic.

Study Limitations

Although our study is a unique one investigating how the frequency of certain conditions changed before and after the COVID-19 diagnosis in patients, it has some limitations. The retrospective nature of the study does not allow for the determination of causal relationships between the results. The data were obtained from a health record system that does not provide precise information about the patient's health status and medication use. It is unknown whether the patient received the treatment in real life, for how long, or the underlying primary cause of the diagnosis recorded in the system. Additionally, the sample size of the study is relatively small. Especially considering the global scale of the pandemic, a larger sample size would have yielded more accurate results.

CONCLUSION

After COVID-19 infection, we found an increase in the rates of anemia, polypharmacy, antidepressant use, antipsychotic use, and analgesic use in patients. Overall, our study demonstrates

that COVID-19 infection may increase the prevalence of anemia and that antipsychotic use after infection is independently associated with this hematologic complication. Given the limited evidence on this topic, our findings provide important clinical insight and emphasize the need for further prospective studies to confirm the mechanisms and guide safer prescribing practices for post-COVID-19 patients.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Gaziantep University (approval number: 2022/10, date: 07.02.2022).

Informed Consent: This is retrospective study.

Fotnootes

Authorship Contributions

Concept: E.S.A., E.Ö., Z.A.Ö., Design: E.S.A., E.Ö., Z.A.Ö., Data Collection or Processing: E.S.A., E.Ö., Z.A.Ö., Analysis or Interpretation: E.S.A., E.Ö., Z.A.Ö., Literature Search: E.S.A., E.Ö., Z.A.Ö., Writing: E.S.A., E.Ö., Z.A.Ö.

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Evaluating The Learning Curve in Suprapatellar Nailing of Tibial Shaft Fractures

Tibial Şaft Kırıklarının Suprapatellar Çivilenmesinde Öğrenme Eğrisinin Değerlendirilmesi

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ABSTRACT

Aim: Tibial shaft fractures constitute approximately 2% of all fractures. Intramedullary nails are considered the gold standard in their treatment due to less soft tissue damage and biomechanical superiority. The learning curve is an expression of how quickly and efficiently a person learns a skill or process in a certain period. Our aim in this study is to analyze the suprapatellar nailing learning curve, using operative time and total fluoroscopy time as outcome measures.

Materials and Methods: Between January 2021 and December 2021, patients aged 18-65 with fresh tibial shaft fractures, no open fractures, who underwent surgery with a senior physician and had complete records, were included in the study. Forty-seven patients met the inclusion criteria. Demographic data of the patients, fracture types, side, surgical duration and total fluoroscopy dose were examined.

Results: Surgeon-1 operated on 23 patients (48.9%) and surgeon-2 on 24 patients (51.1%). The study included 34 males (72.3%) and 13 females (27.7%), with a mean age of 36.11±12.92 years (range: 19-64). Mean operative time was 64.89±10.41 minutes (range: 48-90), and the number of fluoroscopy shots averaged 54.09±13.46 (range: 30-78). No significant differences were observed regarding gender, age, side, or operative time ($p>0.05$). Over time, both surgeons showed a significant decrease in operative duration, with surgeon-1 reducing time by 1.557 minutes per case [beta (ß)=-1.557; $p=0.001$] and surgeon-2 by 0.847 minutes (ß=-0.847; $p=0.001$). No significant learning curve was found for fluoroscopy usage ($p>0.05$).

Conclusion: Our study showed that the suprapatellar intramedullary nailing technique resulted in a significant reduction in operative time and a significant regression curve.

Keywords: Tibia shaft fracture, intramedullary nailing, suprapatellar technique, learning curve

ÖZ

Amaç: Tibial şaft kırıkları tüm kırıkların yaklaşık %2'sini oluşturur. İntramedüller çiviler, daha az yumuşak doku hasarı ve biyomekanik üstünlük nedeniyle tedavilerinde altın standart olarak kabul edilir. Öğrenme eğrisi, bir kişinin belirli bir sürede bir beceriyi veya işlemi ne kadar hızlı ve verimli bir şekilde öğrendiğinin bir ifadesidir. Bu çalışmadaki amacımız, sonuç ölçütleri olarak operasyon süresi ve toplam floroskopi süresini kullanarak suprapatellar çivileme öğrenme eğrisini analiz etmektir.

Gereç ve Yöntem: Ocak 2021 ile Aralık 2021 tarihleri arasında, açık kırığı olmayan, kıdemli bir hekim tarafından ameliyat edilen ve kayıtları tam olan, 18-65 yaş aralığındaki taze tibial şaft kırığı olan hastalar çalışmaya dahil edildi. Kırk yedi hasta dahil etme kriterlerini karşıladı. Hastaların demografik verileri, kırık tipleri, taraf, cerrahi süresi ve toplam floroskopi dozu incelendi.

Bulgular: Cerrah-1 23 hastayı (%48,9) ve cerrah-2 24 hastayı (%51,1) ameliyat etti. Çalışmaya 34 erkek (%72,3) ve 13 kadın (%27,7) dahil edildi ve ortalama yaşları 36,11±12,92 yıldı (aralığı: 19-64). Ortalama ameliyat süresi 64,89±10,41 dakikaydı (aralığı: 48-90) ve floroskopi çekim sayı ortalaması 54,09±13,46'ydı (aralığı: 30-78). Cinsiyet, yaş, taraf veya ameliyat süresi açısından anlamlı bir fark gözlenmedi ($p>0,05$). Zamanla, her iki cerrah da

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operasyon süresinde önemli bir azalma gösterdi; cerrah-1 olgu başına süreyi 1,557 dakika [beta (ß)=-1,557; p=0,001] ve cerrah-2 0,847 dakika (ß=-0,847; p=0,001) azalttı. Floroskopi kullanımında önemli bir öğrenme eğrisi bulunamadı (p>0,05).

Sonuç: Çalışmamız, suprapatellar intramedüller çivileme tekniğinin ameliyat süresinde anlamlı azalma ve belirgin regresyon eğrisi ile sonuçlandığını göstermiştir.

Anahtar Kelimeler: Tibia şaft kırığı, intramedüller çivileme, suprapatellar teknik, öğrenme eğrisi

INTRODUCTION

Tibial shaft fractures constitute approximately 2% of all fractures[1]. Surgical treatment methods such as external fixation osteosynthesis, plate-screw osteosynthesis, and intramedullary nail and screw fixation are available for tibial shaft fractures. Studies and experience have shown that intramedullary fixation methods are more advantageous in terms of success and complications[2,3]. It is accepted as the gold standard due to less soft tissue damage and being biomechanically superior[4]. In addition, fewer wound complications and higher union rates have been reported in the literature with intramedullary nailing[5].

Intramedullary nailing technique for tibial shaft fractures is performed by passing the skin with an infrapatellar incision opened from the inferior part of the patella in knee flexion and passing the patellar tendon with a transverse or parapatellar technique. This method has reported serious union and satisfaction rates in tibial shaft fractures. However, it has been experienced over time that anterior knee pain and joint stiffness complaints affect the quality of life in these patients and are the main source of problems for clinicians during follow-ups[6]. For this reason, it is noteworthy in the literature that the use of the suprapatellar technique is becoming more widespread with the aim of fewer knee joint problems and ease of surgical application[7,8].

Learning an orthopedic surgical procedure requires theoretical knowledge, intraoperative decision-making skill, mastery of fluoroscopy, and technical application that respects soft tissue. In this context, orthopedic surgery includes a certain learning curve and many parameters, such as complication rates, surgical duration, and radiation exposure, are evaluated as an indicator of this process. The learning curve is an expression that indicates how quickly and efficiently a person learns a skill or process in a certain period. The learning curve represents a graphical result between the learning effort and the learning result[9]. Defining a new technique's learning curve is important for instructors and practitioners. It provides information about the surgeon's experience and also provides confidence in the management of complications that may occur. When the suprapatellar nailing method is compared with the infrapatellar method, which is known and whose treatment adequacy is sufficiently proven, it is important to investigate the existence of a learning curve. This study aims to analyze the suprapatellar nailing learning curve by taking the surgery time and the total number of fluoroscopy shots as the outcome measures.

MATERIALS AND METHODS

This retrospective, single-center study was conducted at the Orthopedics and Traumatology Clinic of University of Health Sciences Türkiye, Ümraniye Training and Research Hospital between January and December 2021. Ethical approval was obtained from the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital Ethics Committee (decision number: 50, date: 13.03.2025) in accordance with the Declaration of Helsinki. Two senior orthopedic specialists who had successfully treated at least 30 tibial shaft fractures with intramedullary nailing in the last two years and attended the necessary training and courses [Turkish Orthopedic Association and Arbeitsgemeinschaft für Osteosynthesefragen (AO)] courses were determined. Tibial shaft fractures were operated on randomly by two surgeons. The surgical technique was standardized using the same order in each case. The patient was in the supine position and elevated with a specially prepared sponge pillow under the leg (Figure 1). Trigen, Metanail, semi-extended (Smith & Nephew, London, UK) nails suitable for suprapatellar access were used in all cases. Demographic data of the patients, fracture types, side, surgical time, and total number of fluoroscopy shots were examined.

Inclusion criteria were determined as patients with fresh tibial shaft fractures between the ages of 18-65, no open fracture, operated by a senior physician, and appropriate records kept. Exclusion criteria were determined as cases with open fracture, another surgical team, insufficient records, and cases without appropriate equipment.

Between January and December 2021, 84 patients underwent intramedullary nailing for tibial shaft fractures. After excluding 18 open fractures, 12 cases operated on by another team, 3 treated with different implants, and 4 with incomplete data, 47 patients were included. Of these, 23 were operated on by surgeon-1 and 24 by surgeon-2. The total number of fluoroscopy shots was recorded for all procedures examined. A single X-ray image intensifier (Philips Zenition 50) was used for all procedures examined. Surgical times were determined as the time from the initial incision to the final suture after



Figure 1. Surgical position and incision line

appropriate alignment and fixation of the fracture.

This was a retrospective study using consecutive patients treated during the surgeons' initial experience with the suprapatellar technique. No formal a priori sample-size calculation was performed. Post-hoc power analysis based on the observed effect sizes demonstrated >0.95 power for detecting the learning-curve effect on operative time; however, the power for detecting a moderate learning effect on the number of fluoroscopy shots was only approximately 0.20-0.30. Therefore, the absence of a significant learning curve for the number of fluoroscopy shots should be interpreted with caution, as the study may have been underpowered for this secondary outcome.

Statistical Analysis

Statistical analysis were performed using SPSS 27 (IBM Corp., Armonk, NY, USA). Quantitative variables were expressed as mean ± standard deviation, median, and range (min-max). Qualitative variables were summarized using descriptive statistics, including frequencies and percentages.

Normality of distribution was assessed using the Shapiro-Wilk test and Box-Plot visualizations. For normally distributed data comparing two groups, the independent Student's t-test was applied. Categorical variables were analyzed with the Pearson chi-square test.

To evaluate the learning curve of surgeons, linear regression analysis was conducted for operating time and the number of fluoroscopy shots. All results were interpreted within a 95% confidence interval, and statistical significance was set at $p<0.05$.

RESULTS

A total of 47 patients who underwent suprapatellar intramedullary nailing for tibial shaft fractures between January and December 2021 were included in this study. Surgeries were performed randomly by two senior surgeons: surgeon-1 operated on 23 patients (48.9%) and surgeon-2 on 24 patients (51.1%). The cohort consisted of 34 males (72.3%) and 13 females (27.7%), with a mean age of 36.11±12.92 years (range: 19-64). Fractures involved the right side in 27 patients (57.4%) and the left in 20 patients (42.6%). The mean operative time was 64.89±10.41 minutes (range: 48-90), and the mean number of fluoroscopy shots was 54.09±13.46 shots (range: 30-78) (Table 1).

Fracture types of the patients were determined according to AO/Orthopaedic Trauma Association classification[10]. No statistically significant difference was found between gender, side, and age of the patients according to surgeons ($p>0.05$). No statistically significant difference was found between surgery times according to surgeons ($p>0.05$). The number of fluoroscopy shots of surgeon-2 was found to be statistically significantly longer than surgeon-1($p=0.002$; $p<0.01$) (Table 2).

Table 1. Demographic characteristics

		n (%)
Gender	**Male**	34 (72.3)
	Female	13 (27.7)
Age	Mean ± SD	36.11±12.92
	Median (mini-maximum)	31 (19-64)
Side	**Right**	27 (57.4)
	Left	20 (42.6)
Fracture type	**42A1**	13 (27.6)
	42A2	7 (14.9)
	42A3	8 (17.0)
	42B1	6 (12.8)
	42B2	8 (17.0)
	42B3	5 (10.6)
Duration of surgery (minimum)	Mean ± SD	64.89±10.41
	Median (mini-maximum)	62 (48-90)
Number of fluoroscopy shots	Mean ± SD	54.09±13.46
	Median (mini-maximum)	55 (30-78)
Surgeons	**Senior surgeon-1**	23 (48.9)
	Senior surgeon-2	24 (51.1)

SD: Standard deviation

Table 2. Comparison of data according to surgeons

		Surgeon-1	Surgeon-2	p-value
Gender	**Male**	14 (60.9)	20 (83.3)	0.085
	Famale	9 (39.1)	4 (16.7)	
Age	Mean ± SD	39.39±15.63	32.96±8.88	0.088
	Median (mini-maximum)	33 (19-64)	31 (20-51)	
Side	**Right**	9 (39.1)	18 (75.0)	0.013*
	Left	14 (60.9)	6 (25.0)	
Duration of surgery (minimum)	Mean ± SD	64.17±12.51	65.58±8.12	0.132
	Median (mini-maximum)	60 (48-90)	63.5 (56-90)	
Number of fluoroscopy shots	Mean ± SD	47.74±11.72	60.17±12.34	**0.002****
	Median (mini-maximum)	49 (30-66)	62 (30-78)	

*p<0,05, **p<0,01, Pearson chi-square test, Student's t-test, SD: Standard deviation

Table 3. Learning curve statistical analysis

Dependent variable	Independent variable	ß	SE	%95 CI	p-value	R2
Duration of surgery	Surgeon-1	-1.577	0.209	-2.011/-1.143	0.001**	0.731
	Surgeon-2	-0.847	0.165	-1.190/-0.504	0.001**	0.543
Number of fluoroscopy shots	Surgeon-1	0.197	0.375	-0.540/0.935	0.603	0.040
	Surgeon-2	0.050	0.372	-0.684/0.785	0.893	0.010

**p<0,01, ß: Beta, SE: Standard error, CI: Confidence interval

When the learning outcomes of the surgery duration in surgeons are examined, the surgery duration decreased significantly over time. Surgeon-1 shortened the surgery duration faster than surgeon-2. While the surgery duration of surgeon-1 decreased by an average of 1.557 minutes [beta (ß):-1.557; p=0.001; p<0.01] with each case, this decrease was 0.847 (ß:-0.847; p=0.001; p<0.01) minutes for surgeon-2. These results show that surgeon-1 adapted to the suprapatellar technique faster and reduced the surgery duration more effectively (Table 3). When the regression curve is drawn as a figure, it is seen that there is a significant decrease and that it intersects after approximately 10 cases. After the intersection, it is seen that the duration starts to plateau and the curves tend to be parallel (Figure 2).

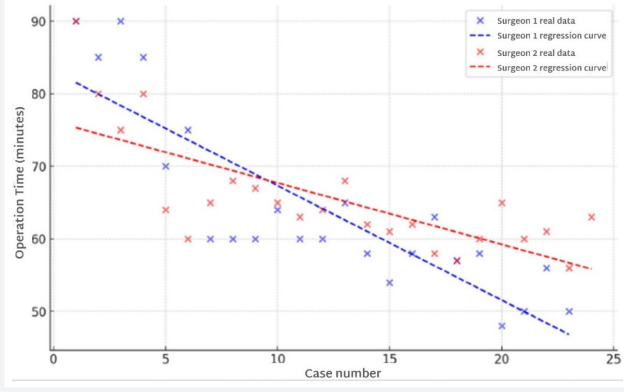


Figure 2. Regression curve of operation time and number of cases

There was no significant learning curve in terms of number of fluoroscopy shots (p>0.05). It was observed that there was no significant decrease in the number of fluoroscopy shots over time. This was the result for both surgeons. As shown in the figure, there is a more parallel curve (Figure 3).

DISCUSSION

The results of this study show that there can be a learning curve in terms of surgery time during the recognition phase of the suprapatellar nailing technique performed by two senior surgeons. After approximately 10 cases, an intersection in the regression curve in terms of surgery time and then a parallel course are shown graphically. It has been shown that there is no significant change in terms of the number of fluoroscopy shots. This shows us that there is no decrease or increase in intraoperative radiation exposure with learning.

In our study, surgery time was determined as the time during which skin-to-skin alignment and fixation were achieved. The first studies conducted with the suprapatellar technique showed that surgery time did not create a significant difference when compared to the infrapatellar technique[11,12]. As the technique developed, studies conducted showed that surgery

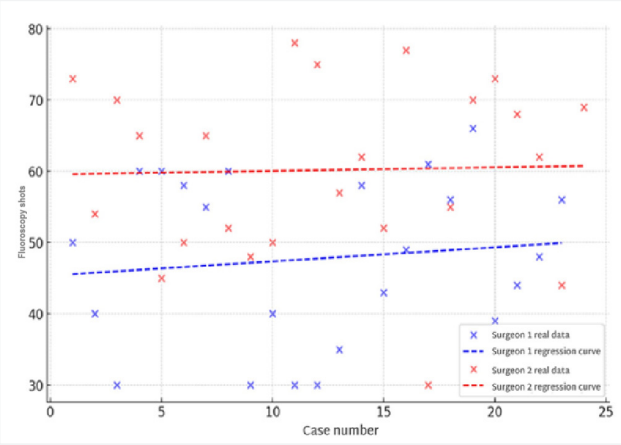


Figure 3. Regression plateau of the number of fluoroscopy shots and the number of cases

times were stated to be much shorter than the infrapatellar technique[7,13]. In the infrapatellar technique, the knee must be in a flexed position. This situation causes reduction problems and entry site problems, especially in proximal fractures, and causes a loss of time. This problem is minimal in the suprapatellar method. Since the entry point is proximal to the patella and the patient is in the supine position, it has been observed that there is less time loss with reduction because the leg is extended. In addition, it is thought that the time loss between the flexion position and the supine position in distal screw locking is due to the less time spent in favor of the supine position. There are many studies indicating patellar tendon and infrapatellar tendon damage with the infrapatellar technique[12,14,15]. Due to the increase in anterior knee pain and pain complaints in patients followed with the infrapatellar technique, the suprapatellar technique has been tried to be developed. However, there is a risk of chondral damage in the patellofemoral joint with this technique[16,17]. Since the studies on this subject are not sufficient, it is open to investigation. With the replacement of open surgeries with minimally invasive and intramedullary techniques, X-ray exposure for fracture reduction and stabilization status control has increased. This poses a risk for people in the operating room during surgery[18]. The potential for increased neoplastic activity, even in the low-dose range used at the time of fluoroscopy, has been known for many years[19]. It has been shown that the risk of cataract increases in people exposed to low-dose ionizing radiation for a long time[20]. Studies have shown that the surgeon's hand faces the highest radiation exposure. The thyroid and eyes are sensitive to radiation and can be affected even at low doses[18].

Although segmental comminuted fractures and open fractures were not included in our study in order to standardize the sample group as much as possible, the results showed that the fluoroscopy period was not included in the learning curve. When we investigated the reason for this, no organic problem was found. However, we think that differences in the surgeons' habits may have caused this. In the first cases, it was observed that surgeons were more meticulous during the entry site problems and the medullary canal during nailing and followed the canal path step by step. It was observed that this habit did not decrease over time and continued. This shows us that fluoroscopy is needed in cases regardless of the number of cases.

Allen et al.[13] mentioned a significant decrease in the number of fluoroscopy shots in their study comparing the suprapatellar technique with the infrapatellar technique. They stated that there was a significant decrease in the duration of surgery and the number of fluoroscopy shots between the two groups. In contrast to these data, recent studies have indicated that no significant difference was observed in terms of the number of fluoroscopy shots[11,21]. We thought that the comparison would not be useful for us in the learning curve during the planning phase. Because we think that comparisons made with the curve of the infrapatellar technique, which has been a successful and proven method for a long time, will not yield significant results.

Study Limitations

Our study has some limitations. Although we standardized the fracture pattern and surgeon experience as much as possible, surgical skill was an uncontrolled variable. Another limitation was the surgeons' habits. The average time between the two surgeons was significantly different ($p=0.002$; $p<0.01$). Although all other conditions were tried to be standardized, there was no standardization of the surgical team (anesthesia team, assistant surgical team, radiology technician).

CONCLUSION

Our study demonstrated that the suprapatellar intramedullary nailing technique was associated with a significant reduction in operative time, accompanied by a distinct regression curve indicative of a measurable learning process. Surgeons adapted rapidly to this technique, showing improved ability in fracture reduction and fixation over time. However, no significant decrease in the number of fluoroscopy shots was observed, nor was there evidence of a corresponding regression curve for this parameter. These findings suggest that while the suprapatellar approach facilitates quicker technical adaptation and enhanced intraoperative efficiency, further prospective studies with larger cohorts and longer follow-up periods are necessary to validate and generalize these results.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital Ethics Committee (decision number: 50, date: 13.03.2025) in accordance with the Declaration of Helsinki.

Informed Consent: This is retrospective, single-center study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.K., Ç.Ö., Concept: Ö.P., Ç.Ö., Design: Ö.P., B.K., S.G.B., Data Collection or Processing: Ö.P., S.G.B., Analysis or Interpretation: Ö.P., A.D., Ç.Ö., Literature Search: Ö.P., A.D., Writing: Ö.P., A.D., B.K., Ç.Ö., S.G.B.

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Silent Struggles in Critical Care: Functional Impact and Predictors of Depression Among ICU Nurses

Yoğun Bakımda Sessiz Mücadeleler: Yoğun Bakım Hemşirelerinde Depresyonun Fonksiyonel Etkisi ve Belirleyicileri

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ABSTRACT

Aim: This study aimed to explore the prevalence of depressive symptoms among nurses working in intensive care units (ICUs) and to analyze how these symptoms relate to both individual (e.g., age) and occupational (e.g., stress level, job satisfaction) variables. Furthermore, it sought to assess the functional consequences of depression using the final item of the Patient Health Questionnaire-9 (PHQ-9) scale.

Materials and Methods: A descriptive cross-sectional design was employed among ICU nurses from a tertiary-level hospital. Data were gathered through a structured questionnaire incorporating the PHQ-9, a job satisfaction measure, and a perceived stress scale. Statistical analyses included descriptive evaluations, correlation tests, and chi-square analyses, with effect sizes computed via Cramer's V where appropriate.

Results: More than half of the nurses (55.4%) demonstrated moderate-to-severe depressive symptoms. Depression scores showed significant associations with age ($r=-0.210$, $p=0.016$), perceived stress ($p<0.001$, Cramer's $V=0.344$), and job satisfaction ($p<0.001$, Cramer's $V=0.297$). According to the PHQ-9 item assessing functional impairment, 36.2% of participants indicated that depressive symptoms had a moderate-to-severe impact on their daily and professional functioning ($p<0.001$, Cramer's $V=0.484$).

Conclusion: Depression appears to be highly prevalent among ICU nurses and is influenced by both personal and workplace factors. The strong link between depressive symptoms and functional impairment highlights the importance of comprehensive mental health evaluations in nursing populations. Early recognition of vulnerable individuals, along with institutional programs addressing stress management and job satisfaction, may enhance nurses' psychological well-being and improve the overall quality of critical care delivery.

Keywords: Depression, intensive care unit nurses, PHQ-9, functional impairment, occupational stress, job satisfaction, mental health

ÖZ

Amaç: Bu araştırma, yoğun bakım ünitesinde (YBÜ) görev yapan hemşirelerde depresyon belirtilerinin görülme sıklığını belirlemeyi; depresyon ile bireysel (yaş) ve mesleki (stres düzeyi, iş doyumu) değişkenler arasındaki ilişkileri incelemeyi; ayrıca Hasta Sağlık Anketi-9 (PHQ-9) ölçeğinin son maddesi aracılığıyla depresyonun günlük işlevsellik üzerindeki etkisini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntem: Tersiyer düzeyde bir hastanede görev yapan YBÜ hemşireleri arasında tanımlayıcı kesitsel bir çalışma tasarımı kullanılmıştır. Veriler, PHQ-9, iş doyumu ölçeği ve algılanan stres ölçeğini içeren yapılandırılmış bir anket formu aracılığıyla toplanmıştır. Elde edilen veriler tanımlayıcı istatistikler, korelasyon analizleri ve ki-kare testleriyle değerlendirilmiş; etki büyüklükleri Cramer's V katsayısı ile hesaplanmıştır.

