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Contents

Original Articles

PARP-1 rs1136410 Polymorphism and Gastrointestinal Cancer Risk: A Meta-Analysis of Cancer-Type and Ethnic-Specific Associations

PARP-1 rs1136410 Polimorfizmi ve Gastrointestinal Kanser Riski: Kanser Türü ve Etnik Spesifik İlişkilerin Meta Analizi

Amirhosein NASERI, Mohammad Hossein ANTIKCHI, Sepideh SOLEYMANI, Mehdi KHOSRAVI-MASHIZI, Rezvan NEZAMESLAMI, Alireza NEZAMESLAMI, Bahareh MEHDIKHANI, Ahmad SHIRINZADEH-DASTGIRI, Seyed Masoud HAGHIGHIKIAN, Mohammad VAKILI-OJAROOD, Amirhossein RAHMANI, Hossein NEAMATZADEH; Tehran, Yazd, Ardabil, Iranshahr, Iran.....116

Clinical Impact of Cerebrospinal Fluid Multiplex Polymerase Chain Reaction (PCR) Testing in Children with Suspected Central Nervous System Infection

Merkezi Sinir Sistemi Enfeksiyonu Şüphesi Olan Çocuklarda Serebrospinal Sıvı Multipleks Polimeraz Zincir Reaksiyonu (PCR) Testinin Hasta Yönetimine Etkisi

Aytac GOKTUG, Idil AK GUNDOGDU, Muhterem DUYU, Esen BESLI; Istanbul, Türkiye 128

Short- and Long-Term Statin Persistence and Determinants in Patients Initiating Statin Therapy

Statin Başlanan Hastalarda Kısa ve Uzun Dönem Statin Persistansı ve Belirleyicileri

Mehmet UZUNLULU, Cundullah TORUN, Erhan EKEN, Gizem AYASGIL ULAS, Lütfullah CASTUR; Istanbul, Türkiye 136

Epidemiological Insights into HACEK Bacteria: A Seven-year Retrospective Analysis at a Tertiary Care Center in Istanbul

HACEK Bakterilerine Yönelik Epidemiyolojik Bulgular: Istanbul'daki Bir Üçüncü Basamak Merkezde Yedi Yıllık Retrospektif Analiz

Tansu DUNDAR, Zafer HABIP, Mucahide Esra KOCOGLU, Tuncer OZEKINCI; Istanbul, Türkiye 143

Impact of Eosinophil Levels on Disease Progression and Clinical Outcomes in Chronic Obstructive Pulmonary Disease (COPD) Patients: A Retrospective Study

Kronik Obstrüktif Akciğer Hastalığı (KOAH) Hastalarında Eozinofil Düzeylerinin Hastalık İlerlemesi ve Klinik Sonuçlar Üzerindeki Etkisi: Retrospektif Bir Çalışma

Burcu ARPINAR YIGITBAS, Esra ERTAN YAZAR, Elif Hazal KARADAG, Elif Yelda OZGUN NIKSARLIOGLU, Coskun DOGAN, Fatma Ceren GUREL; Istanbul, Türkiye..... 150

Is Transient Tachypnea of the Newborn a Risk Factor for Bronchiolitis in the First Two Years of Life? A Population-Based Birth Cohort Study

Yenidoğanın Geçici Takipnesi Yaşamın İlk İki Yılında Bronşiyolit için Bir Risk Faktörü mü? Nüfus Temelli Doğum Kohort Çalışması

Sinem CAN OKSAY, Mustafa Kursat SAHIN, Gulay BILGIN, Deniz MAVI TORTOP, Zeynep Reyhan ONAY, Yetkin AYHAN, Askın KESKIN KAPLAN, Saniye GIRIT; Istanbul, Samsun, Eskisehir, Sanlıurfa, Türkiye 157

MEDJ

Contents

Sex Estimation Using Patellar Morphometry: Evidence from a Late Roman Population in Anatolia

Patellar Morfometri Kullanarak Cinsiyet Tahmini: Anadolu'daki Geç Roma Dönemi Nüfusundan Kanıtlar

Yarenkur ALKAN, Adem TOKPINAR; Kırsehir, Ordu, Türkiye 166

Evaluation of Deaths in Malatya Due to the Earthquake Centered in Kahramanmaras on 6 February 2023

6 Şubat 2023'te Kahramanmaraş Merkezli Depremlerde Malatya'da Meydana Gelen Ölüm Olgularının Değerlendirilmesi

Mucahit ORUC, Mehmet Efdal SAYDAN, Emre BOZ, Abdulkerim YAYLA, Erkal GUMUSBOGA, Mesut YILMAZ, Osman CELBIS; Malatya, Istanbul, Antalya, Türkiye 173

Anti-Nuclear Antibody Staining Patterns in Juvenile Idiopathic Arthritis: Association of AC-1 Pattern and Elevated Titers with Uveitis

Juvenil İdiopatik Artritte Anti-Nükleer Antikor Boyanma Paternleri: AC-1 Paterni ve Yüksek Titrelerin Üveit ile İlişkisi

Lütfiye KORU, Fehim ESEN, Kardelen CELIKEL, Özlem TURKYILMAZ, Feray KAYA, Elif KUCUK, Zelal AYDIN, Eda Nur DIZMAN, Hatice Kübra DURSUN, Merve Özen BALCI, Fatih HASLAK, Kübra OZTURK; Istanbul, Türkiye 180

Review

Exploring the Link between DNA Hypermethylation and HPV in Salivary Gland Tumors

Tükürük Bezi Tümörlerinde DNA Hipermetilasyonu ve HPV Arasındaki Bağlantının Araştırılması

Anzila MEER, Sarina SULONG, Anani Aila MAT ZIN, Rohimah MOHAMUD, Norhafiza MAT LAZIM; Kelantan, Malaysia 187

Case Report

Pi*M Palermo Mutation in Bronchiectasis due to Alpha-1 Antitrypsin Deficiency: A Rare Genetic Cause

*Alfa-1 Antitripsin Eksikliğine Bağlı Bronşektazide Pi*M Palermo Mutasyonu: Nadir Bir Genetik Sebep*

Beyza YILDIRIMLI, Coskun DOGAN, Elif YILMAZ GULEC, Gonul SEVEN YALCIN; Istanbul, Türkiye 193

Letters to the Editor

Comment on "the Role of Combined C-reactive Protein and Albumin Indices in Predicting Prolonged Hospital Stay in Acute Pancreatitis: A Prospective Observational Study"

"Akut Pankreatitte Uzun Süreli Hastane Yatışını Tahmin Etmede Kombine C-Reaktif Protein ve Albümin İndekslerinin Rolü: Prospektif Gözlemsel Çalışma"

Rachana MEHTA, Ranjana SAH; Faridabad, Maharashtra, India 198

Response to the Letter to the Editor Regarding Our Research Article on "The Role of Combined C-reactive Protein and Albumin Indices in Predicting Prolonged Hospital Stay in Acute Pancreatitis: A Prospective Observational Study"

"Akut Pankreatitte Uzamış Hastanede Kalış Süresini Öngörmede Kombine C-reaktif Protein ve Albümin İndekslerinin Rolü: Prospektif Gözlemsel Bir Çalışma" Başlıklı Araştırma Makalemize Gelen Editöre Mektuba Yanıt

Abdullah ALGIN, Serdar OZDEMIR, Abuzer OZKAN, Kaan YUSUFOGLU, Mustafa Ahmet AFACAN; Istanbul, Türkiye 200

Contents

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Medeni Med J 2025;40:116-127

Original Article

PARP-1 rs1136410 Polymorphism and Gastrointestinal Cancer Risk: A Meta-Analysis of Cancer-Type and Ethnic-Specific Associations

PARP-1 rs1136410 Polimorfizmi ve Gastrointestinal Kanser Riski: Kanser Türü ve Etnik Spesifik İlişkilerin Meta Analizi

Amirhosein NASERI[1], Mohammad Hossein ANTIKCHI[2], Sepideh SOLEYMANI[3], Mehdi KHOSRAVI-MASHIZI[3], Rezvan NEZAMESLAMI[4], Alireza NEZAMESLAMI[5], Bahareh MEHDIKHANI[6], Ahmad SHIRINZADEH-DASTGIRI[3], Seyed Masoud HAGHIGHIKIAN[3], Mohammad VAKILI-OJAROOD[7], Amirhossein RAHMANI[8], Hossein NEAMATZADEH[9]

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ABSTRACT

Objective: Poly (ADP-ribose) polymerase-1 (*PARP-1*) is a key enzyme in DNA repair pathways and has been implicated in cancer susceptibility. The rs1136410 polymorphism in the *PARP-1* gene has shown inconsistent associations with gastrointestinal cancer risk across populations. This meta-analysis aimed to evaluate the association between *PARP-1* rs1136410 polymorphism and the risk of colorectal cancer (CRC) and gastric cancer (GC), with a focus on ethnic differences.

Methods: A systematic literature search was performed in PubMed, Scopus, EMBASE, Web of Science, Cochrane Library, BIOSIS, LILACS, CNKI, CBM, Wan Fang, and other regional databases up to February 1, 2025. Eligible case-control studies assessing the association between *PARP-1* rs1136410 polymorphism and CRC or GC were included. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated under five genetic models using Comprehensive Meta-Analysis software.