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Bulgular: Katılımcıların %55,4'ünde orta ile şiddetli düzey arasında değişen depresif belirtiler saptanmıştır. Depresyon puanları ile yaş (r=-0,210, p=0,016), stres düzeyi (p<0,001, Cramer's V =0,344) ve iş doyumu (p<0,001, Cramer's V =0,297) arasında anlamlı ilişkiler bulunmuştur. PHQ-9'un "fonksiyonel etki" maddesi değerlendirildiğinde, hemşirelerin %36,2'si depresyonun günlük yaşam ve mesleki işlevsellik üzerinde orta-şiddetli düzeyde olumsuz etki yarattığını bildirmiştir (p<0,001, Cramer's V =0,484).

Sonuç: Yoğun bakım hemşirelerinde depresyon yaygın bir sorundur ve hem bireysel hem de mesleki etkenlerden etkilenmektedir. Depresyonun işlevsellik üzerindeki belirgin etkisi, hemşirelerin ruhsal durumlarının bütüncül biçimde değerlendirilmesi gereğini ortaya koymaktadır. Erken tanı ve stresle baş etme ile iş doyumunu artırmaya yönelik kurumsal girişimler, hem çalışanların psikolojik iyilik halini hem de verilen bakımın kalitesini geliştirebilir.

Anahtar Kelimeler: Depresyon, yoğun bakım hemşireleri, PHQ-9, fonksiyonel bozulma, mesleki stres, iş memnuniyeti, mental sağlık

INTRODUCTION

Nurses form the cornerstone of healthcare delivery and routinely operate under challenging circumstances that demand emotional engagement, sustained alertness, rotating shifts, and uninterrupted patient contact. These persistent stressors increase their susceptibility to psychological strain, often reflected through conditions such as depression, anxiety, or occupational burnout[1,2]. The occurrence of depressive symptoms among nurses remains notably greater than that observed in the general population. According to recent meta-analyses, approximately one out of every three nurses reports some degree of depression, with particularly elevated prevalence rates reported in studies from Asian regions[3,4]. Such psychological burdens can negatively affect both nurses' personal well-being and the safety of the patients under their care, highlighting the necessity of viewing depression as a significant occupational health issue.

Among all nursing subspecialties, those working in intensive care units (ICUs) encounter some of the most mentally demanding conditions, driven by the high acuity of their patients, the necessity for complex clinical judgments, and the frequent confrontation with ethically challenging situations[5,6]. Workplace stress among ICU nurses is further intensified by factors such as insufficient staffing, irregular shift schedules, and the constant exposure to critically ill or life-threatening patient cases[7]. A recent systematic review demonstrated that nurses working in ICUs experience markedly higher levels of depression and anxiety than their counterparts in general hospital wards[8]. Furthermore, conceptual models such as the Job Demands-Resources framework illustrate that excessive work demands combined with inadequate organizational resources foster burnout and emotional fatigue, which in turn may evolve into depressive symptoms[9].

Psychological resilience has been recognized as an important protective mechanism against mental health deterioration. Evidence from various studies suggests that interventions such as mindfulness practices, emotional regulation training, and organizational well-being initiatives can play a preventive role in reducing the likelihood of depression among nurses[10,11]. Despite the expanding body of international research, depression among intensive care nurses continues to be insufficiently investigated in many middle-income nations, including Türkiye. Most Turkish studies to date have primarily addressed general occupational stress or burnout, rather than employing validated instruments specifically designed to measure depressive symptoms[12,13].

The Patient Health Questionnaire-9 (PHQ-9) is a well-established, self-report instrument developed in accordance with DSM-5 diagnostic criteria. It is commonly utilized to assess both the occurrence and the severity of depressive symptoms in clinical and research settings[14]. The Turkish adaptation of the PHQ-9 has also demonstrated strong reliability and validity, confirming its suitability for use among healthcare professionals in Türkiye[15]. In addition, the final item of the PHQ-9 "How difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?" specifically reflects the degree of functional impairment caused by depressive symptoms, thereby providing a broader understanding of depression that extends beyond mere symptom intensity[14,15].

Given the scarcity of national data on this topic, the present study sought to determine the prevalence of depressive symptoms among intensive care nurses in Türkiye using the PHQ-9 scale. It also aimed to examine how depression relates to demographic variables (such as age, gender, and professional experience) and occupational characteristics (including stress level and job satisfaction). In addition, the research evaluated the functional impact of depression through the final item of the PHQ-9, highlighting the extent to which psychological symptoms interfere with nurses' daily routines and professional performance.

MATERIALS AND METHODS

This study utilized a descriptive and cross-sectional design to examine depressive symptoms among nurses employed in the second- and third-level ICUs of University of Health Sciences Türkiye, Diyarbakır Gazi Yaşargil Training and Research Hospital between February and March 2025. All study procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki. Ethical approval was granted by the University of Health Sciences Türkiye, Gazi Yaşargil Training and

Research Hospital Ethics Committee (decision no: 316, date: 17.01.2025), and written informed consent was obtained from every participant prior to inclusion.

The study population consisted of all nurses aged 18 years or older who were working in the designated ICUs during the study period. A total of 130 nurses who completed the distributed questionnaires in full were included in the final analysis.

Statistical Analysis

Data were collected using a structured questionnaire composed of three integrated sections. The first section gathered sociodemographic and professional characteristics, including variables such as age, gender, educational attainment, total professional experience, duration of ICU-specific employment, type of shift worked, patient load, and any previous history of depression. The second section focused on occupational perceptions, evaluating perceived stress levels and overall job satisfaction over the preceding two weeks using Likert-type items. The third section incorporated the PHQ-9, a validated tool used to assess the presence and severity of depressive symptoms.

The internal consistency of the PHQ-9 scale was examined using Cronbach's alpha coefficient. Depression severity was classified according to internationally recognized thresholds: scores between 0-4 indicated minimal depression, 5-9 mild, 10-14 moderate, 15-19 moderately severe, and 20-27 severe depression.

Prior to statistical evaluation, incomplete responses were excluded using the listwise deletion method, and outliers were identified and removed based on standardized z-scores. Statistical analyses were performed using IBM SPSS Statistics version 25.0. Descriptive statistics, including means, standard deviations, frequencies, and percentages, were calculated to summarize the data. The Kolmogorov–Smirnov test was employed to assess the normality of variable distributions. Correlations between PHQ-9 scores and ordinal variables were analyzed using Spearman's rank correlation test. Relationships between categorical variables and depression levels were examined using Pearson's chi-square, Fisher's exact, and Fisher-Freeman-Halton tests where appropriate. A p-value less than 0.05 was considered statistically significant in all analyses.

RESULTS

A total of 130 nurses working in ICUs participated in the study. The median age of the participants was 31 years (range: 21-45). Among them, 50 nurses (38.5%) were female and 80 (61.5%) were male. The mean total professional experience was 9.2±4.0 years, while the average duration of work in intensive care settings was 6.1±4.1 years. The majority of respondents (82.3%) reported working on rotating shifts, and the median number of patients cared for per shift was four (range: 0-10) (Table 1).

The internal reliability of the PHQ-9 was found to be excellent, with a Cronbach's alpha of 0.894. The mean total PHQ-9 score was 11.2±5.4, indicating a generally moderate level of depressive symptoms. Based on severity categories, 12 nurses (9.2%) showed minimal symptoms, 46 (35.4%) mild, 37 (28.5%) moderate, 24 (18.5%) moderately severe, and 11 (8.5%) severe symptoms of depression. Altogether, 72 participants (55.4%) demonstrated moderate-to-severe levels of depression (Figure 1).

Correlation analyses examining associations between PHQ-9 scores and participant characteristics revealed a significant negative correlation with age (r=-0.210, p=0.016), indicating that younger nurses tended to report higher depressive symptom levels. No significant relationships were detected between PHQ-9 scores and the number of monthly night shifts (p=0.211), total years of professional experience (p=0.136), duration of ICU experience (p=0.177), length of employment in the current unit (p=0.703), number of patients cared for per shift (p=0.548), or monthly shift frequency (p=0.211).

Table 1. Sociodemographic and professional characteristics of participants

Variable	Value
Age, median (range), years	31 (21-45)
Total professional experience, mean ± SD (years)	9.2±4.0
ICU experience, mean ± SD (years)	6.1±4.1
Gender, n (%) Female Male	 50 (38.5%) 80 (61.5%)
Rotating shifts, n (%)	107 (82.3%)
Number of patients cared for, median (range)	4 (0-10)

SD: Standard deviation, ICU: Intensive care unit

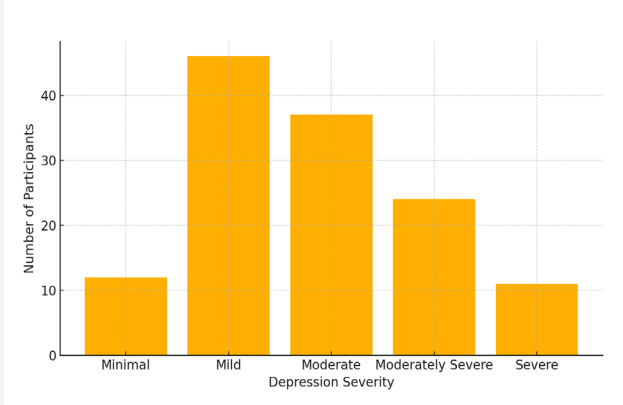


Figure 1. Distribution of participants by PHQ-9 depression severity levels

PHQ-9: Patient Health Questionnaire-9

When occupational variables were examined, a statistically significant relationship was identified between perceived stress levels and depression severity ($p<0.001$). The corresponding effect size, calculated with Cramer's V, was 0.344, suggesting a moderate strength of association. Job satisfaction was likewise significantly related to depressive symptoms ($p<0.001$; Cramer's V =0.297).

No other significant associations were observed between depression levels and demographic or professional characteristics, including gender, educational status, shift schedule, number of patients assigned, history of depression, or use of antidepressant medication. Comprehensive data are provided in Table 2.

Analysis of responses to the final PHQ-9 item, which evaluates the functional consequences of depressive symptoms, showed that half of the participants (50.0%) stated these symptoms had a "slight" effect on their daily lives. Meanwhile, 21 nurses (16.2%) described a "moderate" impact, 16 (12.3%) a "considerable" impact, and 10 (7.7%) reported the effect as "very severe." Only 18 nurses (13.8%) indicated that depression had "no effect at all" on their functioning (Table 3).

A statistically significant association was also detected between responses to the PHQ-9 functional impact item and overall depression severity ($p<0.001$). The strength of this relationship was substantial (Cramer's V= 0.484). As illustrated in Figure 2, higher levels of reported functional difficulty corresponded to a greater proportion of nurses experiencing more severe depressive symptoms.

Table 2. Depression severity by demographic and occupational factors in ICU nurses

	Depression levels					
Variable	**Minimal**	**Mild**	**Moderate**	**Moderately severe**	**Severe**	**p-value**
Gender						
Female	6	26	25	16	7	0.737
Male	6	20	12	8	4	
Education level						
Health high school	1	2	0	0	1	
Associate degree	0	3	3	4	4	0.071
Bachelor's degree	11	36	33	19	6	
Master's degree or above	0	5	1	1	0	
Shift type						
Day	2	9	8	2	1	
Night	0	0	1	0	0	0.731
Rotating	10	37	28	22	10	
History of depression						
Yes	0	1	4	4	1	0.145
No	12	45	33	20	10	
Use of antidepressants						
Yes	0	0	0	1	0	0.362
No	12	46	37	24	11	
Stress level						
Very low	4	6	0	0	0	
Low	5	11	7	1	0	
Moderate	2	22	16	5	1	**<0.001**
High	0	5	10	10	7	
Very high	1	2	4	8	3	
Job satisfaction						
Very low	2	4	6	8	6	
Low	1	9	13	8	3	
Moderate	2	26	12	7	1	**0.001**
High	4	6	5	1	1	
Very high	3	1	1	0	0	

ICU: Intensive care unit

Table 3. Functional impact of depressive symptoms based on the PHQ-9 "difficulty" item		
Difficulty level (1= not at all, 5= extremely)	**Number of participants (n)**	**Percentage (%)**
Not at all	18	13.8%
A little	65	50.0%
Moderately	21	16.2%
Considerably	16	12.3%
Extremely	10	7.7%
PHQ-9: Patient Health Questionnaire-9		

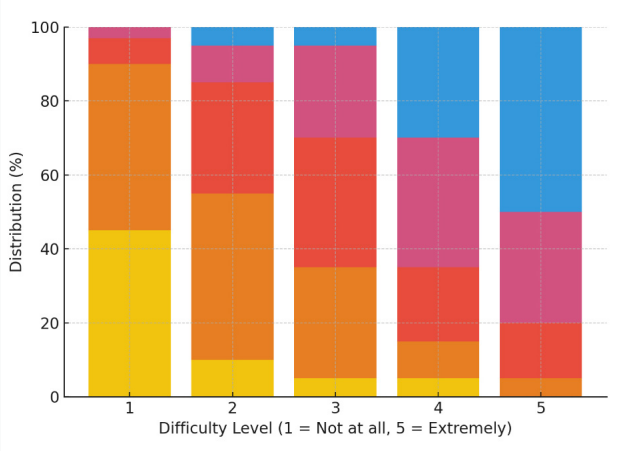


Figure 2. Distribution of depression severity by functional difficulty (PHQ-9 item 10)

PHQ-9: Patient Health Questionnaire-9

DISCUSSION

The present study revealed that depressive symptoms of moderate-to-severe intensity were highly prevalent among intensive care nurses. It also identified significant associations between depression levels, occupational stress, and job satisfaction. These findings are consistent with international evidence suggesting that nurses especially those working in high-acuity environments are at an increased risk of developing mental health problems[1-3,8].

The proportion of nurses in our study who exhibited moderate-to-severe depressive symptoms (55.4%) was higher than global estimates, which typically range between 34% and 45%[3,8,16]. Similarly, Xie et al.[8] reported high rates of depression among ICU nurses, while post-pandemic studies such as that of Labrague[17] also demonstrated elevated psychological distress among nursing staff. These findings collectively indicate that intensive care environments amplify occupational stressors and increase vulnerability to depression.

The significant association identified between PHQ-9 scores and perceived stress highlights the substantial influence of workplace environment and organizational dynamics. Factors such as excessive workload, inadequate social support, and rotating shift schedules are well-documented contributors to emotional exhaustion and psychological strain among nurses[5,9]. Furthermore, the observed link between higher depression severity and lower job satisfaction is consistent with both international and Turkish studies that identify the work environment and organizational climate as key determinants of mental well-being among healthcare professionals[12,13,18]. The Job Demands-Resources framework offers a conceptual basis for understanding these relationships, proposing that elevated job demands combined with insufficient organizational resources lead to increased psychological burden and emotional strain among employees[9].

The recently published multicenter HELLO Trial demonstrated that organizational interventions designed to enhance positive communication significantly reduced burnout among ICU staff. These findings reinforce the relevance of the observed associations between depression, job satisfaction, and stress in our study, highlighting the need for a comprehensive, multidimensional approach to the psychosocial well-being of ICU personnel[19].

Another important observation in this study was the association between age and depressive symptom levels. Younger nurses demonstrated higher PHQ-9 scores, aligning with prior research that identifies early-career nurses as a particularly vulnerable group, largely due to their limited coping strategies and reduced access to professional support systems[20]. Accordingly, targeted approaches such as structured mentorship initiatives and resilience-building programs may strengthen psychological stability and coping capacity among early-career nurses[10,11].

Functional impairment, as evaluated through the final item of the PHQ-9, demonstrated a strong and significant association with the severity of depressive symptoms. This observation is consistent with the seminal work of Kroenke et al.[14] and later validation studies, which established functional disruption as a fundamental marker of clinical depression severity[15]. Accordingly, the present findings emphasize the practical value of incorporating assessments of functional impact into mental health evaluations, particularly in occupational and healthcare contexts.

From a public health standpoint, institutional measures aimed at regulating workload, optimizing shift arrangements, and strengthening psychosocial support systems are essential to protect the mental health of ICU staff. Evidence-based approaches including mindfulness-based interventions, structured debriefing sessions, and programs that foster organizational resilience have consistently been shown to lower rates of depression and burnout among healthcare professionals[10,11,21].

Study Limitations

This study has certain limitations that should be acknowledged. Its cross-sectional design precludes establishing causal relationships, and the use of self-administered questionnaires may have introduced potential reporting bias. Despite these constraints, the research possesses notable strengths, including an adequate sample size, the application of validated psychometric instruments, and a specific focus on ICU nurses a population that remains relatively underrepresented in the context of mental health studies in Türkiye. Taken together, the findings underscore the pressing need for both institutional and individual-level strategies aimed at enhancing psychological resilience and protecting the overall well-being of professionals working in intensive care environments.

CONCLUSION

In summary, this study offers an in-depth examination of the prevalence of depressive symptoms among ICU nurses and explores their associations with both individual factors (such as age) and occupational determinants (including stress level and job satisfaction).

The results suggest that younger nurses, those experiencing greater occupational stress, and those with diminished job satisfaction are more vulnerable to psychological distress. A particularly important finding is the strong association between depressive symptoms and functional impairment, underscoring the need for a more comprehensive and multidimensional approach to the evaluation and management of depression. Nurses serve not only as caregivers but also as vital professionals who ensure patient safety, continuity, and quality of care. In high-intensity environments such as ICUs, their psychological health plays a decisive role in maintaining these standards. Early detection of frequently neglected mental health conditions such as depression along with the reinforcement of individual coping mechanisms, promotion of team-based psychosocial support, and the implementation of organizational strategies to reduce workplace stress are essential for preventing burnout and sustaining high-quality healthcare services.

As one of the few studies conducted in Türkiye employing the PHQ-9 to evaluate depression among ICU nurses, this research makes a valuable contribution to the national and international literature. It emphasizes the critical importance of prioritizing mental health and well-being at both individual and institutional levels within the healthcare system.

These findings underscore the need for ICU administrators to implement structured mental-health support programs, enhance organizational communication, and strengthen resilience-building initiatives to protect staff well-being. Integrating such strategies into routine ICU practice may not only mitigate psychological distress among nurses but also contribute to improved patient safety and care quality.

Future multicenter and longitudinal studies are needed to clarify causal relationships and to evaluate the long-term impact of organizational and individual-level interventions on the mental health of ICU nurses.

Ethics

Ethics Committee Approval: Ethical approval was granted by the University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital Ethics Committee (decision no: 316, date: 17.01.2025).

Informed Consent: Written informed consent was obtained from all participants prior to their inclusion in the study.

Footnotes

Authorship Contributions

Concept: A.D., S.Y., Design: A.D., S.Y., Data Collection or Processing: A.D., S.Y., B.S.K., Analysis or Interpretation: A.D., S.Y., İ.K.U., E.Ç., Literature Search: A.D., B.S.K., E.Ç., Writing: A.D., S.Y., B.S.K., İ.K.U., E.Ç.

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

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ORIGINAL ARTICLE

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Gender Dimorphism in the Impact of Obesity on Chronic Rhinosinusitis Symptoms: An Analysis of SNOT-22 Subitems

Obezitenin Kronik Rinosinüzit Semptomları Üzerindeki Cinsiyet Dimorfizmi: SNOT-22 Alt Kalemlerinin Analizi

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ABSTRACT

Aim: This study aimed to evaluate the effect of obesity on the symptom profile and radiological disease burden in patients with chronic rhinosinusitis (CRS).

Materials and Methods: A total of 123 patients with CRS were retrospectively analyzed. The patients' heights, weights, 22-item Sinonasal Outcome test (SNOT-22) scores, and Lund-Mackay scores were recorded. Patients were grouped into obese (≥30 kg/m^2) and normal (<30 kg/m^2) based on their body mass index (BMI) values. Symptom profiles and radiological disease burden were assessed using SNOT-22 and Lund-Mackay scoring, respectively BMI was correlated with each SNOT-22 question and with the total SNOT-22 score using Spearman's rank correlation, while the Lund-Mackay score was analyzed using Pearson correlation.

Results: Increase in BMI was positively correlated ($p<0.05$) with certain SNOT-22 items; these associations were more pronounced in women with nasal congestion and sleep-related symptoms and in men with postnasal drip. However, no significant differences were found in total SNOT-22 and Lund-Mackay scores between the obese and normal groups.

Conclusion: Obesity may be associated with specific symptom patterns, although it does not significantly increase the total symptom burden. The fact that obesity is associated with obstructive symptoms in women and secretory symptoms in men underscores the need for personalized treatment approaches in this patient group. Large-scale studies are needed to more clearly demonstrate the effect of obesity on CRS symptoms, phenotypes, and radiological findings.

Keywords: Chronic rhinosinusitis, obesity, body mass index, SNOT-22

ÖZ

Amaç: Bu çalışmada, obezitenin kronik rinosinüzitli (KRS) hastalardaki semptom profili ve radyolojik hastalık yükü üzerindeki etkisini değerlendirmek amaçlandı.

Gereç ve Yöntem: Toplam 123 KRS hasta, geriye dönük olarak analiz edildi. Olguların boy, kilo, 22-öğelik Sinonazal Sonuç testi (SNOT-22) testi ve Lund-Mackay skorları not edildi. Hastalar vücut kitle indeksi (VKİ) değerlerine göre obez (≥30 kg/m^2) ve normal (<30 kg/m^2) olarak gruplandırıldı. Semptom profilleri SNOT-22 ile, radyolojik hastalık yükü ise Lund-Mackay skorlaması ile değerlendirildi. VKİ ile SNOT-22'nin her bir sorusu ve toplam SNOT-22 skoru Spearman korelasyon, Lund-Mackay skoru ise Pearson korelasyon analizi ile incelendi.

Bulgular: VKİ artışı bazı SNOT-22 maddeleri ile anlamlı pozitif korelasyon gösterdi; bu ilişkiler kadınlarda burun tıkanıklığı ve uykuya ilişkin semptomlarda, erkeklerde ise geniz akıntısında daha belirgindi. Ancak obez ve normal gruplar karşılaştırıldığında toplam SNOT-22 ve Lund-Mackay skorlarında anlamlı fark saptanmadı.

Sonuç: Obezite, toplam semptom yükünü belirgin olarak artırmamakla birlikte belirli semptom paternleri ile ilişkili olabilir. Kadınlarda obezitenin obstrüktif semptomları, erkeklerde ise sekretuar semptomları ön plana çıkarması, bu hasta grubunda kişiselleştirilmiş tedavi yaklaşımlarının gerekliliğini ortaya koymaktadır. Obezitenin KRS semptomları, fenotipleri ve radyolojik bulguları üzerindeki etkisini daha net ortaya koymak için geniş örneklemli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Kronik rinosinüzit, obezite, vücut kitle indeksi, SNOT-22

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INTRODUCTION

Chronic rhinosinusitis (CRS) is characterized by inflammation of the nasal passages and paranasal sinuses. CRS is a common disease that imposes a substantial burden on individuals and society. A multicenter study in Europe reported a CRS prevalence of 10.9%[1]. Similar prevalence studies have been conducted in different countries, with reported rates of 5.5% in Brazil, 8% in China, 8.4% in South Korea, and 11.9% in the United States (US)[2-5].

CRS can be divided into two subtypes based on endoscopic findings: CRS with nasal polyps and CRS without nasal polyps[6]. It can also be classified based on the predominance of type 2 inflammation[7]. Type 2 inflammation is associated with T helper 2 cell cytokines, including interleukin (IL)-4, IL-5, and IL-13[8]. In Western countries, T helper 2-mediated type 2 inflammation is recognized as the predominant inflammatory profile, affecting approximately 50% of patients without nasal polyps and up to 80% of patients with nasal polyps[9].

According to the World Health Organization (WHO), overweight is defined as a body mass index (BMI) of 25 kg/m^2 or greater, and obesity as a BMI of 30 kg/m^2 or greater. Obesity is a rapidly growing pandemic, affecting more than 1 billion people worldwide[10]. Obesity prevalence is also increasing markedly in the Turkish population. The prevalence of overweight and obesity in Türkiye is higher than in Europe and comparable to that in the US. Obesity prevalence among adults in Türkiye was 18.8% in 1990 and increased to 36% in 2010[11].

Obesity is characterized by low-grade inflammation mediated by inflammatory cytokines and adipokines, including IL-6, TNF-α, and IL-1[12,13]. Asthma, an airway inflammatory disease, is well known to be associated with obesity[14]. In addition, higher BMI has been associated with increased prevalence of CRS; obesity is one of the most common comorbidities among patients with CRS[15,16].

Bapat et al.[17] compared obese and non-obese groups in mouse models of atopic dermatitis and allergic asthma. In the obese group, they observed that Th17-dominant inflammation replaced type 2 helper T (Th2) inflammation and that disease symptoms and findings were exacerbated. Furthermore, while biological therapies targeting Th2 cytokines were effective in the non-obese group, they were found to exacerbate disease severity in the obese group.

Although several studies have examined the relationship between obesity and CRS, the number of studies evaluating symptom-specific associations remains limited. In this study, we aimed to investigate the relationship between obesity and the symptom profile of CRS by analyzing each item of the 22-item Sinonasal Outcome test (SNOT-22). To this end, data from patients diagnosed with CRS at our clinic were retrospectively analyzed.

MATERIALS AND METHODS

This retrospective study included patients aged 18-70 years who presented to our clinic between 2021 and 2024, were diagnosed with CRS according to the EPOS 2020 criteria, and who had complete SNOT-22 forms, paranasal sinus computed tomography (CT) scans, and height and weight measurements. Patients with chronic diseases that could cause systemic inflammation, other than obesity, were excluded from the study; a total of 123 patients were included in the analysis. The study was approved by the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee with (decision number 2024.236.07.11, date: 30.07.2024) and was conducted in accordance with the Declaration of Helsinki.

Symptom severity and quality of life were assessed using the SNOT-22; the Turkish-validated version by Hancı et al.[18] was used. Radiological disease burden was assessed using the Lund-Mackay classification on paranasal sinus CT. The BMI of all patients was calculated from height and weight measurements according to the WHO classification; patients with a BMI <30 kg/m^2 were classified as non-obese, and those with a BMI ≥30 kg/m^2 were classified as obese.

Statistical Analysis

An independent-groups t-test or Mann-Whitney U test was used to compare obese and normal groups, depending on the data distribution. The relationships between BMI and SNOT-22 items and between BMI and CT scores were evaluated using Pearson or Spearman's rank correlation analyses. $p<0.05$ was considered statistically significant, and all analyses were performed using IBM SPSS Statistics v29 (IBM Corp., Armonk, NY).

RESULTS

A total of 123 patients, with or without nasal polyps, were included in the study. Of the participants, 81 (65.9%) were male and 42 (34.1%) were female. The mean age was 41.95±13.46 years, with no significant difference in age distribution between male and female patients ($p=0.332$). Eighty-four patients (68.3%) had nasal polyps, while 39 patients (31.7%) did not. BMI ranged from 17 to 42.90, with a mean BMI of 27.12±4.81. The demographic characteristics of patients in the obese and non-obese groups are presented in Table 1.

Comparison of mean SNOT-22 scores between the obese and normal groups showed no significant difference in total SNOT-22 score ($p=0.867$). Similarly, no significant differences were found between the obese and normal-weight groups across the items of the SNOT-22. Lund-Mackay scores obtained from paranasal sinus CT were also similar between the two groups ($p=0.430$).

When examining the relationships between BMI and SNOT-22 items, some items showed weak but statistically significant correlations with BMI. As BMI in female patients increased, nasal congestion severity increased (p=0.033) and cough scores decreased (p=0.009). Furthermore, among female patients, an increase in BMI was positively and significantly correlated with difficulty falling asleep (p=0.011), nighttime awakenings (p<0.001), and inability to get a good night's sleep (p<0.001). Among male patients, BMI was negatively correlated with sneezing (p=0.001) and positively correlated with postnasal drip (p=0.042).

In the analysis of the entire patient group, some items were also significantly related to BMI. The sneezing item showed a negative correlation with BMI (p=0.005), whereas the poor nighttime sleep quality item showed a positive correlation with BMI (p<0.001). The irritability item showed a positive correlation with BMI in female patients (p=0.012), and this relationship remained significant in the analysis of the entire patient group (p=0.038). The correlation values for SNOT-22 items associated with obesity in the female, male, and entire group are shown in Table 2.

DISCUSSION

In this study, we evaluated the effects of obesity on the symptom profile and radiologic disease burden in patients with CRS. The associations between BMI, SNOT-22 scores, and Lund-Mackay scores were analyzed. Our findings demonstrated no significant differences between obese and non-obese groups in total SNOT-22 or Lund-Mackay scores. However, correlation analyses of individual SNOT-22 items revealed that BMI was associated with specific symptoms.

In female patients, an increase in BMI was associated with higher scores for nasal congestion, difficulty falling asleep, nighttime awakenings, and embarrassed; in male patients, a decrease in sneezing scores and an increase in postnasal drip scores were observed. In the entire patient group, the item "lack of a good night's sleep" showed a positive correlation with BMI. These findings suggest that obesity may affect certain symptom patterns, even if it does not significantly alter the total symptom burden.

These differences in symptom severity are consistent with the upper airway pathophysiology of obesity. In obese patients, increased body weight leads to narrowing of the upper airway, increased airway resistance, and a predisposition to nasopharyngeal collapse, resulting in reduced nasal airflow and more pronounced sleep-related symptoms[19]. For this reason, the positive correlations between BMI and measures of nasal blockage and sleep quality are biologically plausible. The negative correlation between sneezing and BMI may be explained by obesity-related alterations in sensory perception and neuromuscular responses[20]. In addition, the association between BMI and the item assessing embarrassed may reflect the negative impact of obesity on quality of life and the increased psychosocial burden experienced by these patients.

Table 1. Demographic characteristics of obese and normal-weight patients

Age (Mean ± SD)	43.53±12.94	41.4±13.66	0.442
Gender, n (%)	Female: 10 (31.2%) Male: 22 (68.8%)	Female: 32 (35.2%) Male: 59 (64.8%)	0.668
BMI (kg/m²) (Mean ± SD)	33.68±3.10	24.8±2.73	<0.01
Nasal polyp, n (%)	Present: 21 (65.6%) Absent: 11 (34.4%)	Present: 63 (69.2%) Absent: 28 (30.8%)	0.706
Lund-Mackay score (mean)	15.13	16.24	0.43

BMI: Body mass index, SD: Standard deviation

Table 2. Correlation coefficients for SNOT-22 items showing a significant relationship with BMI

SNOT-22 item	Female (rs/p)	Male (rs/p)	Total (rs/p)
Nasal blockage	0.329/0.033*	—	—
Sneezing	—	-0.347/0.001*	-0.250/0.005*
Cough	0.397/0.009*	—	—
Post-nasal discharge	—	0.226/0.042*	—
Difficulty falling asleep	0.388/0.011*	—	—
Wake up at night	0.557/<0.001*	—	—
Lack of a good night's sleep	0.505/<0.001*	—	0.181/0.045*
Embarrassed	0.385/0.012*	—	0.187/0.038*

*: p<0.05, SNOT-22: 22-item Sinonasal Outcome test, BMI: Body mass index

The positive correlation between nasal blockage and BMI observed in female patients may reflect obesity-related, sex-specific hormonal and inflammatory mechanisms affecting the nasal mucosa. Estrogen is known to predispose individuals to nasal congestion through increased acetylcholinesterase activity and alterations in vascular tone[21]. In obesity, peripheral adipose tissue exhibits increased aromatase activity, leading to enhanced conversion of androgens to estrogens. The resulting increase in estrogenic activity in obese female patients may further exacerbate pre-existing edematous changes in the sinonasal mucosa, thereby intensifying the sensation of nasal obstruction.

In addition, adipokines, such as leptin, which play a key role in obesity-associated systemic inflammation, are present at higher levels in females than in males. Leptin receptors have been identified in the nasal mucosa, where leptin can induce the release of pro-inflammatory cytokines[22]. This finding suggests that, in female patients in our study, the effect of obesity on nasal obstruction symptoms is mediated primarily by mucosal inflammation and edema rather than by structural narrowing, as evidenced by the absence of a significant change in Lund-Mackay scores. In male patients, the closer association between obesity and postnasal drip suggests that obesity-characterized by a predominance of visceral adiposity-may be linked to a distinct inflammatory pathway or impaired mucociliary clearance.

To the best of our knowledge, no previous study has performed a detailed, item-level analysis of the relationship between BMI and individual SNOT-22 components; in this respect, our study is unique. In a study by Chen et al.[23] that included 325 patients, symptoms were assessed using the visual analog scale. Although obese and overweight patients demonstrated higher scores for certain symptoms, these differences did not reach statistical significance[23]. In the same study, consistent with our findings, no significant association was observed between obesity and Lund-Mackay scores. Bhattacharyya,[15] in a large-scale US population study, reported a strong association between obesity and CRS and emphasized the need for further research to investigate how obesity influences CRS severity. Similarly, EPOS 2020 identified the impact of obesity on CRS as a research priority. In line with this recommendation, our study contributes to the literature by evaluating obesity-related effects at the symptom level, using individual SNOT-22 items.

Study Limitations

Our study has several limitations. The relatively small number of obese patients, compared with the normal-weight group, may have reduced the statistical power for comparisons between groups. In addition, the predominance of male patients and patients with nasal polyps in our cohort limited the feasibility of detailed subgroup analyses. The SNOT-22 is a subjective assessment tool and may be influenced by emotional or behavioral factors, potentially leading to variability in symptom reporting. Furthermore, due to the cross-sectional design of the study, causal relationships between BMI and symptom severity cannot be established.

CONCLUSION

In patients with CRS, obesity does not appear to directly increase the overall disease burden; however, it may modify the clinical expression of the disease in a sex-specific manner. Obesity is predominantly associated with nasal obstruction and sleep-related disturbances in female patients, whereas postnasal drip appears to be more prominent in male patients. These findings suggest that, in the management of obese patients with CRS, personalized treatment strategies that take sex and BMI into account may be more effective than a standardized approach. To fully elucidate the impact of obesity on CRS, future studies should focus on symptom clusters and the underlying sex-specific inflammatory mechanisms.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee with (decision number 2024.236.07.11, date: 30.07.2024) and was conducted in accordance with the Declaration of Helsinki.

Informed Consent: The study is a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.K., Concept: Ö.K., M.F.E., Design: M.F.E., Data Collection or Processing: Ö.K., M.F.E., Analysis or Interpretation: Ö.K., Literature Search: M.F.E., Writing: Ö.K., M.F.E.

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The Effects of ODD Comorbidity on Internalizing and Externalizing Symptoms in Children and Adolescents with ADHD

DEHB Olan Çocuk ve Ergenlerde KOKGB Komorbiditesinin İçselleştirme ve Dışsallaştırma Semptomları Üzerindeki Etkileri

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ABSTRACT

Aim: Oppositional defiant disorder (ODD) is the most common psychiatric disorder comorbid with attention deficit hyperactivity disorder (ADHD). This study aims to identify the areas of difficulty and to evaluate the internalizing and externalizing symptoms of children and adolescents diagnosed with ADHD with and without ODD comorbidity.

Materials and Methods: The study was conducted with 51 children and adolescents with ADHD and 51 children and adolescents with ADHD+ODD. The Strengths and Difficulties Questionnaire (SDQ) was used to determine the areas of difficulty experienced by the children, and the Revised Child Anxiety and Depression scale was used to assess depression and anxiety levels, rated separately by the children and their parents.

Results: In children's statements, emotional problems, peer problems, internalization, total difficulty, and de-pression scores were significantly higher in the ADHD+ODD group compared to the ADHD group ($p<0.05$). In the ADHD+ODD group, behavioral problems, attention-deficit/hyperactivity, externalization, total difficulties, anxiety, and depression scores were found to be higher on the parental scales compared to the ADHD group. In the binary logistic regression analysis examining the predictive factors related to the presence of ODD comor-bidity, it was found that externalizing symptoms such as conduct problems and attention-deficit/hyperactivity obtained from the parent-rated SDQ sub-scales predicted ODD.

Conclusion: Our findings suggest that the presence of ODD comorbidity leads to an increase in internalizing and externalizing symptoms in children with ADHD and that children's and parents' reports of subjective expe-riences may differ from each other.

Keywords: Attention deficit hyperactivity disorder, oppositional defiant disorder, internalizing, externalizing, symptoms

ÖZ

Amaç: Bu çalışmada, karşıt olma karşı gelme bozukluğu (KOKGB) komorbiditesi olan ve olmayan dikkat eksikliği hiperaktivite bozukluğuna (DEHB) tanılı çocuk ve ergenlerin güçlük yaşadıkları alanların tespit edilmesi, içselleştirme ve dışsallaştırma semptomlarının değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya DEHB olan 51 çocuk ve ergen ve DEHB+KOKGB olan 51 çocuk ve ergen dahil edildi. Katılımcılar okul çağı çocukları için duygulanım bozuklukları ve şizofreni çizelgesi, şimdi ve yaşam boyu versiyonu kullanılarak değerlendirildi. Çocukların yaşadıkları zorluk alanlarını belirlemek için Güçler ve Güçlükler Anketi (GGA), depresyon ve anksiyete düzeylerini değerlendirmek çocuklar için kaygı ve depresyon ölçeği-yenilenmiş kullanıldı.

Bulgular: Çocuk bildirimlerinde DEHB+KOKGB grubunda duygusal sorunlar, akran sorunları, internalizasy-on, toplam güçlük ve depresyon puanlarının DEHB grubuna göre anlamlı derecede yüksek olduğu saptanmıştır. DEHB+KOKGB grubunda DEHB grubuna kıyasla ebeveyn ölçeklerinde davranış sorunları, dikkat eksikliği/aşırı hareketlilik, eksternalizasyon, toplam güçlük, anksiyete ve depresyon puanlarının daha yüksek olduğu bulunmuştur. KOKGB komorbiditesi varlığı ile ilgili yordayıcı faktörlerin incelendiği binary lojistik regresyon analizinde, ebeveyn tarafından derecelendirilen GGA

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alt ölçeklerinden elde edilen davranış sorunları ve dikkat eksikliği/aşırı hareketlilik gibi dışsallaştırma semptomlarının KOKGB'nu öngördüğü tespit edilmiştir.

Sonuç: Bulgularımız, KOKGB komorbiditesi varlığının DEHB'li çocuklarda içselleştirme ve dışsallaştırma semptomlarında artışa neden olduğunu ve çocukların ve ebeveynlerin öznel deneyimler yoluyla verdikleri rapor-ların birbirlerinden farklılık gösterebileceğini düşündürmektedir.

Anahtar Kelimeler: Dikkat eksikliği hiperaktivite bozukluğu, karşıt olma-karşı gelme bozukluğu, içselleştirme, dışsallaştırma, belirtiler

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that is characterized by developmentally inappropriate levels of inattention, hyperactivity and impulsivity[1]. Although the age of onset is in childhood, persistence in adolescence and adulthood is common. ADHD is more common in males, and both externalizing and internalizing disorders are quite prevalent[2]. Oppositional defiant disorder (ODD) is the most common comorbid disorder among children with ADHD[3]. The high comorbidity of ADHD and ODD is evident even in the early years of life[4]. ADHD and ODD alone can cause significant impairments in family, school, and social functioning. Additionally, functional impairments worsen when these disorders co-occur[5]. It has been reported that when ADHD and ODD symptoms are observed together, they result in different and more negative clinical manifestations than the effects of ADHD symptoms alone[6]. In addition, it has been shown that the association of ADHD and ODD leads to higher behavioral and emotional difficulties[7]. Symptoms of oppositional defiant behavior, inattention, and hyperactivity are more severe when ADHD and ODD co-occur[8]. Another study showed that the ADHD+ODD group exhibited higher anxiety/depression scores, more problems in peer relationships, and lower academic achievement than the ADHD group[9].

The existing literature reports that the relationship between ADHD and ODD is associated with academic performance, depressive symptoms, peer rejection, and peer bullying in childhood and that this association may lead to more negative outcomes at later ages[10]. Most of these studies were based on symptoms reported by parents. Studies focusing on symptom assessment indicate that reports given by children and parents through subjective and objective experiences differ from each other[11]. It is known that parent-reported measures can report more accurately about a child's objective experiences, whereas child self-report measures provide important information about subjective experiences that cannot be obtained from other sources such as parents. Considering all these, it seems to be a more accurate approach to make use of both parent- and child-reported measures in symptom assessment.

This study aimed to identify areas of difficulty and assess depression and anxiety levels in children and adolescents with ADHD with and without ODD. Our aim was not only to focus on externalizing and internalizing symptoms but also to assess how children and parents perceive symptoms using both self-report and parent-reported measures. We hypothesized that parents are a better source of information than children for externalizing symptoms due to their more observable nature and that children are a better source of information for internalizing symptoms because they experience less observable emotional difficulties.

MATERIALS AND METHODS

Participants

The study sample consisted of children and adolescents aged 8-17 years with ADHD with and without comorbidity of ODD who were admitted to the Child and Adolescent Psychiatry Outpatient Clinic between June 2022 and October 2022. The study groups were divided into ADHD without comorbidity and ADHD with ODD. Participants with psychiatric disorders other than ADHD and ODD (specific learning disorder, intellectual disability, other neurodevelopmental disorders including autism spectrum disorder, conduct disorder, major depression, anxiety disorders) were excluded. Participants with a history of used psychiatric medications in the last 6 months were also excluded from thestudy.

All study procedures were planned according to the Declaration of Helsinki, and the local laws and regulations. The research was carried out by taking the necessary permissions from the Ethics Committee of the Clinical Research, Atatürk University Faculty of Medicine (decision no: B.30.2.0.01.00/475, date: 02.06.2022).

The study procedure was explained to the participants and their parents, and written informed consent was obtained from parents who agreed to participate in the study. In addition, the children and their parents verbally agreed to participate in the study.

Procedure

Sociodemographic and clinical data were obtained using a questionnaire prepared by the researchers. All children and adolescents in the study group were evaluated by a certified child and adolescent psychiatrist using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-

Present and Lifetime Version (K-SADS-PL)[12], and ADHD and ADHD+ODD were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria[1]. The K-SADS-PL has been used to support ADHD and ODD diagnoses according to the DSM-5 criteria, as well as to identify possible psychiatric comorbidities, through clinical interviews. The Strengths and Difficulties Questionnaire (SDQ)[13] was used to determine the areas of difficulty experienced by the subjects, and the Revised Child Anxiety and Depression scale (RCADS)[14] was completed by the subjects themselves and their parents to assess depression and anxiety levels. Psychiatric diagnoses were made by child and adolescent psychiatrists through clinical interviews, while internalizing and externalizing symptoms were assessed using scales.

Materials

Sociodemographic Data Form: The questionnaire prepared within the scope of the study was used to collect information about the demographic data (age, gender, grade, duration of maternal and paternal education, income status, etc.) of the children and their parents.

Schedule for Affective Disorders and Schizophrenia for School- Age Children-Present and Lifetime version DSM-5 November 2016 - Turkish Adaptation: This schedule was developed by Kaufman et al.[12]. The same interview chart was renewed to comply with the DSM-5 diagnostic criteria. In the first part of the interview schedule, basic information about the sociodemographic characteristics, complaints, developmental history, and general functionality of the child and his/her family was questioned in an unstructured interview. The second part included screening questions that assessed more than 200 specific symptoms, both past and present (in the last two months). The third part consisted of assessment and observation results to confirm the DSM-5 diagnoses. At the end of the interview, the clinician's observations and information received were evaluated and scored together. Its Turkish validity and reliability study was conducted by Ünal et al.[15].

Strengths and Difficulties Questionnaire: The SDQ was developed by Goodman[13] for use in screening mental health problems in children and adolescents. The subscales of the scale consist of attention deficit/hyperactivity, behavioral problems, emotional problems, peer problems, and social behaviors. Each subscale can be evaluated separately, and a score can be obtained for each scale. Alternatively, a total difficulty score can be calculated with the sum of the first four subscales. The Turkish version of the scale adapted to Turkish by Güvenir et al.[16] was found to be valid and reliable.

Revised Child Anxiety and Depression Scale: It was developed by Chorpita et al.[14]. The 47-item scale comprises six subtests: separation anxiety disorder, generalized anxiety disorder, panic disorder, social phobia, obsessive-compulsive disorder, and major depressive disorder. Total anxiety and total internalization scores were obtained by combining subscale scores. Both the child and parent versions were adapted into Turkish by Gormez et al.[17,18].

Statistical Analysis

The data obtained in this study were evaluated using the SPSS 24.0 statistics software package (SPSS Inc., Chicago, IL, USA). All variables were evaluated using the Shapiro-Wilk test to determine whether the distribution was normal. Student's t or Mann-Whitney U tests were used to compare the differences between the two groups according to their distribution. Pearson's chi-square and Fisher's exact tests were used to evaluate the distribution of categorical variables. Binary logistic regression analysis was used to examine the variables that predicted the presence of ODD comorbidity. Statistical significance was set at $p<0.05$.

RESULTS

The study was conducted with 51 children and adolescents (36 boys and 15 girls) with ADHD and 51 children and adolescents (45 boys and 6 girls) with ADHD+ODD. There was a statistically significant difference between the groups in terms of sex ($p=0.022$). The mean age was 13.34±2.77 in the ADHD group, and 11.62±2.31 in the ADHD+ODD group, and a significant difference was found between these values ($p=0.028$). There was no statistically significant difference between the two groups in terms of age, educational level, and family income of the parents ($p>0.05$). Demographic characteristics of the participants are presented in Table 1.

A comparison of the SDQ and RCADS children's scores in the study groups is shown in Table 2. The total difficulty and internalization scores were significantly higher in the ADHD+ODD group than in the ADHD group ($p=0.003$, $p=0.001$ respectively). The SDQ subscale analysis revealed that emotional problems and peer problems scores were significantly higher in the ADHD+ODD group ($p=0.017$, $p=0.028$ respectively). In terms of the RCADS children's scores, only the RCADS depression score was found to be significantly higher in the ADHD+ODD group than in the ADHD group ($p=0.005$).

A comparison of the SDQ and RCADS parental scores of the study groups is shown in Table 3. The total difficulty and externalization scores were significantly higher in the ADHD+ODD group than in the ADHD group ($p=0.006$, $p=0.001$ respectively). In the SDQ subscale analysis, conduct problems and attention-deficit/hyperactivity scores were significantly higher in the ADHD+ODD group ($p=0.001$, $p=0.005$, respectively). In terms of the RCADS parental scores, the total RCADS internalization score was significantly higher in the

Table 1. Sociodemographic characteristics of the participants

	ADHD (n=51)	ADHD+ODD (n=51)	p
Age (years)			
Mean ± SD	13.34±2.77	11.62±2.31	**0.028**[a]
Gender, n (%)			
Male	36 (70.6)	45 (88.2)	**0.022**[c]
Female	15 (29.4)	6 (11.8)	
Mothers' age (years)			
Mean ± SD	39.09±6.52	38.96±6.30	0.945[a]
Mothers' schooling (years)			
Mean ± SD	8.39±4.42	6.26±3.65	0.082[b]
Mother's psychiatric disorder, n (%)	12 (23.5)	49 (17.6)	0.463[c]
Fathers' age (years)			
Mean ± SD	44.51±6.83	44.14±7.22	0.869[a]
Fathers' schooling (years)			
Mean ± SD	9.64±4.18	8.55±3.62	0.360[b]
Father's psychiatric disorder, n (%)	11 (21.6)	14 (27.5)	0.489[c]
Family income, n (%)			
Low	24 (47.1)	31 (60.8)	
Middle	19 (37.3)	14 (27.5)	0.380[c]
High	8 (15.7)	6 (11.8)	

p<0.05 statistically significant; bold p-values highlight where the comparisons between

[a]: Student's t-test, [b]: Mann-Whitney U test, [c]: Chi-square test, ADHD and ADHD+ODD groups are statistically significant

ADHD: Attention deficit hyperactivity disorder, ODD: Oppositional defiant disorder, SD: Standard deviation

Table 2. Comparison of the study groups' SDQ and RCADS child scores

	ADHD (n=51)	ADHD+ODD (n=51)	p
SDQ Emotional symptoms	2.96±2.07	4.43±1.95	**0.017**[a]
SDQ conduct problems	3.01±1.53	3.96±2.03	0.079[b]
SDQ hyperactivity-inattention	5.22±1.38	5.17±1.96	0.931[a]
SDQ peer problems	3.96±1.63	5.03±1.47	**0.028**[a]
SDQ prosocial	7.17±2.08	6.65±2.41	0.436[b]
SDQ internalizing difficulties	6.91±2.48	9.52±2.60	**0.001**[a]
SDQ externalizing difficulties	8.22±1.99	9.13±2.68	0.198[a]
SDQ total difficulties	15.13±3.61	18.65±3.88	**0.003**[a]
RCADS SoP	10.17±5.31	9.43±5.57	0.647[b]
RCADS PD	4.01±3.46	5.91±5.03	0.141[b]
RCADS SAD	3.57±3.01	4.83±3.79	0.219[b]
RCADS GAD	5.87±3.07	6.09±3.61	0.827[a]
RCADS OCD	4.87±2.61	5.35±3.41	0.596[a]
RCADS MDD	6.96±4.95	11.09±4.40	**0.005**[a]
RCADS anxiety total score	28.48±12.71	31.61±16.57	0.476[a]
RCADS total (internalizing) score	35.43±16.31	42.70±18.43	0.164[a]

p<0.05 statistically significant; bold p-values highlight where the comparisons between

[a]: Student's t-test, [b]: Mann-Whitney U test

ADHD and ADHD+ODD groups are statistically significant

ADHD: Attention deficit hyperactivity disorder, ODD: Oppositional defiant disorder, SDQ: Strengths and Difficulties Questionnaire, RCADS: Revised Child Anxiety and Depression scales, SoP: Social phobia, PD: Panic disorder, SAD: Separation anxiety disorder, GAD: Generalized anxiety disorder, OCD: Obsessive-compulsive disorder, MDD: Major depressive disorder

ADHD+ODD group compared to the ADHD group (p=0.041). The subscale analysis of the RCADS revealed that separation anxiety disorder, generalized anxiety disorder, and depression scores were significantly higher in the ADHD+ODD group (p<0.05).

A regression model was created to independently determine the predictors of ODD. The relationship between the subscale scores that were found to be statistically significant as a result of pairwise comparisons and the presence of ODD comorbidity was estimated using binary logistic regression analysis. The conduct problems sub-scale [odds ratio (OR)=3.353, p=0.020] and the attention-deficit/hyperactivity sub-scale (OR=2.765, p=0.021) of the SDQ parent form were found to be significantly correlated with the presence of ODD comorbidity. The binary logistic regression analysis of the predictors of ODD presence is presented in Table 4.

Table 3. Comparison of the study groups' SDQ and RCADS parent scores

	ADHD (n=51)	ADHD+ODD (n=51)	p
SDQ emotional symptoms	3.26±1.86	4.00±2.69	0.285[b]
SDQ conduct problems	2.70±1.39	4.35±1.72	**0.001**[a]
SDQ hyperactivity-inattention	4.83±1.11	6.04±1.63	**0.005**[a]
SDQ peer problems	5.43±1.72	5.04± 2.21	0.506[b]
SDQ prosocial	7.52±2.06	6.30±2.40	0.072[b]
SDQ internalizing difficulties	8.70±2.58	9.04±4.11	0.733[b]
SDQ externalizing difficulties	7.52±1.81	10.39±2.29	**<0.001**[a]
SDQ total difficulties	16.22±3.24	19.43±4.25	**0.006**[a]
RCADS SoP	9.35±5.17	8.61±4.79	0.618[a]
RCADS PD	2.52±2.39	3.96±3.51	0.112[b]
RCADS SAD	3.65±2.97	6.26±4.27	**0.021**[a]
RCADS GAD	3.61±1.99	5.39±3.11	**0.026**[a]
RCADS OCD	2.74±3.12	4.26±3.16	0.108[b]
RCADS MDD	7.74±3.92	11.04±5.07	**0.017**[a]
RCADS anxiety total score	21.87±11.56	28.48±14.01	0.088[b]
RCADS total (internalizing) score	29.61±14.50	39.52±17.27	**0.041**[a]

p<0.05 statistically significant; bold p-values highlight where the comparisons between

[a]: Student's t-test; [b]: Mann-Whitney U test

ADHD and ADHD+ODD groups are statistically significant

ADHD: Attention deficit hyperactivity disorder, ODD: Oppositional defiant disorder, SDQ: Strengths and Difficulties Questionnaire, RCADS: Revised Child Anxiety and Depression scales, SoP: Social phobia, PD: Panic disorder, SAD: Separation anxiety disorder, GAD: Generalized anxiety disorder, OCD: Obsessive-compulsive disorder, MDD: Major depressive disorder

Table 4. Binary logistic regression predictors of the presence of ODD comorbidity

	B	SE	Wald	OR	95% CI-OR	p
SDQ emotional symptoms-child	0.434	0.343	1.601	1.543	0.788-3.020	0.206
SDQ peer problems-child	0.314	0.312	1.017	1.369	0.743-2.522	0.313
RCADS MDD-child	-0.070	0.139	0.252	0.933	0.710-1.225	0.616
SDQ conduct problems-parent	1.210	0.519	5.434	3.353	1.212-9.274	**0.020**
SDQ hyperactivity-inattention-parent	1.017	0.441	5.326	2.765	1.166-6.560	**0.021**
RCADS MDD-parent	0.283	0.170	2.758	1.327	0.950-1.853	0.097
RCADS SAD-parent	0.435	0.258	2.853	1.546	0.933-2.562	0.091
RCADS GAD-parent	-0.405	0.311	1.694	0.667	0.363-1.227	0.193

p<0.05 statistically significant; bold p-values highlight where it is statistically significant

SE: Standard error, OR: Odds ratio, CI: Confidence interval (lower limit-upper limit)

ODD: Oppositional defiant disorder, SDQ: Strengths and Difficulties Questionnaire, RCADS: Revised Child Anxiety and Depression scales, MDD: Major depressive disorder, SAD: Separation anxiety disorder, GAD: Generalized anxiety disorder

DISCUSSION

This study assessed the areas of difficulty, internalizing, and externalizing symptoms in children and adolescents with ADHD with and without ODD comorbidity. To the best of our knowledge, this is the first study to assess the internalizing and externalizing symptoms of children and adolescents with ADHD with and without ODD comorbidity in detail using both self-reported and parent-reported measures. Our findings suggest that the presence of ODD comorbidity leads to an increase in internalizing and externalizing symptoms in children with ADHD and that children's and parents' reports of subjective experiences differ from each other. Additionally, our findings also indicate that externalizing symptoms, such as conduct problems and attention-deficit/hyperactivity obtained from the parent-rated SDQ sub-scales predicted ODD.

In the present study, a significant difference was found between the two groups in terms of age and gender of the children. Similarly, in many studies, it has been observed that the vast majority of children with both ADHD and ODD are male, and the age of children with ODD comorbidity tends to be younger[7,19]. In this respect, the results of our study are in line with the literature.

In the present study, when children's SDQ scores were examined, it was observed that emotional problems, peer problems, internalization, and total difficulty scores were significantly higher in the ADHD+ODD group than in the ADHD group. Sobanski et al.[20] found that emotional problems were more closely related to ODD comorbidity than to core symptoms of ADHD. Likewise, Biederman et al.[21] showed that worsening anxiety/depressive, attention, and aggression subscales of the child behavior checklist (CBCL) were strongly associated with higher rates of ODD. Moreover, in a study comparing the executive functions of children with ADHD and children with ADHD with comorbidity of ODD, more severe deficits in emotional control were found in children with ADHD and ODD[22]. In addition, ODD symptoms in middle childhood and adolescence are associated with peer problems such as peer rejection and peer bullying[23]. It has been reported that children with ODD assessed with the SDQ have a higher risk of emotional symptoms and peer problems[24]. In line with the literature, it was found that children in the ADHD+ODD group experienced more emotional problems and peer problems. On the other hand, the RCADS depression sub-scale scores of the children were significantly higher in the group with ODD. Consistent with this result, it has been shown that ODD symptoms in adolescence are directly related to depressive symptoms assessed through self-reported scales[25]. Indeed, these findings show that the comorbidity of ODD, which is defined as an externalizing disorder, in children and adolescents with ADHD poses a high risk of internalizing symptoms.

Another main finding of the study was that the ADHD+ODD group had higher scores for conduct problems, attention deficit/hyperactivity, anxiety, and depression on the SDQ and RCADS parent subscales compared to the ADHD group. The differences in these parental sub-scales suggest that ADHD cases with ODD tend to suffer from both internalizing and externalizing symptoms more than those without ODD. The association between ODD and ADHD is consistent with the findings of previous studies indicating that they experience more severe emotional and behavioral difficulties[6,26]. In a study, it was shown that comorbidity of ADHD and ODD was associated with higher scores in both attention deficit and hyperactivity/impulsivity scores compared to the group with ADHD alone, according to parental reports[8]. In another study, similar to our study, anxiety/depression, attention problems, behavioral problems, and externalizing scores of the CBCL scale rated by the parents were found to be higher in the ADHD+ODD group compared to the ADHD-only group[9]. In addition, it has been reported that ODD in adolescents is associated with internalizing symptoms such as anxiety and depression as well as externalizing symptoms such as behavior problems[27]. These results provide evidence that ODD comorbidity harms the clinical symptoms of children with ADHD. In addition, it is believed that the recognition of ODD among children and adolescents with ADHD and the addition of appropriate intervention methods will reduce the negative effects that may occur in the future.

As experiencing and observing symptoms are separate situations, differences may be observed in children's and parents' reports. Since children's self-report measures provide important information about their less observable subjective experiences, parental reports may lead to inaccurate estimates, especially for assessing internalizing symptoms[14]. In contrast, parents have been shown to report more accurate reports about observable, objective experiences, and parental reports have been shown to provide a more precise estimate, especially in assessing externalizing symptoms[14,28]. In addition, it has been reported that parents are the best source of information for externalizing disorders, whereas children and adolescents are the best sources of information for internalizing disorders[29]. In the present study, the finding of a significant difference between the two groups in internalizing scores on the SDQ child form and, conversely, the finding of higher externalizing scores in the SDQ parental form in the ADHD+ODD group supports the finding that child and parent reports may differ from each other. Even if children's and parents' reports differ from each other, it is a fact that ODD leads to an increase in internalizing and externalizing symptoms of children with ADHD. Indeed, the results of our study are consistent with those of studies in which the ADHD+ODD group showed significantly more internalizing and externalizing problems compared to pure ADHD[19].

The results of the binary logistic regression analysis examining the predictive factors for the presence of ODD comorbidity showed that externalizing symptoms, such as conduct problems and attention-deficit/hyperactivity obtained from the parent-rated SDQ sub-scales predicted ODD. In a related study, it was reported that the SDQ parental form could be used as an appropriate screening tool to identify the comorbidity of ODD in an ADHD sample and that the SDQ had sufficient accuracy in predicting the comorbidity of ODD[19]. It has been reported that comorbid ODD increases the severity of symptoms of inattention and hyperactivity/impulsivity in children and adolescents diagnosed with ADHD[30]. According to reports from parents, children with both ADHD and ODD have been found to have higher scores for both inattention and hyperactivity/impulsivity than those with ADHD alone[8]. The same study also found that parental reports could help clinicians to identify suspected ODD in children and adolescents with ADHD. Having difficulty with parent-rated behavioral control in the presence of comorbid ODD in young people with ADHD was found to be an important predictor of social functioning[31]. Additionally, compared to the ADHD-only group, the ADHD+ODD group exhibited greater anxiety and depression, more aggressive behavior, and poorer emotional control in daily life[32]. When all of these results are considered together, the results of the present study are in line with the literature.

Study Limitations

Our results should be evaluated considering the various limitations. First, there was no healthy control group in this study. However, our main aim was to evaluate the effect of ODD comorbidity on internalizing and externalizing symptoms in children with ADHD compared to children with pure ADHD. Therefore, it is clear that having a healthy control group in the study would not have contributed to our results. Therefore, the absence of a control group was considered reasonable. Second, another limitation is the wide age range of the children included in the study. This affects the generalizability of the results of the present study to children and adolescents. Third, this study was limited by the lack of ADHD-specific measures. Consequently, it was not possible to assess the severity of ADHD and ODD symptoms. Many studies have shown that children with a co-diagnosis of ADHD and ODD have more severe inattention, hyperactivity/impulsivity, and oppositional symptoms than children with ADHD alone. Therefore, when designing the research, the areas in which the children had difficulties and other conditions that may accompany these symptoms, especially anxiety and depression were considered rather than core symptoms. Taking into account all these together, it should be considered a more accurate approach to not use ADHD-specific measurements in the study in line with our aim.

CONCLUSION

Our study showed that the presence of ODD comorbidity leads to an increase in internalizing and externalizing symptoms in children with ADHD. Moreover, children's and parents' reports of subjective experiences may differ. Evidence has shown that the comorbidity of ODD has a negative effect on the clinical symptoms of children with ADHD. In addition, early diagnosis of ODD comorbidity in children and adolescents with ADHD is critical as it causes worsening of internalizing and externalizing symptoms. Planning future studies with larger sample sizes and evaluating children and adolescents separately will make a significant contribution to the literature.

Ethics

Ethics Committee Approval: The research was carried out by taking the necessary permissions from the Ethics Committee of the Clinical Research, Atatürk University Faculty of Medicine, (decision no: B.30.2.0.01.00/475, date: 02.06.2022).

Informed Consent: Written informed consent was obtained from all participants.

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Footnotes

Authorship Contributions

Concept: M.A.A., E.Y.D., Design: M.A.A., B.T., Data Collection or Processing: M.A.A., E.Y.D., Analysis or Interpretation: M.A.A., B.T., Literature Search: M.A.A., B.T., Writing: M.A.A.

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

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ORIGINAL ARTICLE

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The Relationship between the rs5918 Polymorphism Serum GPIIb/IIIa Levels, and Biochemical Parameters in Pulmonary Embolism Associated with COVID-19 Infection

COVID-19 Enfeksiyonuna Bağlı Pulmoner Embolide rs5918 Polimorfizmi ile Serum GPIIb/IIIa Düzeyleri ve Biyokimyasal Parametreler Arasındaki İlişki

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ABSTRACT

Aim: This study aimed to investigate the potential associations among serum levels of the glycoprotein Glycoprotein IIb/IIIa (GPIIb/IIIa) which plays a critical role in platelet function the rs5918 polymorphism in the *ITGB3* gene, and biochemical parameters (platelet count, D-dimer, fibrinogen, mean platelet volume, C-reactive protein) in patients who developed pulmonary embolism (PE) due to coronavirus disease 2019 (COVID-19) infection.

Materials and Methods: The study was conducted using DNA and serum samples from 80 adult patients diagnosed with COVID-19 between 2020 and 2022. Patients were divided into two groups as PE positive PE(+) and PE negative PE(-). The rs5918 polymorphism was analyzed using the TaqMan single nucleotide polymorphism genotyping method on a real-time polymerase chain reaction system. Serum GPIIb/IIIa levels were measured using the ELISA method. Demographic, hematological, and biochemical data were retrieved from patient records and analyzed statistically.

Results: Platelet count ($p=0.007$) and D-dimer levels ($p<0.001$) were found to be significantly higher in the PE(+) group. No significant difference was observed in GPIIb/IIIa serum levels between the PE(+) and PE(-) groups ($p=0.42$). There was no statistically significant difference between the groups regarding rs5918 genotype and allele distributions. However, significant differences in D-dimer levels were observed across genotypes.

Conclusion: Although rs5918 polymorphism and GPIIb/IIIa serum levels were not directly associated with the development of PE in COVID-19 patients, the findings suggest that genetic variation may influence certain coagulation parameters. These results indicate the need for further studies to explore genetic determinants of thrombotic processes associated with COVID-19.

Keywords: COVID-19, pulmonary embolism, platelet, rs5918, GPIIb/IIIa

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ÖZ

Amaç: Bu çalışma, koronavirüs hastalığı 2019 (COVID-19) enfeksiyonu nedeniyle pulmoner emboli (PE) gelişen hastalarda, platelet fonksiyonunda kritik rol oynayan glikoprotein Glikoprotein IIb/IIIa (GPIIb/IIIa) serum seviyeleri ile *ITGB3* genindeki rs5918 polimorfizmi ve biyokimyasal parametreler (platelet sayısı, D-dimer, fibrinojen, ortalama platelet hacmi, C-reaktif protein) arasındaki olası ilişkileri araştırmayı amaçlamıştır.

Gereç ve Yöntem: Çalışma, 2020-2022 yılları arasında COVID-19 tanısı konmuş 80 yetişkin hastadan alınan DNA ve serum örnekleri kullanılarak yürütülmüştür. Hastalar PE pozitif PE(+) ve PE negatif PE(-) olmak üzere iki gruba ayrılmıştır. rs5918 polimorfizmi, gerçek zamanlı polimeraz zincir reaksiyonu sistemi üzerinde TaqMan tek nükleotid polimorfizmi genotipleme yöntemi ile analiz edilmiştir. Serum GPIIb/IIIa seviyeleri ELISA yöntemi ile ölçülmüştür. Demografik, hematolojik ve biyokimyasal veriler hasta kayıtlarından elde edilerek istatistiksel olarak analiz edilmiştir.

Bulgular: PE(+) grubunda platelet sayısı (p=0,007) ve D-dimer seviyeleri (p<0,001) anlamlı derecede yüksek bulunmuştur. GPIIb/IIIa serum seviyeleri açısından PE(+) ve PE(-) grupları arasında anlamlı bir fark gözlenmemiştir (p=0,42). Gruplar arasında rs5918 genotip ve allel dağılımları açısından istatistiksel olarak anlamlı bir fark bulunmamıştır. Bununla birlikte, D-dimer seviyelerinde genotiplere göre anlamlı farklılıklar gözlenmiştir.

Sonuç: Rs5918 polimorfizmi ve GPIIb/IIIa serum seviyeleri COVID-19 hastalarında PE gelişimi ile doğrudan ilişkili olmasa da, genetik varyasyonun bazı koagülasyon parametrelerini etkileyebileceği düşünülmektedir. Bu bulgular, COVID-19 ile ilişkili trombotik süreçlerin genetik belirleyicilerini araştıran daha kapsamlı çalışmalara ihtiyaç olduğunu göstermektedir.

Anahtar Kelimeler: COVID-19, pulmoner emboli, platelet, rs5918, GPIIb/IIIa

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has been reported to induce hypercoagulability and to lead to both arterial and venous thromboembolic events[1,2]. In cases of pulmonary embolism (PE) associated with COVID-19 infection, increased platelet activation and a corresponding rise in the tendency of blood to clot have been demonstrated[3]. In addition, studies in the literature have shown that blood fibrinogen levels are significantly elevated following COVID-19 infection[4].

Platelets, which originate from megakaryocytes in the bone marrow and have a diameter of 2-4 µm, are the smallest cellular components in the blood and play a central role in hemostasis and thrombosis[5]. On the platelet surface, the Glycoprotein IIb/IIIa (GPIIb/IIIa) receptor, a member of the integrin family, exhibits high-affinity binding to fibrinogen[6]. This receptor is formed by the combination of the GPIIb and GPIIIa subunits[7,8] and is the most abundant receptor on the platelet surface[9]. The GPIIIa subunit contains extensive polymorphic regions[10].

The rs5918 polymorphism located within this region leads to the substitution of leucine by proline in the GPIIIa protein, resulting in a conformational change in the protein structure[10]. This rs5918 variant is defined as the T1565C polymorphism in the human platelet antigen-1 system. It is a single nucleotide polymorphism (SNP) in exon 2 of the *ITGB3* gene on chromosome 17 and causes a Leu33Pro (L33P) amino acid substitution in the GPIIb/IIIa protein on the platelet surface. The rs5918 genotypes are classified as follows: TT (homozygous wild-type, Leu33), TC (heterozygous), and CC (homozygous mutant, Pro33)[10].

Previous studies have suggested that this polymorphism increases the binding affinity of the GPIIb/IIIa receptor for fibrinogen and facilitates platelet aggregation, thereby potentially enhancing thrombotic susceptibility[11,12]. Although endothelial damage and disturbances in coagulation mechanisms have been reported in patients with PE due to COVID-19 infection, the relationships between cellular interactions and the underlying molecular and biochemical mechanisms have not been fully elucidated. To date, no study has been identified in the literature that investigates the relationship between GPIIb/IIIa levels and the rs5918 polymorphism in patients with PE associated with COVID-19 infection.

Therefore, in this study, we aimed to investigate, in addition to parameters involved in coagulation mechanisms, the association between serum GPIIb/IIIa levels and the rs5918 polymorphism in patients with PE secondary to COVID-19 infection.

MATERIALS AND METHODS

This retrospective case-control study was conducted using DNA and serum samples obtained from 80 individuals aged 18 years or older with laboratory-confirmed COVID-19 infection. During the study period (2020-2022), the diagnosis of COVID-19 was established in accordance with the interim guidance of the World Health Organization and the national COVID-19 management guidelines issued by the Turkish Ministry of Health that were in effect at the time. Confirmation of SARS-CoV-2 infection was achieved by real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis of nasopharyngeal and/or oropharyngeal swab specimens collected at hospital admission. Only patients with at least one documented positive RT-PCR result were included in the study.

Ethical approval for the study was obtained from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University, Faculty of Medicine (protocol no: 2023.90.05.09, date: 30.05.2023).

Patient Study Group

This study included DNA and serum samples obtained from 80 individuals aged 18 years and older who had laboratory-confirmed COVID-19 infection between 2020 and 2022. The study population consisted of patients who developed PE and those who did not. All participants had at least one documented positive SARS-CoV-2 RT-PCR test result in their medical records. PE was diagnosed by computed tomography pulmonary angiography (CTPA) and defined as the presence of a new intraluminal filling defect within the pulmonary arterial tree. Patients without radiological evidence of PE on CTPA were assigned to the PE negative PE(-) group.

Blood Collection, DNA Isolation, and Serum Preparation

For genetic analyses, 2 mL of peripheral venous blood was collected from each participant into tubes containing ethylenediaminetetraacetic acid as an anticoagulant. Genomic DNA was extracted from peripheral blood mononuclear cells using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The concentration and purity of the isolated DNA were determined spectrophotometrically by measuring absorbance at 260/280 nm using a NanoDrop Lite Plus spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA).

For biochemical analyses, 2 mL of peripheral venous blood was collected into serum separator (plain) tubes. The samples were allowed to clot at room temperature for 20-30 minutes and were then centrifuged at 4000 rpm for 10 minutes. The resulting serum was carefully separated and aliquoted into polypropylene tubes to prevent repeated freeze-thaw cycles. Serum samples were stored at -80 °C until analysis. Each sample was thawed only once and immediately used for the determination of GPIIb/IIIa levels by enzyme-linked immunosorbent assay (ELISA).

SNP Genotyping

Genotype Analysis

Detection and analysis of the rs5918 SNP were performed on human genomic DNA isolated from patients' peripheral blood samples using a real-time PCR system (Bio-Rad CFX Connect Real-Time PCR Detection System, California 94547, USA). The TaqMan SNP genotyping method was employed to genotype the polymorphic region at codon 33 of exon 2 (T1565C, dbSNP ID: rs5918) of the *ITGB3* gene on chromosome 17. Specifically, the ITGB3 rs5918 (T1565C) polymorphism was genotyped using a commercially available TaqMan SNP genotyping assay (Assay ID: C_818008_30, Thermo Fisher Scientific, Waltham, MA, USA) on the Bio-Rad CFX Connect Real-Time PCR System (Bio-Rad Laboratories, Hercules, CA, USA).

According to the manufacturer's information, the polymorphic region in exon 2 of the *ITGB3* gene was amplified using gene-specific primers [context sequence: [(VIC/FAM)GCTCCTGTCTTACAGGCCCTGCCTC (C/T) GGGCTCACCTCGCTGTGACCTGAAG] together with allele-specific fluorogenic probes that had been predesigned and analytically validated by the manufacturer. Because the primers and probes were provided as part of this commercial assay, no additional primer design was performed in this study. The reaction mixture and cycling conditions were applied according to the kit datasheet, with only minor laboratory-specific optimizations when necessary.

Each real-time PCR reaction was carried out in a final volume of 20 µL containing 100 ng of genomic DNA, 1.6 µL $MgCl_2$, 1 µL LightSNiP reagent mix (Applied Biosystems TaqMan Drug Metabolism Genotyping Assays, Massachusetts, USA), 2 µL LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics GmbH, Mannheim, Germany), and variable amounts of H_2O to adjust the final volume. The PCR cycling conditions were as follows: initial denaturation at 95 °C for 10 minutes; 40 cycles of denaturation at 95 °C for 40 seconds, annealing at 62 °C for 30 seconds, and extension at 76 °C for 15 seconds; followed by a final extension at 76 °C for 5 minutes. Bio-Rad CFX Maestro software was used for the analysis of amplification curves and allele discrimination generated by the TaqMan SNP genotyping assay.

Hardy-weinberg Equilibrium Analysis

The genotype distribution of the ITGB3 rs5918 (T1565C) polymorphism was assessed for conformity with Hardy-Weinberg equilibrium (HWE) in the overall study population, as well as separately in the (PE+) and (PE-) subgroups. Observed and expected genotype frequencies were compared using the chi-square (χ^2) goodness-of-fit test. A p-value <0.05 was considered indicative of deviation from HWE. All analyses were performed using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA).

ELISA Method

Serum GPIIb/IIIa concentrations were determined using the ELISA method. The analysis was performed in duplicate using a commercially available ELISA kit (E-EL-H2202; Elabscience, Houston, USA), in accordance with the manufacturer's instructions. Briefly, serum samples and standards were added to microplate wells pre-coated with antibodies specific for human GPIIb/IIIa and incubated at 37 °C for 60 minutes. After incubation, the wells were washed to remove unbound material, and a horseradish peroxidase-conjugated detection antibody was added, followed by a 30-minute incubation.

Following an additional wash step, tetramethylbenzidine substrate solution was added to each well, and the enzymatic reaction was allowed to proceed until sufficient color development was observed. The reaction was then terminated by the addition of sulfuric acid. Absorbance was measured at 450 nm using a microplate reader (BioTek ELx800). GPIIb/IIIa concentrations were calculated from a standard curve generated using known standard concentrations, and the results were expressed as ng/mL. Serum GPIIb/IIIa concentrations were determined using the ELISA procedure as previously described by Ayaz et al.[13].

Demographic and Clinical Laboratory Data

Demographic and other clinical laboratory parameters of the patients were retrieved from their medical records.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 18.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics, including mean, standard deviation, median, interquartile range, frequency, and percentage, were used to summarize the personal characteristics (e.g., age, gender) of the patients included in the study.

The distribution of continuous variables was first evaluated using normality tests. For comparisons between two independent groups, the independent samples t-test was applied when the assumptions of normality were met; otherwise, the Mann-Whitney U test was used. For comparisons among three or more independent groups, ANOVA was conducted when parametric test assumptions were satisfied, while the Kruskal-Wallis test was used in the absence of these assumptions. For post-hoc subgroup analyses, the Tukey test was employed following ANOVA, and Tamhane's T2 test was used when variance homogeneity was not present.

The chi-square test was used to compare categorical variables between groups. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS

Patient Characteristics

The study group consisted of 80 patients known to be COVID-19 PCR positive. Of the entire patient group, 55 (68.75%) were female and 25 (31.25%) were male. Based on information from patient records, the 80 patients were divided into two groups according to their PE status. Among the COVID-19 positive patients, 54 (67.5%) were in the PE pozitive PE(+) group, while 26 (32.5%) were in the PE(-) group. When examining the demographic data, among the 54 individuals in the PE(+) group, 13 (24.1%) were male and 41 (75.9%) were female. The PE(-) group consisted of 26 individuals, of whom 14 (53.8%) were female and 12 (46.2%) were male. The ages of the PE(+) group ranged from 19 to 74 years (mean= 52.86±13.98), while the ages of the PE(-) group ranged from 22 to 77 years (mean= 47.16±14.08), and the mean ages of the PE(+) and PE(-) groups were similar (p=0.1) (Table 1).

Table 2 shows the genotype distribution of the ITGB3 rs5918 (T1565C) polymorphism according to PE status in COVID-19 patients.

The genotype distribution of the ITGB3 rs5918 (T1565C) polymorphism in the total study population was consistent with the HWE (χ^2= 0.59, p=0.44). When the groups were analyzed separately, the genotype distribution remained in HWE in the PE(+) subgroup (χ^2= 0.84, p=0.36), whereas a significant deviation from HWE was observed in the PE(-) subgroup (χ^2= 4.35, p=0.037).

DISCUSSION

One of the most critical pathological processes associated with COVID-19 is excessive coagulation. It is well established that SARS-CoV-2 disrupts hemostatic balance, thereby increasing the risk of hypercoagulability and both arterial and venous thromboembolic events[14]. A substantial increase in the incidence of PE associated with COVID-19 has been documented in the literature[15,16]. Although several studies have demonstrated dysregulation of coagulation pathways in patients who develop PE secondary to COVID-19 infection, the molecular mechanisms underlying the resultant hypercoagulable state remain incompletely elucidated. In the present study, we aimed to investigate the role of the rs5918 genetic polymorphism, which is potentially associated with the function of GPIIb/IIIa a receptor predominantly expressed on the platelet surface and critically involved in coagulation pathways in patients with and without PE secondary to COVID-19 infection.

Previous studies have indicated that this polymorphism enhances the binding of the GPIIb/IIIa receptor to fibrinogen and promotes platelet aggregation, thereby potentially serving as a contributing factor to thrombotic tendency[11,12]. In a study conducted by Lapić et al.[10], the relationship between COVID-19 severity and the rs5918 polymorphism was examined. They reported that individuals carrying the homozygous mutant genotype experienced more severe COVID-19 infection and identified the mutant genotype as an independent risk factor for disease severity. In a study conducted by Lehmann et al.[17] using next-generation sequencing, genotype results of COVID-19 positive and COVID-19 negative individuals were compared. It was demonstrated that rs5918 homozygous wild-type individuals may be protected against COVID-19 through platelet-associated immune mechanisms, whereas rs5918 heterozygous individuals showed increased susceptibility to

infection. The rs5918 polymorphism (Leu33Pro in the *GPIIb/IIIa* gene) suggests that the Pro33 homozygous mutant variant may increase the binding capacity of SARS-CoV-2, thereby elevating the risk of infection, whereas the Leu33 homozygous wild-type variant may exert a protective effect[16]. In the study conducted by Fiorentino et al.[18] the rs5918 genetic polymorphism was compared between (PE+) and (PE-) groups in patients with COVID-19. Similar to our study, they did not find a significant difference between the groups according to genotypes. These comparable results suggest that the rs5918 variant alone may not be a decisive genetic determinant for PE in the context of COVID-19, but could still act as a modulating factor when combined with inflammatory and hemostatic imbalances.

In our study, D-dimer levels were significantly higher in the PE(+) group, reflecting enhanced fibrin degradation and increased coagulation activity typical of COVID-19 related thrombotic processes. This finding aligns with that of Fiorentino et al.[18] who also noted D-dimer elevation in PE(+) cases. However, while Fiorentino's subgroup analysis by genotype showed no difference[17], our study indicated that genotype-related variation might subtly influence D-dimer behavior (Table 3). This suggests that although rs5918 does

Table 1. Demographic and biochemical parameters in PE(+) and PE(-) groups

Patient groups	COVID-19(+) PE(+) n=54	COVID-19(+) PE(-) n=26	Total n=80	p-value
Gender				
Female Male	41 (75.9%) 13 (24.1%)	14 (53.8%) 12 (46.2%)	55 (68.75%) 25 (31.25%)	 0.082
Age	52.86±13.98	47.16±14.08		0.1
Biochemical parameters				
MPV (fl)	8.25±0.73	8.44±0.90		0.34
PLT (10^3/µL)	314.24±90.81	260.75±72.24		0.007*
WBC (10^3/UI)	7.47±1.60	6.81±1.99		0.12
Fibrinogen (mg/dL)	432.56±72.8	394.28±128.6		0.19
CRP (mg/L)	4.67±5.4	2.98±2.95		0.15
D-dimer(mg/L)	1.16±0.80	0.46±0.32		0.000***
GPIIb/IIIa (ng/mL)	0.46±0.88	0.40±0.23		0.42
Comorbidities				
Hipertansion				
Present	12	4		
Absent	42	22		0.444
Diabetes mellitus				
Present	8	1		
Absent	46	25		0.194
Lung diseases				
Present	10	4		
Absent	44	22		0.559
Cardiovascular diseases				
Present	5	2		
Absent	49	24		0.587
Hypothyroidism				
Present	5	1		
Absent	49	25		0.404

Demographic data in the PE+ and PE- groups were analyzed using Pearson's chi-square test. Biochemical parameters were analyzed using the student's t-test. Significance levels are indicated as *p<0.05, **p<0.01, and ***p<0.001

PLT: Platelet count, MPV: Mean platelet volume, WBC: White blood cells, CRP: C-reactive protein, GPIIb/IIIa: Glikoprotein IIb/IIIa, PE(+): Pulmonary embolism positive, PE(-): Pulmonary embolism negative, n: Number of individuals, COVID-19: Coronavirus disease 2019

Table 2. Genotype distribution according to PE(+) and PE(-) status in COVID-19 patients

Gen	COVID-19(+) PE(+), n (%)	COVID-19(+) PE(-), n (%)	p-value
T1565C, dbSNP ID: rs5918 polymorphism	54	26	
Genotypes			
TT	42 (78)	20 (77)	
TC	12 (22)	4 (15)	**0.76**
CC	0	2 (8)	**0.12**
Alleles			
T	89	85	
C	11	15	**0.40**

In COVID-19 patients, according to the presence (+) or absence (-) of PE, genotype distribution is expressed as follows: TT homozygous wild-type genotypes, TC heterozygous genotypes, and CC homozygous mutant-type genotypes

PE(+): Pulmonary embolism positive, PE(-): Pulmonary embolism negative, COVID-19: Coronavirus disease 2019

Table 3. Comparison of parameters according to T1565C, rs5918 polymorphism genotypes in PE(+) and PE(-) groups with COVID-19 infection

Parameters	COVID-19(+), PE(+) group T1565C, rs5918 polymorphism			COVID-19(+), PE(-) group T1565C, rs5918 polymorphism		
	TT (n=42)	TC (n=12)	CC (n=0)	TT (n=20)	TC (n=4)	CC (n=2)
MPV (fl)	8.34±0.76	7.90±0.41	-	8.38±0.86	8.70±1.36	8.60±0.71
PLT (10^3/µL)	313.81±97.76	315.67±65.99	-	266.34±79.55	247.25±38.11	232.00±53.74
WBC (10^3/UI)	**7.32±1.61**[d*]	**7.95±1.52**[e*]	-	7.07±2.11	6.58±1.12	4.65±0.50
Fibrinogen(mg/dL)	438.64±71.74	411.62±76.75	-	389.59±121.33	446.72±176.68	331.67±132.07
CRP (mg/L)	5.06±5.94	3.40±2.96	-	3.41±3.19	1.79±1.38	1.06±1.44
GPIIb/IIIa (ng/mL)	0.47±0.31	0.42±0.19	-	0.36±0.17	0.68±0.36	0.30±0.05
D-dimer (mg/L)	**1.12±0.85**[a**]	**1.30±0.63**[b*]	-	**0.49±0.35**[c***]	0.39±0.31	0.33±0.19

The data are presented as mean ± SD. Bold values indicate statistical significance. Significance refers to comparisons between: aTT genotypes in the PE(+) group and TT genotypes in the PE(-) group, bTC genotypes in the PE(+) group and TC genotypes in the PE(-) group, cTT genotypes in the PE(-) group and TC genotypes in the PE(+) group, dTT genotypes in the PE(+) group and CC genotypes in the PE(-) group, and eTC genotypes in the PE(+) group and CC genotypes in the PE(-) group. The significance thresholds were set at *p<0.05, **p<0.01, and ***p<0.001 (student's t-test or Mann-Whitney U test). PLT: Platelet count, MPV: Mean platelet volume, WBC: White blood cells, CRP: C-reactive protein, GPIIb/IIIa: Glikoprotein IIb/IIIa, PE(+): Pulmonary embolism positive, PE(-): Pulmonary embolism negative, N: number of individuals, SD: Standard deviation, COVID-19: Coronavirus disease 2019

not directly cause PE, it may contribute to prothrombotic phenotypes by modulating coagulation reactivity under infection-induced stress.

In this study, C-reactive protein (CRP) levels, as an acute-phase reactant, showed no significant differences between genotypes or groups. The results of the study by Fiorentino et al.[18], which investigated CRP levels, are similar to those of our study. This supports the notion that systemic inflammation during COVID-19 likely overshadows subtle genetic influences on inflammatory markers. Similarly, Ghaffari et al.[19] observed no genotype-dependent differences in platelet or inflammatory parameters among COVID-19 patients.

Osikov et al.[20] emphasized that platelet dysfunction and genetic polymorphisms, including ITGB3 variants, play an important role in determining both disease severity and thrombotic complications in COVID-19 patients. Consistent with that view, our study supports the idea that rs5918 may not independently trigger coagulation disturbances but could interact with platelet activation pathways in genetically predisposed individuals.

GPIIb/IIIa, the most abundant receptor on the platelet surface, provides essential binding sites for fibrinogen and mediates platelet aggregation. The absence of a significant relationship between rs5918 genotypes and fibrinogen or GPIIb/IIIa serum levels in our study suggests that this polymorphism does not substantially alter receptor expression or fibrinogen-binding efficiency at the systemic level. This observation adds new information to the literature, as no previous research has simultaneously analyzed GPIIb/IIIa serum levels and rs5918 status in COVID-19 related PE[18].

Analysis of platelet function indicators, including mean platelet volume and platelet count (PLT), revealed higher PLTs in the PE(+) group. This increase may represent a compensatory response to ongoing coagulation activation rather than a direct genetic effect. The literature contains conflicting evidence: Osikov et al.[20] reported lower PLTs but increased platelet reactivity in severe COVID-19, whereas Fiorentino et al.[18], similar to our findings, noted elevated PLTs in PE(+) patients[19].These discrepancies highlight the multifactorial regulation of platelet behavior in COVID-19, involving inflammation, endothelial injury, and host genetic background.

Finally, our analysis found no difference in serum GPIIb/IIIa concentrations between PE(+) and PE(-) groups or among genotypes. Given the absence of prior studies addressing this association, our findings suggest that circulating GPIIb/IIIa levels are unlikely to serve as a biomarker for COVID-19 related PE but merit further investigation in larger, longitudinal cohorts.

Taken together, our data indicate that while rs5918 polymorphism and GPIIb/IIIa levels do not directly predict PE development in COVID-19, they may subtly influence coagulation dynamics in the context of systemic inflammation and viral-mediated endothelial injury. These results reinforce the multifactorial nature of COVID-19 associated thrombosis, where genetic, inflammatory, and environmental factors converge to shape individual thrombotic risk.

Study Limitations

The small sample size of our study, the absence of a healthy control group, and its single-center design may have influenced the statistical power of our analyses.

CONCLUSION

Although the rs5918 polymorphism and GPIIb/IIIa levels were not found to have a direct effect on the development of PE associated with COVID-19, it was demonstrated that genetic variations may influence certain coagulation parameters. These findings underscore the need for further studies to investigate the genetic determinants of COVID-19 related thrombotic processes.

Ethics

Ethics Committee Approval: The ethical approval for this study was granted by the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University, Faculty of Medicine (protocol no: 2023.90.05.09, date: 30.05.2023).

Informed Consent: This research is a retrospective case-control study conducted using pre-existing DNA samples.

Footnotes

Authorship Contributions

Concept: G.A., N.E., E.A., Design: G.A., B.G., Data Collection or Processing: N.E., T.M., Analysis or Interpretation: T.A.K., B.T., B.Ç.Ö., Literature Search: A.J.Y., B.Ş., B.N.K., Ş.Ş., A.B.T., P.P., H.C., Z.A., I.Ö., N.N.A., H.G., M.S.K., Z.N.K., İ.A.K., E.Ç., E.N.A., Writing: G.A., T.A.K., A.B.T.

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ORIGINAL ARTICLE

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Apoptotic Effects on HepG2 Cells and Antifungal Activity Against *Candida* spp. of *Lactobacillus paracasei* Postbiotics

Lactobacillus paracasei Postbiyotiklerinin HepG2 Hücrelerinde Apoptotik Etkisi ve *Candida* spp.'ye Karşı Antifungal Aktivitesi

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ABSTRACT

Aim: This study aimed to evaluate the antiproliferative effects of postbiotics derived from *Lactobacillus paracasei subsp. paracasei* (*L. paracasei*) on the HepG2 cell line and their antifungal activity against *Candida* species.

Materials and Methods: Cell-free supernatants (CFS) were obtained from *L. paracasei* isolates, and their cytotoxic effects on HepG2 cell lines were assessed using the MTT assay. Fluorescent staining and cell cycle analyses were performed on the cells at the determined inhibitory concentration 50 (IC_{50}). The antifungal activities of the postbiotics were evaluated by determining the minimum IC_{50} and minimum fungicidal concentration values on *Candida albicans* (*C. albicans*) ATCC 90028 and *Candida glabrata* (*C. glabrata*) ATCC 2950 strains.

Results: Live and heat-inactivated *L. paracasei* CFS were found to exhibit significant cytotoxic effects on HepG2 cells. Inactivated CFS produced stronger antiproliferative effect with lower IC_{50} value (live: 20.13%; inactive: 10.81% ± standard deviation). Acridine orange/propidium iodide staining revealed an increase in apoptotic cells, while cell cycle analysis revealed a significant increase in the Sub-G phase (control 0.67%; live CFS 6.47%; inactivated CFS 12.67%). In antifungal tests, both CFS strains were found to be effective against *C. albicans and C. glabrata*. While inactivated CFS (6.25%) was more potent in *C. albicans*, live CFS (12.5%) was found to be more effective in *C. glabrata*.

Conclusion: This study demonstrates that *L. paracasei* CFSs possess anticancer and antifungal effects. The fact that these effects vary depending on the CFS form and target yeast species suggests that the postbiotic response is biologically driven and specific. Elucidating the active components of CFS will significantly contribute to the development of new postbiotic-based treatment strategies.

Keywords: *L. paracasei*, HepG2, *Candida*, anticancer, antifungal

ÖZ

Amaç: Bu çalışma, *Lactobacillus paracasei subsp. paracasei* (*L. paracasei*) elde edilen postbiyotiklerin HepG2 hücre hattı üzerindeki antiproliferatif etkilerini ve *Candida* türlerine karşı antifungal aktivitelerini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: *L. paracasei* izolatlarından hücresiz süpernatantlar (CFS) elde edilmiş ve HepG2 hücre hatları üzerindeki sitotoksik etkileri MTT testi kullanılarak değerlendirilmiştir. Belirlenen inhibitör konsantrasyon 50 (IC_{50}) hücreler üzerinde floresan boyama ve hücre döngüsü analizleri gerçekleştirilmiştir. Postbiyotiklerin antifungal aktiviteleri, *Candida albicans* (*C. albicans*) ATCC 90028 ve *Candida glabrata* (*C. glabrata*) ATCC 2950 suşları üzerinde minimum IC_{50} ve minimum fungisidal konsantrasyon değerleri belirlenerek değerlendirilmiştir.

Bulgular: Canlı ve ısıyla inaktif edilmiş *L. paracasei* CFS'lerinin HepG2 hücreleri üzerinde anlamlı sitotoksik etkiler gösterdiği bulunmuştur. İnaktif edilmiş CFS, daha düşük IC_{50} değeri ile daha güçlü antiproliferatif etki oluşturmuştur (canlı: %20,13; inaktif: %10,81 standart sapma). Akridin oranj/propidyum iyodür boyaması apoptotik hücrelerde artış olduğunu gösterirken, hücre döngüsü analizi Sub-G fazında anlamlı bir artış olduğunu

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ortaya koymuştur (kontrol %0,67; canlı CFS %6,47; inaktif CFS %12,67). Antifungal testlerde her iki CFS'nin *de C. albicans ve C. glabrata'*ya karşı etkili olduğu belirlenmiştir. İnaktif CFS (%6,25) *C. albicans* üzerinde daha güçlü bulunurken, canlı CFS'nin (%12,5) *C. glabrata* üzerinde daha etkili olduğu saptanmıştır.

Sonuç: Bu çalışma, *L. paracasei* CFS'lerinin antikanser ve antifungal etkilere sahip olduğunu göstermektedir. Bu etkilerin CFS formuna ve hedef maya türüne bağlı olarak değişmesi, postbiyotik yanıtın biyolojik olarak yönlendirildiğini ve özgül olduğunu düşündürmektedir. CFS'nin aktif bileşenlerinin aydınlatılması, yeni postbiyotik temelli tedavi stratejilerinin geliştirilmesine önemli katkılar sağlayacaktır.

Anahtar Kelimeler: *L. paracasei,* HepG2, *Candida*, antikanser, antifungal

INTRODUCTION

Cancer is a serious disease that develops when cells multiply uncontrollably and spread to different parts of the body, causing high mortality rates worldwide[1]. Cancer, which has many different subtypes such as breast, liver, skin, and prostate cancer, continues to be a significant public health problem as it causes one in every six deaths today[2]. Although treatment approaches such as radiotherapy, chemotherapy, and immunotherapy are widely used among treatment options, the wide side effect profile of chemotherapeutic agents, the development of drug resistance, and the risk of relapse constitute important limitations in treatment[3]. Liver cancer is among the most common malignancies, with hundreds of thousands of new cases worldwide each year. Hepatocellular carcinoma (HCC), which constitutes the majority of primary liver cancers, has a high mortality rate due to its aggressive course and lack of early symptoms[4]. Challenges in the diagnosis and treatment of HCC include the lack of specific biomarkers and the limited efficacy of current treatment options. HepG2 cell line is one of the widely used models in liver cancer research due to its high proliferation capacity and ease of culturing[5,6].

Probiotics are defined as live microorganisms that provide benefits to the host health when consumed in adequate amounts. *Lactobacillus* and other lactic acid bacteria species are one of the most commonly used probiotic groups due to their microbiota-supporting properties[7,8]. Postbiotics are metabolites or cell-free supernatants (CFS) produced by probiotics that do not contain living cells and are considered a safer alternative in immunosuppressed individuals[9,10]. Although the exact mechanisms of these components are not yet clear, they are thought to support host health. Studies have indicated that some *Lactobacillus* strains suppress tumor development through antiproliferative effects, apoptosis induction, and anti-inflammatory mechanisms[11,12]. Apoptosis is a programmed cell death mechanism involved in maintaining tissue homeostasis. Cancer cells generally suppress apoptosis and continue uncontrolled proliferation[13]. Therefore, reactivation of apoptosis is considered an important target in cancer treatment. Postbiotic components are reported to promote cell death by triggering apoptosis in cancer cells through their cytotoxic properties[14-16]. In addition to this therapeutic potential, postbiotics are also considered important biological agents in combating the increasing number of fungal infections in recent years. Opportunistic fungal pathogens, especially *Candida albicans* (*C. albicans*) and *Aspergillus fumigatus*, cause life-threatening infections in conditions where the immune system is weakened, such as AIDS or immunosuppression after organ transplantation, and the incidence of these infections is increasing. Although the innate immune response plays a critical role in the early stages of fungal infections, the emergence of resistant strains limits the effectiveness of current antifungal agents[17]. In recent years, the antifungal activities of *Lactobacillus paracasei* (*L. paracasei*) strains have attracted attention. Probiotic metabolites and postbiotic compounds from *L. paracasei* have been shown to inhibit biofilm formation, especially against *Candida* species, suppress hyphal growth, and disrupt fungal cell wall integrity by lowering the pH of the medium[18-20]. It has also been reported that postbiotics obtained from *L. paracasei* have an antifungal effect by causing structural damage to the *C. albicans* cell wall[21,22]. In this study, it was aimed to investigate the antiproliferative effects as well as antifungal activities of CFSs from *L. paracasei subsp. paracasei* (*L. paracasei*) on HepG2 cell line.

MATERIALS AND METHODS

This study was reviewed and approved by the Tekirdağ Namık Kemal University, University Non-Interventional Clinical Research Ethics Committee (approval number: 2025.58.03.16, date: 25.03.2025).

Bacterial Culture and Inactivation

The *L. paracasei* isolate was incubated in de Man-Rogosa-Sharpe (MRS Broth, Biolife, Milan, Italy) broth medium at 37 °C in an anaerobic environment for 24-48 hours. Then, single colony culture was performed on MRS (Agar, Biolife, Milano, Italy) solid medium under the same conditions for 24 hours. A single colony obtained from the purified *L. paracasei* isolate was seeded in MRS broth and incubated. After growth was observed, the culture was inactivated in a water bath at 100 °C for 30 minutes. After the incubation period was completed, the cultures were centrifuged at 4000 rpm for 15 minutes to obtain the supernatant[12]. This liquid phase was sterilized by passing through a 0.22 µm membrane filter (Isolab, Eschau, Germany) and stored at -80 °C.

Cell Culture

HepG2 cells used in this study were cultured in Dulbecco's Modified Eagle Medium (DMEM, Euroclone, Pero (MI), Italy] containing 10% fetal bovine serum (FBS) (FBS, Capricorn Scientific, South America) and 1% penicillin-streptomycin at 37 °C, 5% CO_2, and under humidified conditions.

MTT Viability Test

Passaging was performed when the cell density covered approximately 80-90% of the T75 (Tissue Culture Flask 75, TPP, Switzerland) culture flask surface and cell viability was assessed with trypan-blue (Gibco, New York, USA). Then, cells were added to 96-well flat-bottom cell culture plates (Tissue Culture Test Plate 96F, TPP, Switzerland) containing 1×10^4 cells per well[23,24]. Plates were incubated for 24 hours at 37 °C, 5% CO_2 and 95% relative humidity. Cell adherence to the surface was observed using an inverted microscope. Afterwards, serial dilutions of live and inactivated CFSs (0.39-100%) were applied to the cells and incubated for 24 hours. For the MTT assay, 10 µL of a 5 mg/mL solution of reagent (Merck, Darmstadt, Germany) was prepared and added to each well, and the plates were incubated at 37 °C for 4 hours. The medium formed after incubation was removed and 100 µl DMSO (Dimethyl Sulfoxide, Biofroxx, Germany) was added to each well and gently shaken for 5 minutes. Each test was performed in triplicate, and absorbance values were measured at 570 nm wavelength using a microplate reader (BioTek-800-TS absorbance reader, Agilent, Santa Clara, United States). Cell viability rates were calculated using the obtained data. Inhibitory concentration 50 (IC_{50}) values were determined using GraphPad Prism 8.0 software[25].

Cell Cycle Analysis

To investigate the cell cycle, 6-well cell plates were seeded with 5×10^5 cells per well. Cells were treated with CFSs at the determined IC_{50} concentrations for 24 hour. At the end of the incubation period, cells were lifted with 0.25% trypsin (ThermoFisher, Paisley, United Kingdom) and centrifuged at 1200 rpm for 3 minutes. After washing, the supernatant was removed and the pellet was resuspended in 1 mL Dulbecco's Phosphate-Buffered Saline (D-PBS) [Euroclone, Pero (MI), Italy]. Then, 8 mL of 70% cold ethanol was added dropwise on the vortex. Following vortexing, the cells were incubated overnight at +4 °C for fixation. After incubation, the fixed cells were centrifuged and transferred to tubes suitable for flow cytometry analysis. 500 µL propidium iodide (PI) (PI, Invitrogen, United States of America) and staining buffer (1 mL D-PBS, 5 µL of 20% Triton X, 6.6 µL RNase A and 20 µL PI) were added to the cells. This mixture was incubated for 30 minutes in dark conditions[12]. In the final step, cells were analyzed by flow cytometry using a FACSCalibur (BD Biosciences) device.

(AO/PI) Fluorescent Staining

For the evaluation of live and dead cells, a staining solution was prepared by carefully mixing 10 g sodium-ethylenediaminetetraacetic acid , 4 mg PI, 50 mL FBS and 4 mg acridine orange (AO) (dissolved in 2 mL 99% ethanol). A homogeneous mixture was achieved by adding sterile distilled water to a final volume of 200 mL. Cells were seeded in 96-well plates with 2×10^4 cells per well in triplicate. Plates were incubated for 24 hours at 37 °C in an environment containing 5% CO_2 to allow cells to adhere to the surface. Cells were treated with CFSs at the determined IC_{50} concentrations, and after the incubation period was completed, 10 µL of AO/PI staining mixture was added to each well and left for 5 minutes[26]. Then, apoptotic cells were examined morphologically under a fluorescence microscope (version 7.5; Genetix; Leica Microsystems).

Assessment of Antifungal Activity

Antifungal susceptibilities of yeast strains were examined using the broth microdilution method according to CLSI guidelines. *C. albicans* ATCC 90028 and *Candida glabrata* (*C. glabrata)* ATCC 2950 were used as reference strains. Serial dilutions of live and heat-inactivated *L. paracasei* CFSs were prepared and the final concentration range was adjusted to 0.39-100%. Each yeast strain was standardized to 1.5×10^3 colony forming unit/mL and 10 µL of the suspension was added to microplate wells containing different concentrations of CFS. Microbial growth was visually assessed after incubating the microplates at 35 °C for 24 hours. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were determined for each strain. For MFC determination, samples at the concentration were plated on sabouraud dextrose agar. The concentration at which no growth was observed was designated as MFC[27]. The antifungal effects of CFSs were compared with the reference antifungal agent Micafungin (8 µg/mL). All experiments were repeated in triplicate.

Statistical Analysis

All experiments were conducted with three independent replicates, and results are presented as the mean ± standard deviation Graphs were created using Microsoft Office 365 Excel and GraphPad Prism 8. Statistical analysis of the data was performed using ANOVA, and post-hoc Tukey test was applied for multiple comparisons. Statistical significance was determined using p-values; results with $p\leq0.05$ were considered significant and are indicated with asterisks in the graphs.

RESULTS

Comparative Cytotoxicity of Live and Heat-inactivated *L. paracasei* CFSs Against HepG2 Cells

To evaluate the cytotoxic potential of *L. paracasei* isolate on HepG2 human hepatocarcinoma cells, both live and heat-inactivated CFSs were applied in the concentration range between 0.39% and 100%. Cell viability was determined by MTT assay after 24 hour incubation period. The findings showed that both forms of CFS decreased cell viability in HepG2 cells in a dose-dependent manner. In particular, inactivated CFS was found to have a significantly stronger cytotoxic effect compared to the live form (****p<0.0001). IC_{50} values were calculated as 20.13±0.22% for live CFS and 10.81±0.13% for inactivated CFS (Figure 1).

Apoptosis Detection by AO/PI Dual Staining

Morphological changes in HepG2 cells treated with IC_{50} concentrations of live and heat-inactivated *L. paracasei* CFS for 24 hours were examined under a fluorescence microscope. AO/PI dual staining method was used to distinguish viable and apoptotic cells. Green fluorescent cells represent AO-stained live cells (white arrows), while orange/red fluorescent cells represent PI-stained apoptotic cells (yellow arrows). A higher number of apoptotic cells were observed in HepG2 cells treated with both live and inactivated CFS of *L. paracasei* compared to the control group (Figure 2). Furthermore, inactivated CFS was found to have a relatively greater apoptotic effect compared to live CFS.

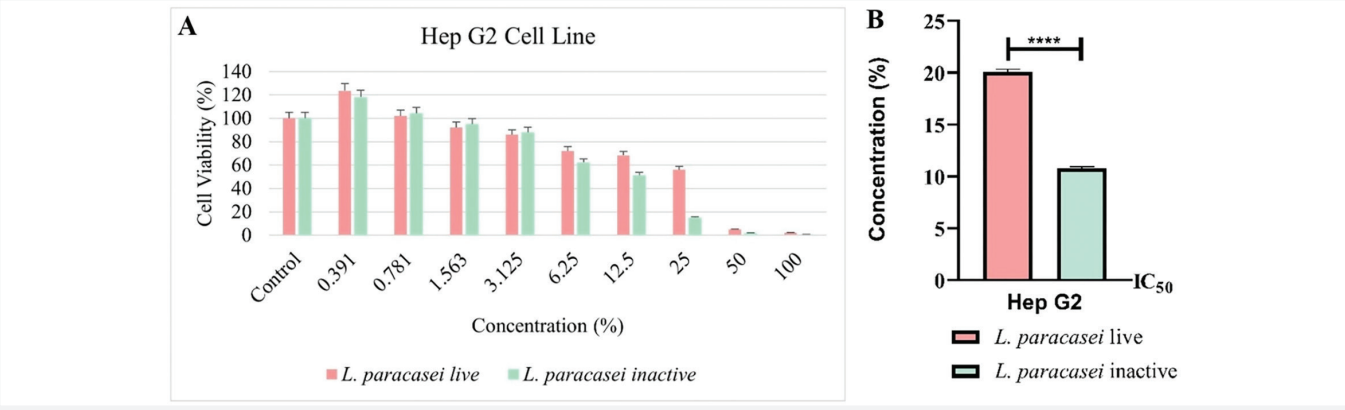


Figure 1. Percentages (A) and IC_{50} values (B) of live and inactivated *L. paracasei* CFS in HepG2 cell after 24 hours of treatment

IC_{50}: Inhibitory concentration 50, CFS: Cell-free supernatants, L. paracasei: Lactobacillus paracasei

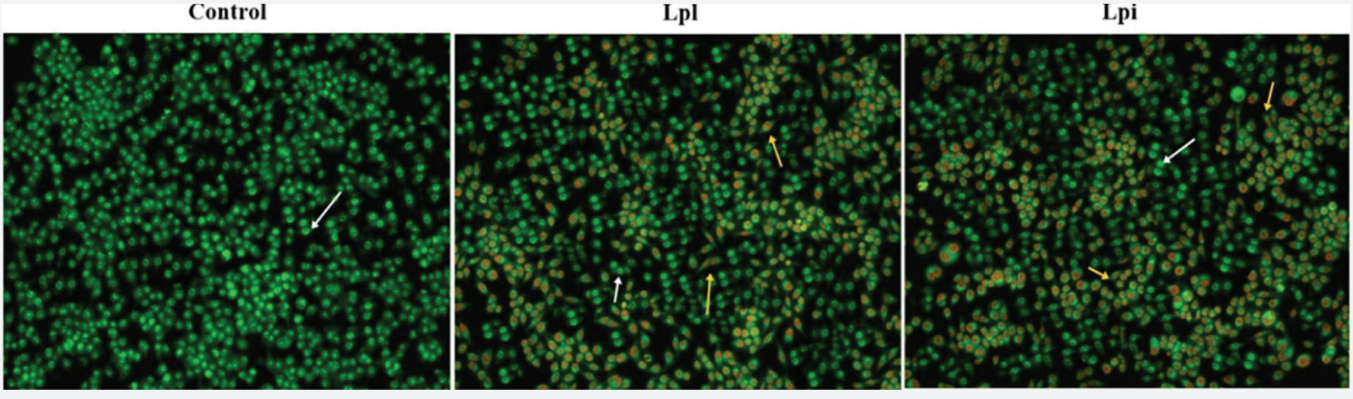


Figure 2. Fluorescence microscope images (20x) of HepG2 cells treated with live and heat-inactivated *L. paracasei* CFSs at IC_{50} concentrations for 24 hours. White arrow shows AO-stained live cells (green), while the yellow arrow indicates PI-stained apoptotic cells (orange/red)

CFSs: Cell-free supernatants, IC50: Inhibitory concentration 50, AO: Acridine orange, L. paracasei: Lactobacillus paracasei, LPL: L. paracasei live, LPI: L. paracasei inactive

Cycle Analysis in HepG2 Cells

Cell cycle analyses were performed by flow cytometry and cell distributions in Sub G, G0-G1, S and G2-M phases were evaluated (Figure 3). According to the findings, while the proportion of cells in Sub G phase was 0.67% in the control group, this proportion increased to 6.47% in cells treated with live *L. paracasei* CFS and to 12.67% in cells treated with inactive CFS. This increase indicates that both treatments caused a significant accumulation of cells in the Sub G phase. While the proportion of cells in the G0-G1 phase was 69.41% in the control group, it slightly increased to 71.49% in the live CFS treatment and decreased to 65.27% in the inactive CFS group. In contrast, the cell population in the S phase was 13.51% in the control group, but decreased to 8.68% and 8.24% after live and inactive CFS applications, respectively. Similarly, the G2-M phase decreased from 15.22% in the control group to 11.27% in the live CFS group and 9.29% in the inactivated CFS group. These findings indicate that *L. paracasei* CFS suppresses cell cycle progression and arrests a significant portion of cells in the sub G phase, inducing apoptosis. The significant increase in the sub G phase and the decrease in the G2-M phase observed with inactivated CFS suggest that this form triggers cell death mechanisms more potently than live CFS.

Antifungal Effects of Live and Inactive *L. paracasei* CFSs

In this study, the antifungal activities of live and inactivated CFS obtained from *L. paracasei* were evaluated against the clinically important strains *C. albicans* 90028 and *C. glabrata* 2950 (Table 1; Figure 4). Antifungal activity was measured by MIC and MFC values, and the results revealed that both forms of CFS exhibited significant antifungal activity on these pathogens. Against *C. albicans*, the MIC and MFC values of inactivated CFS were 6.25% and 12.5% for the live form, respectively. For *C. glabrata*, the MIC and MFC values of inactivated CFS were 25% and 12.5%

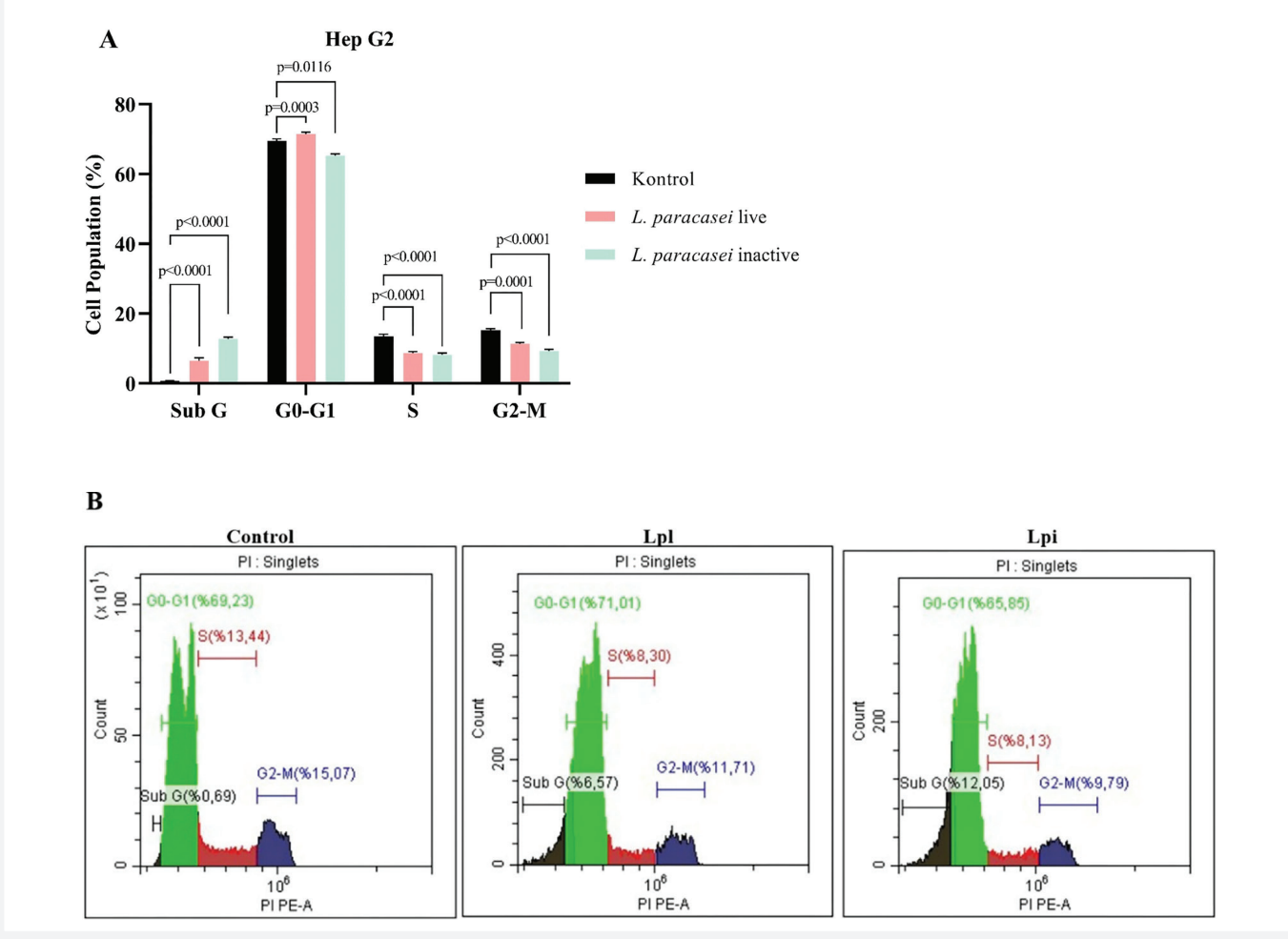


Figure 3. Cell cycle distribution of HepG2 cells treated with IC_{50} concentrations of live and inactive supernatants from *L. paracasei*, showing changes (A) Sub G, G0-G1, S and G2-M phases; (B) Flow cytometry image

IC_{50}: Inhibitory concentration 50, L. paracasei: Lactobacillus paracasei, LPL: L. paracasei live LPI: L. paracasei inactive

Table 1. MIC and MFC values (%) of *L. paracasei* live and inactivated CFSs against *Candida strains*

Fungi strains	Live		Inactive		MF	
	(%)		(%)		(µg/mL)	
	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans ATCC 90028	12.5	12.5	6.25	6.25	0.015	0.015
Candida glabrata ATCC 2950	12.5	12.5	25	25	0.015	0.03

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration, MF: Micafungin, *L. paracasei*: *Lactobacillus paracasei*

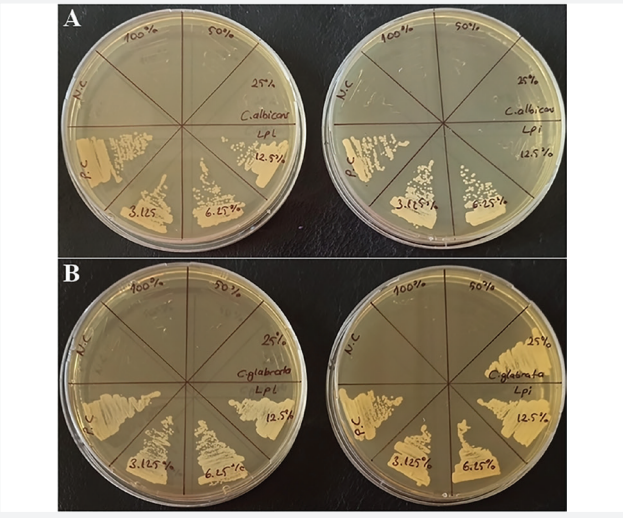


Figure 4. MFC petri dish images of live and inactive *L. paracasei* CFSs. (A) *Candida albicans* 90028. (B) *Candida glabrata* 2950

MFC: Minimum fungicidal concentration, CFSs: Cell-free supernatants, L. paracasei: Lactobacillus paracasei, NC: Negative control; PC: Positive control

for the live form. These results indicate that both forms have antifungal potential, but the level of efficacy varies depending on the *Candida* species.

DISCUSSION

Cancer continues to be one of the diseases with the highest mortality rate worldwide, and current treatment options often cause serious toxic effects on healthy tissues. Although conventional chemotherapeutic agents target tumor cells, their low selectivity leads to increased side effects during treatment. This situation increases the need for safer and more effective alternative therapeutic strategies[1,28,29]. In recent years, intensive research has been carried out on the anticancer potential of probiotic microorganisms and their CFSs. Studies have shown that CFSs derived from probiotic bacteria, such as *Lactobacillus* spp., can suppress tumor cell proliferation, induce apoptosis, and regulate the cell cycle, leading to anticancer effects[29-33]. These findings suggest that *Lactobacillus*-derived CFS may be considered as a potential anticancer agent not only in colon cancer but also in HCC, breast cancer and other types of cancer. HCC is the most common primary liver malignancy worldwide and represents a significant health problem in terms of cancer-related mortality[34]. The fact that HCC mostly develops on the background of chronic liver diseases such as cirrhosis, viral hepatitis, alcohol consumption and metabolic steatohepatitis and that early diagnosis is rare limits the treatment options in the advanced stages. However, the frequent occurrence of chemotherapy resistance in HCC makes treatment success difficult. Different studies demonstrate the anticancer potential of probiotics and postbiotics on HepG2 cells. Mubeen et al.[35] revealed that sea buckthorn and monk fruit beverage fermented with lactic acid bacteria exhibited high antioxidant capacity and significant cytotoxic activity in HepG2 cells. Similarly, it has been reported that cranberry proanthocyanidins increased mitochondrial pathway-dependent cytotoxicity and inhibited HepG2 cell proliferation in a dose- and time-dependent manner as a result of biotransformation by *Lactobacillus rhamnosus (L. rhamnosus)*[36].

Additionally, *L. rhamnosus* GG-derived extracellular vesicles have been shown to induce apoptosis and create cytotoxicity by increasing the Bax/Bcl-2 ratio[37]. Similarly, *Lactobacillus fermentum* BGHV110 postbiotics were reported to reduce acetaminophen-induced hepatotoxicity and provide cytoprotective effects by activating PINK1-dependent autophagy[38]. In addition, it has been reported that exopolysaccharides from lactic acid bacteria and Bifidobacterium have a cytotoxic effect on HepG2 cells and increase the expression of Bax, Caspase-3/8 and p53[39].

In vivo studies have also demonstrated the beneficial effects of probiotics on hepatocytes. For example, *L. paracasei* HY7207 was shown to suppress genes associated with lipogenesis and apoptosis in palmitic acid (PA)-treated HepG2 cells and to reduce inflammation, fibrosis, and hepatic steatosis in non-alcoholic fatty liver disease (NAFLD) mouse models[40]. Similarly, *Lactobacillus plantarum* (*L. plantarum*) MG4296 and *L. paracasei* MG5012 were reported to alleviate insulin resistance in PA-induced HepG2 cells and improve metabolic parameters in mice on a high-fat diet[41]. Additionally, *L. plantarum* LP158, *Lactobacillus helveticus* HY7804 and *L. paracasei* LPC226 strains were shown to suppress lipogenesis genes in PA-treated HepG2 cells, increase β-oxidation and reduce fatty liver and inflammation in NAFLD mouse models[42].

In this study, the cytotoxic effects of live and inactive CFS obtained from *L. paracasei* isolates on HepG2 cells were evaluated by MTT assay and it was determined that both CFS forms caused significant cytotoxicity in cancer cells. Analyses

using AO/PI fluorescent staining methods revealed that CFS applications significantly increased the number of cells undergoing apoptosis in HepG2 cells.

Cell cycle analyses are reported in a limited number of studies in the literature[12,43-47]. Dehghani et al.[44] showed that *L. rhamnosus* CFSs decreased IC_{50} values in human colon cancer (HT-29) cells in a dose- and time-dependent manner and arrested the cells in the G0-G1 phases of the cell cycle[44]. Similarly, Erfanian et al.[45] reported that *Lactobacillus acidophilus* (*L. acidophilus*) CFSs produced antiproliferative and anti-migration effects in HT-29 cells by arresting the cell cycle in the G1 phase and leading to a reduction in the S and G2-M phases. In another study, *L. plantarum* UL4 strain was reported to cause cell accumulation in the G0-G1 phase in breast cancer cells[43].

In two different studies, Liu et al.[46] revealed that fermented grape skin extracts stopped the cell cycle in HepG2 cells and inhibited proliferation by inducing apoptosis[47]. Erdal et al.[12] reported that *L. paracasei* live and inactive CFSs stopped apoptosis and cell cycle in the Sub G phase in glioma cells (U-87); also indicated that inactivated CFSs exhibited a more selective anticancer effect against normal human embryonic kidney (HEK293T) cells.

In this study, live and inactivated *L. paracasei* CFSs applied to HepG2 cells significantly affected cell cycle progression and induced apoptosis. Both CFS forms accumulated in the Sub G phase, while mild changes were observed in the G0-G1 phase, and a decrease in the S and G2-M phases indicated suppression of DNA synthesis and mitotic division. In particular, inactivated CFS induced apoptotic cell death more strongly than live CFS. These findings indicate that probiotic-derived CFSs have the capacity to arrest the cell cycle and induce apoptotic processes in HepG2 cells, and are consistent with the results reported in the literature. Studies in the literature demonstrate that probiotic-derived CFSs are not limited to anticancer effects but also possess potent antifungal properties. For example, one study showed that *L. acidophilus* and *L. plantarum* CFSs suppressed the growth of oral *Candida* species isolated from HIV/AIDS patients, and provided particularly significant inhibition on *Candida* krusei[48]. Similarly, it has been reported that *L. plantarum* and *Lactobacillus* coryniformis metabolites isolated from rice washing water exhibited strong antifungal activity against Aspergillus species and this activity was mainly due to organic acids and fatty acids[49].

Dube et al.[50] showed that *L. rhamnosus* cell-free extract suppressed hyphae formation, protease/phospholipase production, and drug efflux pumps *in C. albicans*, thus reducing both virulence and antifungal drug resistance. Rossoni et al.[19] reported that clinical *Lactobacillus* isolates strongly inhibited *C. albicans* biofilms and this activity was associated with the downregulation of biofilm-related genes such as ALS3, HWP1, EFG1 and CPH1. Additionally, Coman et al.[22] demonstrated that the combination of *L. rhamnosus* IMC 501, *L. paracasei* IMC 502 and SYNBIO® provided broad-spectrum inhibition against both bacterial and fungal pathogens. In another study by García-Gamboa et al.[21], it was determined that *L. paracasei* and *L. plantarum* CFSs combined with inulin-type fructans significantly reduced both growth and biofilm formation in *C. albicans*. In a study conducted on multidrug-resistant *Candida auris*, it was reported that postbiotic fractions derived from *L. paracasei* 28.4 strongly inhibited all planktonic, biofilm and persister cells and exhibited therapeutic potential by enhancing the host immune response in in vivo models[51]. Additionally, Spaggiari et al.[20] showed that CFSs from different *Lactobacillus species* significantly reduced the capacity of *Candida parapsilosis* (*C. parapsilosis*) to adhere to epithelial cells and establish infection in both monolayer and transwell models.

Erdal et al.[12] evaluated the antifungal effects of live and inactivated CFSs from *L. paracasei* on *C. albicans* 10231 and *C. parapsilosis* ATCC 22019 and reported that inactivated CFSs, in particular, showed significant fungistatic and fungicidal activity at lower concentrations. In this study, the antifungal activities of live and inactivated CFSs obtained from *L. paracasei* isolates were investigated on *C. albicans* 90028 and *C. glabrata* 2950 strains, and both forms were determined to exhibit significant activity. Activity levels were observed to vary among species, with the inactivated form exhibiting stronger antifungal activity on *C. albicans*, while the live form exhibited stronger antifungal activity on *C. glabrata*.

Study Limitations

This study has several limitations. The data obtained were evaluated only in the HepG2 cell line, which limits its generalizability to different tumor models. Furthermore, the specific bioactive compounds responsible for the observed antitumor and antifungal activity were not isolated, and mechanisms could not be analyzed at the proteomic or metabolomic level.

CONCLUSION

In this study, the bioactive effects of live and inactivated CFSs obtained from *L. paracasei* isolates on both the HepG2 liver cancer cell line and *Candida species* were comprehensively evaluated. The findings reveal that CFSs carry dual therapeutic potential in terms of both cancer biology and microbial pathogen control. The cytotoxicity, increased apoptosis, and cell cycle arrest observed in HepG2 cells suggest that inactivated CFSs are more effective than the live form. Similarly, the antifungal effects observed on *C. albicans* and *C. glabrata* indicate that live and inactivated CFSs exhibit varying levels of activity depending on the species. These findings suggest that the stable and safe structures of CFSs may offer innovative

and applicable biological strategies in both anticancer and antifungal therapies.

Ethics

Ethics Committee Approval: This study was reviewed and approved by the Tekirdağ Namık Kemal University, University Non-Interventional Clinical Research Ethics Committee (approval number: 2025.58.03.16, date: 25.03.2025).

Informed Consent: No human participants were involved; therefore, informed consent was not required.

Footnotes

Authorship Contributions

Concept: B.Y., B.E., Design: B.Y., B.E., Data Collection or Processing: B.Y., D.E., B.D., S.D., S.Z.Ç., Z.Ü., B.E., Analysis or Interpretation: B.Y., D.E., B.D., S.D., S.Z.Ç., Z.Ü., B.E., Literature Search: B.Y., D.E., B.D., S.D., S.Z.Ç., Z.Ü., B.E., Writing: B.Y., B.E.

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

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CASE REPORT

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A Case of Composite Lymphoma with Coexisting Papillary and Medullary Thyroid Carcinomas in a Patient with a History of Four Primary Malignancies

Dört Farklı Primer Malignite Öyküsü: Kompozit Lenfomaya Eşlik Eden Tiroid Papiller ve Medüller Kanser Olgusu

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ABSTRACT

Composite lymphoma refers to the simultaneous presence of two or more morphologically and immunophenotypically distinct lymphomas within the same tissue or organ. While cases of composite lymphoma consisting of classical Hodgkin lymphoma (cHL) and B-cell lymphoma have been reported, the combination of splenic marginal zone B-cell lymphoma (SMZL) and cHL is extremely rare. In this report, we present a case initially treated with insufficient response for SMZL, later experiencing recurrence, followed by a diagnosis of cHL. Additionally, thyroid tissue from the same patient revealed the presence of thyroid papillary and medullary cancers with a poor prognosis. This report presents a case of composite lymphoma involving four different primary malignancies occurring simultaneously.

Keywords: Hodgkin lymphoma, non-Hodgkin lymphoma, B-cell neoplasms

ÖZ

Kompozit lenfoma, aynı doku veya organda morfolojik ve immünofenotipik olarak farklı iki veya daha fazla lenfomanın eş zamanlı varlığına işaret eder. Klasik Hodgkin lenfoması (cHL) ve B-hücreli lenfoma içeren kompozit lenfoma olguları bildirilmiş olmasına rağmen, splenik marginal zon B-hücreli lenfoma (SMZL) ve cHL kombinasyonu son derece nadirdir. Bu raporda, başlangıçta SMZL için yetersiz yanıt ile tedavi edilen, daha sonra nüks eden ve ardından cHL tanısı konan bir olgu sunulmaktadır. Ayrıca, aynı hastanın tiroid dokusunda kötü prognozlu tiroid papiller ve medüller kanserleritespit edilmiştir. Bu rapor, aynı anda dört farklı primer malignitenin yer aldığı bir kompozit lenfoma olgusunu sunmaktadır.

Anahtar Kelimeler: Hodgkin lenfoma, non-Hodgkin lenfoma, B-hücreli neoplazmlar

INTRODUCTION

Composite lymphoma refers to the simultaneous presence of two or more lymphomas with different morphological and immunophenotypic features in the same tissue or organ[1]. Although cases of composite lymphoma consisting of classical Hodgkin lymphoma (cHL) and B-cell lymphoma have been reported, the combination of splenic marginal zone B-cell lymphoma (SMZL) and cHL is extremely rare[2]. This report presents a case where a patient initially treated for SMZL with inadequate response was later diagnosed with cHL following a repeat biopsy. Additionally, a biopsy of thyroid tissue from the same patient revealed a rare papillary thyroid carcinoma (PTC) and medullary thyroid carcinoma (MTC).

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Informed consent was obtained from the patient involved in this case report. They were informed about the medical procedures and the scientific purpose of this study.

CASE REPORT

A 69-year-old male farmer with a known history of diabetes mellitus and cholelithiasis presented with fever, weight loss, night sweats, and left upper quadrant pain. This table summarizes key laboratory results from the patient's clinical report. Abdominal ultrasound revealed multiple lymphadenopathies in the liver hilum, with the largest node measuring 3.2 cm, and splenomegaly with a spleen size of 17 cm. A bone marrow biopsy was performed, and the immunohistochemical findings (positive for CD20, CD23, CD3, BCL 2) along with the morphological assessment were consistent with SMZL (Figure 1 presents the results of immunohistochemical staining). On the positron emission tomography/computed tomography (PET/CT) scan, the patient had a 47 mm [standardized uptake value (SUV) 6.2] nodular mass in the right lobe of the thyroid gland, a 12 mm (SUV 2.4) mass in the anterior mediastinum, 15 mm (SUV 2.1) bilateral axillary lymphadenopathy, multiple perisplenic lymphadenopathies with the largest being 20 mm, and splenomegaly with a spleen size of 19 cm. After 6 cycles of rituximab treatment, the PET/CT response evaluation showed no significant morphological changes in the hypermetabolic mass lesion in the right lobe of the thyroid gland (Figure 2a), with partial metabolic regression observed. Increased fluorodeoxyglucose (FDG) uptake was noted in the right axillary fossa (Figure 2b), subcutaneous tissue adjacent to the gluteal and paraspinal muscle planes, and bilateral inguinal regions. Complete regression was observed in lymph nodes in the periportal and perisplenic regions, while millimetric lymph nodes showing FDG uptake under mediastinal vascular structures were classified as Deauville score 2. Splenic size was found to be normal. Bone marrow biopsy did not show lymphoma infiltration. Due to a suspicious nodule, the patient underwent total thyroidectomy and an excisional lymph node biopsy from the right axilla. The thyroidectomy material showed MTC in the right lobe, PTC of the follicular variant with capsule invasion in the left lobe, and the right axillary lymph node biopsy revealed mixed cellularity type cHL. The lymph node biopsy showed positive results for CD30, CD15, MUM1, and PAX-5; while CD20, CD45, and EBER were negative (Figure 3 presents the results of immunohistochemical staining). The endocrinology team recommended monitoring with calcitonin and carcinoembryonic antigen every 3 months without treatment. A follow-up neck ultrasound revealed lymphadenopathy in the cervical chain, with the largest node

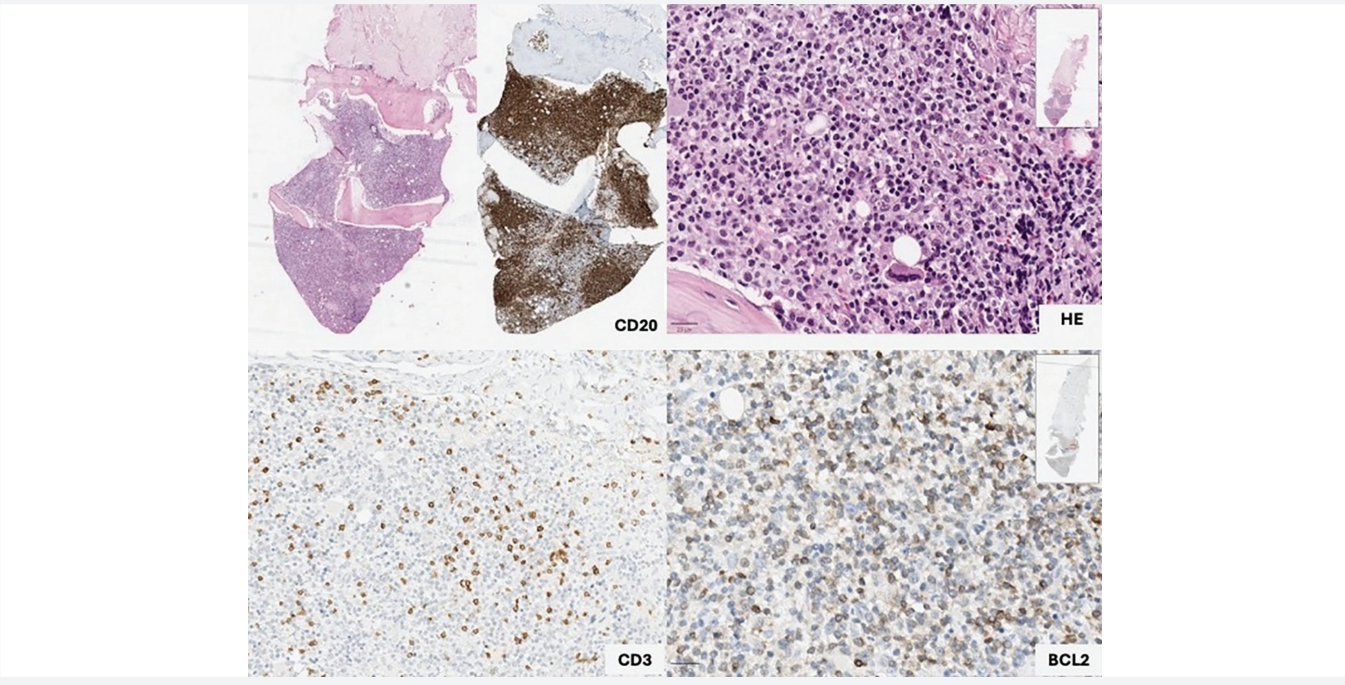


Figure 1. Bone marrow involvement by low-grade B-cell lymphoma/leukemia. Top left: H&E staining shows lymphoid infiltration in the bone marrow with diffuse CD20 positivity. Top right: High-magnification view reveals infiltration of small to medium-sized atypical B-lymphocytes. Bottom left: CD3 immunohistochemical staining highlights sparsely scattered accompanying T lymphocytes. Bottom right: BCL2 immunohistochemical staining shows weak but positive expression in the neoplastic B-cells

H&: Hematoxylin and eosin

measuring 15x6 mm in the right supraclavicular region, and additional nodes in the right parotid cortex. The patient was diagnosed with stage 3A cHL and began doxorubicin (adriamycin), bleomycin, vinblastine, dacarbazine therapy. Genetic testing from the lymph node biopsy using next-generation sequencing (NGS) panel identified possible pathogenic variants: CIITA p.C83fs (loss-of-function) with a variant allele frequency (VAF) of 10% and TCF3 p.R525W with a VAF of 2.9%. After two cycles, the patient achieved a complete response on PET/CT but unfortunately passed away due to pneumonia during the course of treatment.

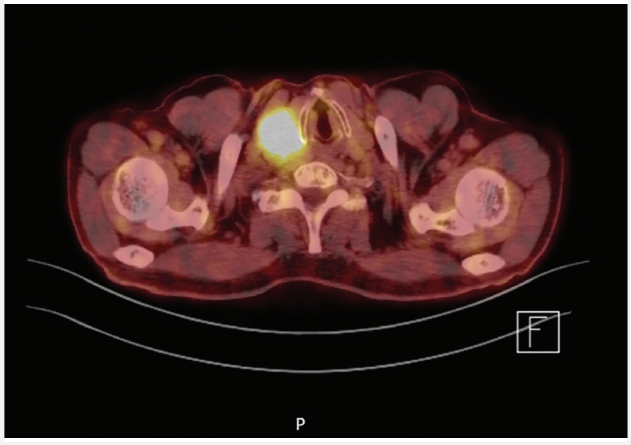


Figure 2a. PET/CT findings: There is a hypermetabolic mass lesion in the right lobe of the thyroid gland, hypodense in nature, measuring 4.6x3.5 cm at its widest, with heterogeneous characteristics (SUV_{max}: 7.7)

SUV_{max}: Maximum standardized uptake value, PET/CT: Positron emission tomography/computed tomography

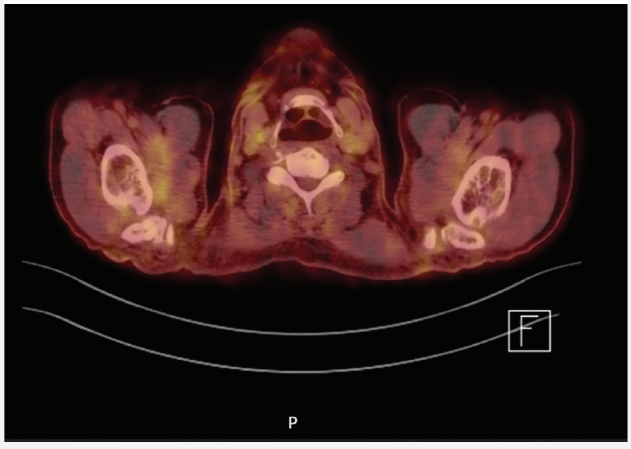


Figure 2b. PET/CT findings: There is a reticulonodular density in the right axillary fossa showing increased FDG uptake (SUV_{max}: 4.9)

SUV_{max}: Maximum standardized uptake value, PET/CT: Positron emission tomography/computed tomography, FDG: Fluorodeoxyglucose

DISCUSSION

In this report, we present a case of composite lymphoma with four different primary malignancies occurring simultaneously. The coexistence of SMZL and cHL is rare, given that cHL typically presents in lymph nodes[2]. To our knowledge, there are very few reported cases of cHL developing in a patient with SMZL. In one case study, PCR and sequencing analyses revealed that amplified rearranged immunoglobulin genes originated from the same clone[3]. In another case, where both cHL and mantle cell lymphoma were present, the findings were clonally unrelated[4]. According to a published review, more than 70% of patients with composite lymphoma are ≥55 years old, and the majority are male. The most commonly associated lymphomas are cHL with follicular lymphoma or diffuse large B-cell lymphoma, with over 130 cases reported. The cHL group is frequently of the mixed cellularity type, and compared to other types, it often shows focal/weak CD20 expression, suggesting a distinct pathophysiology. Both groups can share similar IgH/IgK rearrangements and the same pathogenic variants, which supports the hypothesis of a common clonal origin. Thus, composite lymphomas appear to support a common clonal origin and transdifferentiation process during lymphoma pathogenesis[5]. A study of 20 cases with both HL and non-HL (NHL), including three cases diagnosed with SMZL, found that the HL that developed later was usually more aggressive and at a more advanced stage[6]. In most of these cases, malignant clones develop separately from a common precursor cell, often a germinal center B-cell. Thus, this suggests a scenario where malignant precursor cells undergo transformation resulting in the development of two different lymphomas through distinct and repeated transformation events within the germinal center microenvironment. Molecular findings support this view[7]. PTC is the most common endocrine malignancy. The simultaneous occurrence of HL and MTC has been rarely reported. The coexistence of MTC and PTC in the same thyroid gland is classified into two forms. The first form consists of two distinct tumors separated by non-neoplastic thyroid tissue. The other form is a mixed tumor. Our case fits the first form. In thyroid cancers, metastases are usually observed as lymph nodes in the cervical and anterior mediastinal regions, similar to the widespread involvement seen in HL[8]. HL treatment was initiated after total thyroidectomy in our case. In our case, NGS of the lymph node revealed a significant loss-of-function mutation in CIITA p.C83fs with a VAF of 10%. Hematopoietic cancer cells can also present antigens in the context of HLA class II. It has been shown that CIITA methylation can lead to transcriptional down regulation of *HLA II* genes, allowing these cells to evade antigen presentation. The *CIITA* gene often breaks and fuses in some lymphomas, playing a key role in how these cancers evade

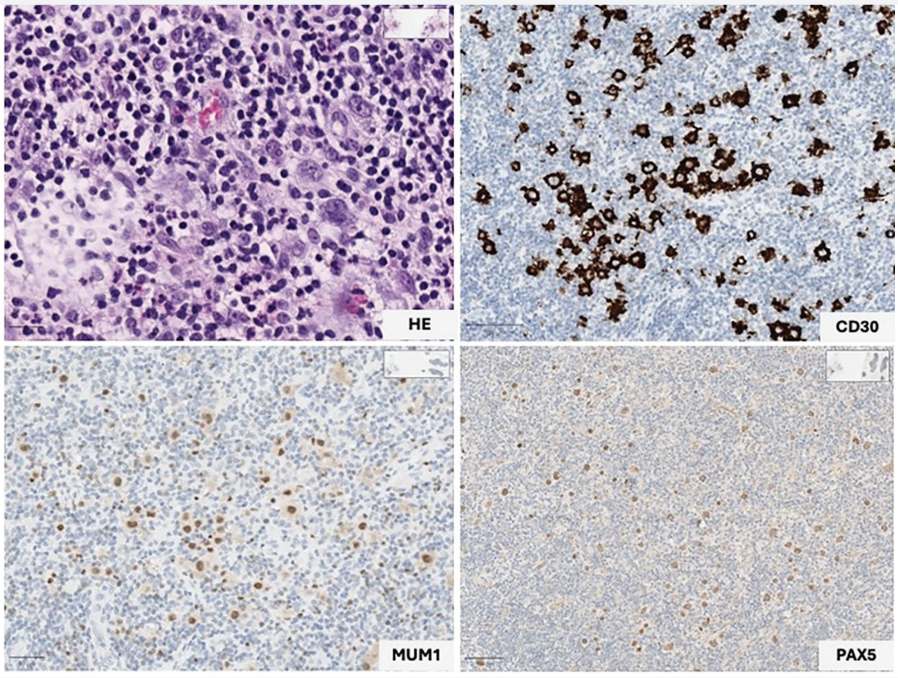


Figure 3. Lymph node involvement by classical Hodgkin lymphoma in the same patient. Top left: H&E staining shows a characteristic nodal architecture with scattered large atypical Reed-Sternberg cells in an inflammatory background. Top right: CD30 immunohistochemical staining highlights strong membranous and Golgi-associated positivity in Reed-Sternberg cells. Bottom left: MUM1 immunohistochemical staining shows moderate positivity in neoplastic cells. Bottom right: PAX5 immunohistochemical staining demonstrates weak but detectable nuclear expression in Hodgkin cells

H&E: Hematoxylin and eosin

the immune system. In a study of 263 patients with B-cell lymphoma, 15% of those with cHL showed recurring genomic breakages in CIITA[9]. Inactivating CIITA mutations are found in cancers including mediastinal large B-cell lymphoma, cHL, and gastric and colorectal cancers[10]. Loss of CIITA function can lead to impaired antigen presentation due to reduced MHC-II expression[11]. CIITA rearrangements can result in the loss of CIITA function, deletion of tumor suppressors, or overexpression of oncogenic fusion partners[9].

CONCLUSION

This case provides clinical experience in the management of both HL and NHL when occurring simultaneously, demonstrating appropriate treatment strategies for both. Further research is needed to elucidate the molecular mechanisms that lead to the simultaneous occurrence of multiple malignancies. To the best of our knowledge, this appears to be the first reported case of composite lymphoma coexisting with two distinct primary thyroid carcinomas, resulting in four synchronous primary malignancies.

Ethics

Informed Consent: Informed consent was obtained from the patient involved in this case report. They were informed about the medical procedures and the scientific purpose of this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Z.A.B., A.G., D.D., M.H., N.S., F.V., Concept: Z.A.B., A.G., D.D., F.V., Design: Z.A.B., F.V., Data Collection or Processing: Z.A.B., A.G., F.V., Analysis or Interpretation: Z.A.B., A.G., D.D., M.H., N.S., F.V., Literature Search: Z.A.B., A.G., D.D., M.H., N.S., F.V., Writing: Z.A.B.

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CASE REPORT

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Bee Sting-induced Status Epilepticus: Case Report

Arı Sokmasına Bağlı Gelişen Status Epileptikus: Olgu Sunumu

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ABSTRACT

A bee sting can provoke a spectrum of clinical symptoms. Status epilepticus, though rare, is a serious and potentially life-threatening complication associated with bee stings, which can be fatal if not treated promptly. This case report describes an adult patient who developed status epilepticus following a bee sting. The individual presented to the emergency department with generalized swelling and erythema after the sting and experienced a seizure within minutes. When seizures could not be managed with diazepam and levetiracetam, intravenous midazolam was administered at a dose appropriate for general anesthesia, leading to the patient's admission to the intensive care unit. It is essential to remember that status epilepticus, though rare, can develop as a consequence of a bee sting. Physicians should closely monitor patients in the emergency department for four to 12 hours to facilitate the early detection of potential complications.

Keywords: Bee sting, status epilepticus, emergency department

ÖZ

Arı sokması, çeşitli klinik semptomların ortaya çıkmasına neden olabilir. Status epileptikus, nadir görülmesine rağmen arı sokmalarıyla ilişkili ciddi ve potansiyel olarak yaşamı tehdit eden bir komplikasyondur ve zamanında tedavi edilmezse ölümcül olabilir. Bu olgu sunumu, arı sokması sonrası status epileptikus gelişen bir yetişkin hastayı anlatmaktadır. Hasta, arı sokmasının ardından genel şişlik ve eritem ile acil servise başvurmuş ve dakikalar içinde nöbet geçirmiştir. Nöbetler diazepam ve levetirasetam ile kontrol altına alınamadığında, genel anestezi için uygun bir dozda intravenöz midazolam uygulanmış ve hasta yoğun bakım ünitesine kabul edilmiştir. Status epileptikusun nadir de olsa arı sokmasının bir sonucu olarak gelişebileceği unutulmamalıdır. Hekimler, olası komplikasyonların erken tespitini kolaylaştırmak için acil servisteki hastaları dört ila 12 saat boyunca yakından izlemelidir.

Anahtar Kelimeler: Arı sokması, status epileptikus, acil servis

INTRODUCTION

Bee stings can result in a wide range of clinical presentations, from mild to severe. Factors such as the patient's age, underlying health conditions, and the number of stings can affect these presentations[1]. Clinically, reactions are categorized as local inflammatory responses (e.g., pain, swelling, itching, rash), allergic reactions (e.g., urticaria, angioedema), anaphylactic shock, or systemic toxic effects (e.g., myocardial injury, hypertension, liver damage, rhabdomyolysis, hemolysis, coma, and acute renal failure)[2]. Neurological symptoms, though rare, may also occur after bee stings[3]. These symptoms can arise without accompanying systemic reactions, vary in severity, and often pose diagnostic challenges. Here, we present a case of status epilepticus following a bee sting.

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CASE REPORT

A 50-year-old male patient presented to the emergency department with erythema and swelling in the abdominal and dorsal regions following multiple bee stings. He denied experiencing dyspnea, abdominal pain, chest pain, nausea, or vomiting. His past medical history was unremarkable, with no chronic conditions, previous surgeries, or regular medication use. Upon admission, his vital signs were as follows: body temperature 36.5 °C, heart rate 112 beats per minute, blood pressure 126/79 mmHg, respiratory rate 19 breaths per minute, peripheral oxygen saturation 99%, and blood glucose 84 mg/dL. Physical examination revealed multiple areas of erythema and swelling in the abdomen and back, consistent with bee stings, while the systemic examination showed no abnormalities. During the physical examination, the patient suddenly developed jaw locking, altered consciousness, and generalized muscle contractions, consistent with a generalized tonic-clonic seizure. Airway, breathing, and circulation were promptly evaluated, and oxygen therapy was initiated via mask along with continuous monitoring. Immediate treatment was provided for both anaphylaxis and the seizure. The patient received intramuscular 0.5 mg adrenaline, intravenous (IV) diazepam at a dose of 0.15 mg/kg, and IV corticosteroids. As the seizure continued, additional doses of adrenaline and diazepam were administered. Due to the inability to control the seizure, an IV loading dose of 20 mg/kg levetiracetam was administered. The patient was deemed to be in status epilepticus after failing to stabilize following levetiracetam administration and was subsequently intubated. An IV bolus of midazolam was administered at a dose of 0.1 mg/kg, followed by a continuous IV infusion at 0.1 mg/kg/hour. The administration of midazolam resulted in the cessation of the patient's seizures. Comprehensive laboratory evaluations, including a complete blood count, biochemical panel, coagulation parameters, and venous blood gas analysis, were conducted. The laboratory findings revealed leukocytosis (18.62×10^3/mL) and lactic acidosis (pH: 7.22, lactate: 8.1 mmol/L), while other parameters remained within normal limits: creatinine 1.1 mg/dL, blood urea nitrogen 11 mg/dL, sodium 138 mmol/L, potassium 3.6 mmol/L, calcium 8.4 mg/dL, magnesium 2.5 mg/dL, glucose 122 mg/dL, and hemoglobin 14 g/dL. The patient's electrocardiogram demonstrated a normal sinus rhythm. To exclude differential diagnoses, non-contrast cranial computed tomography and diffusion-weighted magnetic resonance imaging were performed, both of which revealed no intracranial pathologies. An electroencephalogram conducted 48 hours after admission showed normal results, with no evidence of epileptiform activity. The patient was extubated six days after being admitted to the intensive care unit for status epilepticus and was subsequently discharged in good health.

DISCUSSION

Status epilepticus is characterized by the occurrence of two or more consecutive seizures lasting more than 30 minutes, with incomplete recovery of consciousness between seizures[4]. Potential etiologies for status epilepticus include cerebrovascular diseases, central nervous system infections, neurodegenerative disorders, intracranial tumors, cortical dysplasias, head trauma, poisonings, autoimmune disorders, metabolic disturbances, and mitochondrial diseases[5]. Notably, bee stings are not classified as one of these etiological factors.

The management of status epilepticus begins with a stabilization phase, which focuses on ensuring airway patency, as well as adequate respiration and circulation. The subsequent medical treatment is divided into three phases. The first phase is initiated when the seizure duration reaches 5 minutes, with benzodiazepines (midazolam, lorazepam, or diazepam) recommended as the first-line therapy. The second phase begins at the 20-minute mark, with treatment options including fosphenytoin, valproic acid, and levetiracetam. The third phase is initiated when the seizure duration reaches 40 minutes, at which point either the second-line treatment is repeated, or anesthetic doses of thiopental, midazolam, pentobarbital, or propofol are administered[6].

Various clinical symptoms can arise following a bee sting. In a multicenter study conducted by Xie et al.[7] which examined 1091 cases of bee stings, it was reported that all patients exhibited a local inflammatory reaction, with 22% experiencing anaphylactic shock, 23% demonstrating two or more organ dysfunctions, and 1.2% presenting with seizures. The literature also includes various case reports of seizures triggered by bee stings[8,9]. However, the development of status epilepticus has only been reported in a single case, which was associated with allergic encephalitis[10]. To the best of our knowledge, this is the first reported case of status epilepticus induced by bee venom without an underlying allergic or encephalitic process. This case highlights the potential neurotoxic effects of bee venom in the central nervous system, suggesting a direct role in the pathogenesis of status epilepticus. These findings underscore the need to consider a broader spectrum of neurological complications associated with bee stings than previously recognized.

Cases involving the central or peripheral nervous system related to bee stings have been documented, including encephalitis, acute inflammatory polyradiculoneuropathy (Guillain-Barré syndrome), acute ischemic stroke, seizures, and intracranial hemorrhage[9,11-13]. The proposed pathological mechanisms underlying these cases include vasoconstriction and platelet aggregation triggered by exposure to bee venom, direct neurotoxic effects leading to neuronal membrane damage, and delayed autoimmune mechanisms initiated by antigen exposure[3,14].

Bee venom comprises at least 18 components, including peptides (melittin, apamin, and adolapin), enzymes (phospholipase A2 and hyaluronidase), biologically active amines (histamine and epinephrine), and non-peptides (lipids, carbohydrates, and free amino acids)[15]. These components contribute to the local and systemic reactions observed following a bee sting. Melittin, which constitutes 52% of the peptides, plays a pivotal role in causing prolonged pain, hyperalgesia, and local inflammation[16]. Hyaluronidase, accounting for 1-3% of the peptides, facilitates the spread of inflammation by dilating capillaries; histamine, which comprises 0.5-2%, is implicated in allergic reactions; and dopamine and norepinephrine, making up 1-2%, contribute to increased heart rate[6].

Certain components of bee venom, such as apamin and phospholipase A2, exhibit neurotoxic properties affecting receptors, carriers, and ion channels in neural tissue[17]. Florea et al.[18] reported that bee venom induces widespread and irreversible structural changes in both neurons and glial cells in a dose-dependent manner, resulting in cellular death. Gandolfo et al.[19] demonstrated that experimental injection of the phospholipase A2 component of bee venom into mice led to the emergence of neurotoxic symptoms, including stereotypic movements and seizures, which could not be effectively managed with standard antiepileptic medications, gamma-aminobutyric acid receptor blockers, or calcium channel blockers. Similarly, in our case, seizures could not be controlled with conventional antiepileptic therapies.

CONCLUSION

This case highlights status epilepticus as a rare but serious complication of bee stings. Clinicians should remain vigilant for life-threatening conditions such as status epilepticus and anaphylaxis, in addition to the more common local reactions. Therefore, patients should be closely monitored for four to 12 hours after a bee sting, depending on their risk factors, to ensure early detection of potential complications.

Ethics

Informed Consent: Informed consent form was obtained from the patient.

Footnotes

Authorship Contributions

Concept: Y.Ç., Design: Y.Ç., S.G., Data Collection or Processing: Y.Ç., S.G., Analysis or Interpretation: Y.Ç., S.G., Literature Search: Y.Ç., S.G., Writing: Y.Ç., S.G.

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REVIEW

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Glial Fibrillary Acidic Protein as a Biomarker in Acute Ischemic Stroke: Diagnostic Value and Prognostic Implications

Akut İskemik İnmede Biyobelirteç Olarak Glial Fibriller Asidik Protein: Tanısal Değer ve Prognostik İpuçları

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ABSTRACT

Stroke is one of the leading causes of mortality and long-term disability worldwide, with ischemic stroke representing the most common subtype. Despite significant progress in neuroimaging and reperfusion therapies, rapid and accurate diagnosis remains a major challenge in clinical practice. Glial fibrillary acidic protein, an intermediate filament protein specific to astrocytes, has recently gained attention as a promising blood-based biomarker. Following astrocytic damage and disruption of the blood-brain barrier, glial fibrillary acidic protein is released into the extracellular space and enters the circulation. Although its levels rise rapidly in hemorrhagic stroke, the increase in ischemic stroke occurs more gradually, typically becoming measurable within the first 24 to 48 hours after the onset of symptoms. Clinical investigations have demonstrated that blood concentrations of glial fibrillary acidic protein are significantly higher in patients with ischemic stroke compared to healthy controls and that these elevations correlate with neurological severity, infarct volume, and functional outcomes. Thus, glial fibrillary acidic protein provides valuable prognostic insights and may support patient stratification. However, variability in cut-off values, differences in detection methods, and delayed kinetics remain important limitations. Future developments include the integration of glial fibrillary acidic protein into multimarker panels and the use of ultrasensitive point-of-care assays that may enable rapid decision-making in emergency settings. Overall, glial fibrillary acidic protein has the potential to serve as a complementary biomarker for diagnosis, prognosis, and monitoring in ischemic stroke, thereby contributing to improved patient care and individualized therapeutic strategies.

Keywords: Ischemic stroke, glial fibrillary acidic protein, biomarker, neuroinflammation, prognosis

ÖZ

İnme, dünya genelinde önde gelen ölüm ve kalıcı sakatlık nedenlerinden biridir ve en sık görülen alt tipi iskemik inmedir. Nörogörüntüleme yöntemleri ve reperfüzyon tedavilerindeki önemli ilerlemelere rağmen, hızlı ve doğru tanı klinik uygulamada hala büyük bir güçlük oluşturmaktadır. Astrositlere özgü bir ara filament proteini olan glial fibriller asidik protein, son yıllarda kan temelli umut verici bir biyobelirteç olarak öne çıkmıştır. Astrosit hasarı ve kan-beyin bariyerinin bozulmasını takiben glial fibriller asidik protein ekstrasellüler alana ve dolaşıma salınmaktadır. Hemorajik inmede düzeyler hızla yükselirken, iskemik inmede artış daha yavaş olmakta ve genellikle semptomların başlamasından sonraki ilk 24 ila 48 saat içerisinde ölçülebilir seviyelere ulaşmaktadır. Klinik araştırmalar, iskemik inme hastalarında kanda glial fibriller asidik protein düzeylerinin sağlıklı bireylere kıyasla belirgin şekilde yüksek olduğunu ve bu artışın nörolojik şiddet, enfarktüs hacmi ve fonksiyonel sonuçlarla ilişkili olduğunu göstermiştir. Bu nedenle glial fibriller asidik protein, prognostik öngörüler sunarak hasta sınıflamasına katkıda bulunabilir. Ancak, eşik değerlerdeki değişkenlik, ölçüm yöntemlerindeki farklılıklar ve gecikmiş kinetik önemli sınırlılıklar oluşturmaktadır. Gelecekte glial fibriller asidik proteinin multimarker panellere entegrasyonu ve ultrasensitif hızlı testlerin geliştirilmesi, acil servislerde daha etkin karar süreçlerini mümkün kılabilir. Genel olarak glial fibriller asidik protein, iskemik inmede tanı, prognoz ve izlem için tamamlayıcı bir biyobelirteç olma potansiyeline sahiptir.

Anahtar Kelimeler: İskemik inme, glial fibriller asidik protein, biyobelirteç, nöroenflamasyon, prognoz

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INTRODUCTION

Stroke is recognized as one of the leading causes of mortality and morbidity worldwide. As of 2021, approximately 12 million new stroke cases were reported globally, highlighting the growing burden of stroke on healthcare systems each year. Currently, it is estimated that one in four individuals will experience at least one stroke during their lifetime[1,2].

Acute stroke is characterized by the sudden onset of a focal neurological deficit affecting a specific vascular territory of the brain, retina, or spinal cord. Clinically, it is classified into two main subtypes: hemorrhagic and ischemic stroke (IS). Among these, IS accounts for approximately 85% of all stroke cases, making it the most prevalent form[3]. Acute ischemic stroke (AIS) typically occurs as a result of the occlusion of a cerebral vessel lumen due to either a thrombus or an embolus, leading to an interruption of regional blood flow[4]. Thrombotic strokes are most commonly associated with large-vessel atherosclerotic obstructions, whereas embolic strokes usually occur when clots formed in the cardiac chambers migrate distally into the cerebral vasculature. Lacunar infarcts, on the other hand, develop as a consequence of small-vessel disease, characterized by occlusion of penetrating arteries in subcortical regions with poor collateral circulation[5].

Pathophysiology of Acute Ischemic Stroke

Under normal physiological conditions, cerebral blood flow is maintained constant by autoregulatory mechanisms involving vessel diameter regulation and vasodilators such as nitric oxide[6]. This system operates within a mean arterial pressure range of 60-150 mmHg; however, in pathological conditions such as stroke, autoregulation is impaired, and brain tissue responds passively to decreases in perfusion pressure. This decline in perfusion pressure first results in the cessation of protein synthesis, followed by reduced glucose utilization, activation of anaerobic metabolism, and ultimately neuronal dysfunction[7]. Within the ischemic territory, an infarct core forms in areas supplied by a single artery, surrounded by partially perfused but still viable tissue maintained through collateral circulation, termed the “ischemic penumbra.” Preservation of this region requires early recanalization, which underscores the principle that “time is brain” in IS therapy[7,8].

The cellular damage following IS is not limited to apoptosis but results from the interplay of multiple regulated cell death mechanisms, including ferroptosis, necroptosis, pyroptosis, parthanatos, phagoptosis, and autophagy. These pathways are driven by shared upstream processes such as oxidative stress, metabolic failure, inflammatory signaling, and immune activation, which collectively contribute to irreversible tissue injury within both the infarct core and the ischemic penumbra. Although these mechanisms have distinct molecular features, they converge in amplifying cellular dysfunction and structural damage within the ischemic brain[9-13].

Importantly, these regulated cell death pathways do not exclusively affect neurons but also involve glial cells within the ischemic microenvironment, particularly astrocytes, which play a central role in maintaining metabolic support and blood-brain barrier integrity. The extent and pattern of astroglial involvement may therefore reflect the severity and progression of ischemic injury[12,13].

Diagnosis and Treatment

AIS is a neurological emergency characterized by the sudden onset of focal neurological deficits and requires prompt recognition and intervention. Common clinical manifestations include unilateral weakness, speech and visual disturbances, sensory deficits, imbalance, nausea, and sudden severe headache. Although classical symptoms occur with similar frequency in both sexes, atypical presentations may be more frequent in women, contributing to diagnostic challenges[14,15].

Due to the heterogeneity of clinical presentation, early and accurate diagnosis is a critical determinant of effective stroke management. Timely identification of AIS directly influences treatment eligibility and clinical outcomes, as delays in diagnosis are associated with increased neurological damage and poorer prognosis. Therefore, rapid diagnostic evaluation remains a cornerstone of modern stroke care[16,17].

The primary goal of AIS treatment is the preservation of at-risk brain tissue through timely reperfusion. Treatment strategies and patient management depend largely on early recognition, appropriate triage, and prompt initiation of therapy. Delays in the pre-hospital and early in-hospital phases significantly reduce the effectiveness of therapeutic interventions and negatively affect functional recovery[18-20].

Despite advances in acute stroke management, variability in clinical course and outcomes remains substantial. Early prognostic assessment is essential for guiding treatment decisions, optimizing patient selection, and predicting neurological recovery. In this context, there is a growing need for reliable biomarkers that can support early diagnosis, reflect the extent of brain injury, and provide prognostic information, particularly in the hyperacute phase of AIS[14,21-24].

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is a unique intermediate filament protein of astrocytes in the central nervous system (CNS), playing a critical role in maintaining the structural integrity of these cells, providing mechanical resilience, and sustaining homeostasis within neural tissue. Astrocytes constitute approximately 30-40% of CNS cells and, beyond their role in the

blood-brain barrier (BBB), they engage in extensive interactions with neurons and other glial cells. They are central to fundamental processes such as synaptic transmission, ion balance, and metabolic support. Structurally, GFAP is composed of three main domains: an N-terminal head region, a central rod domain, and a C-terminal tail domain, all of which are functionally important for filament assembly and stabilization[25,26]. GFAP is expressed in mature astrocytes located in both gray and white matter, in the cerebellum, subventricular and subgranular zones, and in Müller cells of the retina. Moreover, GFAP expression can also be observed in Schwann cells, enteric glia, and hepatic stellate cells. To date, ten distinct isoforms of GFAP have been identified in the nervous system, with GFAPα being the most common and extensively studied variant[26]. GFAP is not merely a structural protein but also serves as an important biomarker reflecting glial cell responses. Its levels rise markedly in conditions characterized by impaired gliovascular integrity, disrupted glymphatic clearance, or astrocytic injury[27]. For instance, in post-mortem brain tissue of individuals with CADASIL, a hereditary small-vessel disease, an accumulation of GFAP-positive clasmatodendritic astrocytes was observed in perivascular regions of the deep white matter, indicating disruption of the gliovascular unit and significant impairment of glymphatic function[28].

The recognition of GFAP as a clinically relevant biomarker has been enabled by advances in highly sensitive detection methods in biological fluids such as cerebrospinal fluid (CSF), vitreous humor, and amniotic fluid. Although conventional assays like enzyme-inked immunosorbent assay lack the sensitivity to detect its low plasma concentrations, elevated GFAP levels can nonetheless be observed in conditions such as traumatic brain injury and neuromyelitis optica[26,29]. More recently, ultrasensitive platforms, particularly Simoa, have allowed for reliable quantification of GFAP in both healthy individuals and neurological disorders, with portable devices even enabling results within minutes[30]. The mechanisms by which GFAP enters the circulation under pathological conditions remain incompletely understood, but current evidence indicates a multifactorial process. Proposed pathways include bulk flow through arachnoid villi, the glymphatic system and cervical lymphatic drainage, as well as bidirectional exchange across the BBB and the blood-CSF barrier[26,27,31]. In addition to pre-analytical factors, the "hook effect" caused by protein aggregation represents a technical limitation, potentially compromising assay reliability. Remarkably, GFAP has been shown to remain stable within aggregate structures for extraordinary durations, as evidenced by the "Heslington Brain" specimen, which preserved GFAP integrity for thousands of years under exceptional conditions. In living tissues, however, such aggregate accumulation has been associated with fatal neurological disorders, including Alexander disease[32].

In conclusion, GFAP has emerged as a versatile biomarker not only for elucidating astrocyte biology but also for its growing significance in the diagnosis, prognosis, and monitoring of neurological diseases. Owing to its structural features at the molecular level and its association with the glymphatic system, GFAP provides a valuable parameter for assessing astrocytic responses in both clinical and research contexts.

Glial Fibrillary Acidic Protein in Acute Ischemic Stroke

GFAP is an intermediate filament protein exclusively expressed in astrocytes within the CNS. During acute AIS, disruption of astrocytic integrity and the BBB leads to the release of GFAP into the extracellular space and subsequently into the circulation. This process renders GFAP a promising biomarker reflecting astroglial injury in AIS. While GFAP rises rapidly and to high levels in hemorrhagic stroke, its increase in AIS is more gradual, typically reaching measurable levels within the first 24-48 hours after symptom onset[33,34]. Several clinical studies have demonstrated that GFAP levels in AIS are significantly elevated compared to healthy controls and strongly correlate with clinical severity and outcome measures. Ferrari et al.[35] reported that serum GFAP concentrations peaked within the first 24 hours and showed significant associations with National Institutes of Health Stroke scale (NIHSS) scores, modified Rankin scale and three-month functional outcomes. Similarly, Amalia[34] observed a positive correlation between GFAP levels and NIHSS scores, with markedly higher values in patients with large vessel occlusion compared to small vessel occlusion. These findings indicate that GFAP not only reflects acute astroglial injury but also provides prognostic insights into stroke severity and recovery potential. GFAP has also been linked to infarct volume and neurovascular status in AIS. Wunderlich et al.[36] reported that elevated serum GFAP levels were associated with larger infarct volumes and impaired neurovascular integrity. Furthermore, the multicenter BE FAST India study by Kalra et al.[37] demonstrated that GFAP, at defined cut-off values (0.33-0.57 µg/L), exhibited high diagnostic accuracy and could support clinical decision-making particularly in the early phase. In addition, recent evidence suggests that GFAP can be evaluated not only in its soluble protein form but also in association with circulating immune cells. van den Bossche et al.[38] showed that the proportion of circulating $GFAP^{+}CD16^{+}$ monocytes significantly increased within the first 2-8 hours after AIS and correlated with infarct volume. This cellular approach represents an innovative method that may capture lesion size earlier and more precisely than soluble GFAP. It should be noted that reported GFAP cut-off values may vary depending on the analytical assay and measurement platform used. Key studies evaluating GFAP for early stroke differentiation and prognostic assessment are summarized in Table 1. Taken together, these findings demonstrate that GFAP possesses both diagnostic and prognostic potential in AIS. GFAP reflects astroglial damage, correlates with neurological deficit and infarct burden, and predicts functional recovery. Although its slower rise compared

Table 1. Key studies evaluating GFAP for early stroke differentiation and prognosis

Study (references)	Study design/population	Time window from symptom onset	Correlations	Main findings
Ferrari et al.[35]	Prospective observational study, AIS patients	≤24 h	NIHSS, mRS, 3-month outcome	GFAP levels correlated with stroke severity and functional outcome
Amalia[34]	Observational cohort, AIS patients	≤24-48 h	NIHSS, vessel occlusion	Higher GFAP levels observed in large vessel occlusion compared to small vessel disease
Wunderlich et al.[36]	Cohort study, AIS patients	≤72 h	Infarct volume, neurovascular integrity	Elevated GFAP associated with larger infarct volume and impaired neurovascular status
Kalra et al.[37]	Multicenter prospective study	≤6 h	NIHSS, clinical diagnosis	GFAP showed high diagnostic accuracy and supported early clinical decision-making
van den Bossche et al.[38]	Observational study, AIS patients	2-8 h	Infarct volume	Circulating GFAP-positive monocytes increased early and correlated with lesion size

AIS: Acute ischemic stroke, GFAP: Glial fibrillary acidic protein, NIHSS: National Institutes of Health Stroke scale, mRS: Modified Rankin scale

to hemorrhagic stroke limits its utility in very early diagnosis, GFAP holds substantial clinical relevance in assessing disease severity, predicting outcomes, and serving as a complementary component of multiparametric biomarker panels in AIS.

Future Perspectives and Current Challenges

GFAP has emerged as a promising biomarker in AIS, yet important challenges remain before it can be translated into routine clinical practice. Variability in reported cut-off values, differences in assay platforms, and the absence of standardized protocols continue to hinder comparability across studies. Furthermore, the relatively delayed rise of GFAP in IS limits its usefulness as a very early diagnostic marker. Clinical interpretation may also be complicated by comorbid neurological conditions or prior cerebrovascular events. In particular, chronic neurodegenerative diseases, traumatic brain injury, or other conditions associated with astroglial damage may elevate baseline GFAP levels and thereby confound stroke-related measurements. Looking ahead, technological advances such as rapid point-of-care assays could enable bedside testing and shorten decision-making times in emergency settings. Integrating GFAP into multimarker panels may provide higher diagnostic and prognostic accuracy than using it alone. In addition, innovative cellular approaches, including the assessment of GFAP-positive monocytes, offer new perspectives for estimating lesion burden and monitoring tissue injury in the hyperacute phase. These developments highlight the potential of GFAP to complement existing diagnostic and prognostic strategies in acute AIS.

CONCLUSION

GFAP reflects astrocytic injury and offers valuable insights into disease severity, infarct volume, and functional outcome in acute IS. While its temporal dynamics restrict its role in ultra-early diagnosis, GFAP remains highly relevant for prognostic assessment and patient stratification. Importantly, this review provides a unified framework by integrating the early diagnostic utility of GFAP with its prognostic relevance across the acute and recovery phases of IS. By bridging early stroke differentiation with longitudinal outcome assessment, this perspective highlights GFAP as a versatile biomarker that extends beyond single-purpose applications. With further methodological refinement and integration into multimarker strategies, GFAP is likely to become an important complementary tool in the modern management of acute IS.

Footnote

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