Results: Thirteen case-control studies were included, comprising 3.591 patients and 5.433 controls. For GC (8 studies; 1,784 cases and 2,521 controls), significant associations were observed under multiple genetic models: allele comparison (C vs. T: OR=2.01, 95% CI 1.04-3.91, p=0.039), homozygous comparison (CC vs. TT: OR=1.77, 95% CI 1.24-2.52, p=0.002), heterozygous comparison (CT vs. TT: OR=1.36, 95% CI 1.18-1.57, p<0.001), and recessive comparison (CC vs. CT+TT: OR=1.54, 95% CI 1.08-2.20, p=0.017). No significant association was detected for CRC (5 studies; 1.807 cases and 2.912 controls). Ethnic subgroup analysis revealed a protective

ÖZ

Amaç: Poli (ADP-riboz) polimeraz-1 (*PARP-1*), DNA onarım yolaklarında önemli bir enzimdir ve kansere yatkınlıkla ilişkili olduğu düşünülmektedir. *PARP-1* genindeki rs1136410 polimorfizmi, farklı popülasyonlarda gastrointestinal kanser riski ile tutarsız ilişkiler göstermiştir. Bu meta-analiz, etnik farklılıklara odaklanarak *PARP-1* rs1136410 polimorfizmi ile kolorektal kanser (CRC) ve mide kanseri (GC) riski arasındaki ilişkiyi değerlendirmeyi amaçlamıştır.

Yöntemler: PubMed, Scopus, EMBASE, Web of Science, Cochrane Library, BIOSIS, LILACS, CNKI, CBM, Wan Fang ve diğer bölgesel veritabanlarında 1 Şubat 2025 tarihine kadar sistematik bir literatür taraması yapıldı. *PARP-1* rs1136410 polimorfizmi ile CRC veya GC arasındaki ilişkiyi değerlendiren uygun olgu-kontrol çalışmaları dahil edildi. Comprehensive Meta-Analysis yazılımı kullanılarak beş genetik model altında birleştirilmiş odds oranları (OR) ve %95 güven aralıkları (CI) hesaplandı.

Bulgular: 3.591 hasta ve 5.433 kontrolü içeren on üç olgu-kontrol çalışması dahil edildi. GC için (8 çalışma; 1.784 olgu ve 2.521 kontrol), çoklu genetik modeller altında anlamlı ilişkiler gözlemlenmiştir: alel karşılaştırması (C vs. T: OR=2,01, %95 CI 1,04-3,91, p=0,039), homozigot karşılaştırması (CC vs. TT: OR=1,77, %95 CI 1,24-2,52, p=0,002), heterozigot karşılaştırması (CT vs. TT: OR=1,36, %95 CI 1,18-1,57, p<0,001) ve resesif karşılaştırması (CC vs. CT+TT: OR=1,54, %95 CI 1,08-2,20, p=0,017). CRC için anlamlı bir ilişki tespit edilmemiştir (5 çalışma;

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effect against CRC in Caucasians but increased susceptibility in Asians.

Conclusion: The *PARP-1* rs1136410 polymorphism is associated with elevated GC risk but not CRC, with ethnicity-dependent effects suggesting differential genetic susceptibility. These findings highlight the importance of considering population-specific genetic backgrounds in gastrointestinal cancer risk assessment, prevention, and precision medicine strategies.

Keywords: Colorectal cancer, Gastric cancer, *PARP-1*, rs1136410 polymorphism, meta-analysis, genetic susceptibility, ethnic variation

1.807 olgu ve 2.912 kontrol). Etnik alt grup analizi, Kafkasyalılarda CRC'ye karşı koruyucu bir etki olduğunu, ancak Asyalılarda duyarlılığın arttığını ortaya koymuştur.

Sonuçlar: *PARP-1* rs1136410 polimorfizmi, GC riskiyle ilişkili olmakla birlikte CRC ile ilişkili değildir ve etnik kökene bağlı etkiler, farklı genetik duyarlılığa işaret etmektedir. Bu bulgular, gastrointestinal kanser risk değerlendirmesi, önleme ve hassas tıp stratejilerinde popülasyona özgü genetik arka planların dikkate alınmasının önemini vurgulamaktadır.

Anahtar kelimeler: Kolorektal kanser, mide kanseri, *PARP-1*, rs1136410 polimorfizmi, meta-analiz, genetik duyarlılık, etnik varyasyon

INTRODUCTION

Gastrointestinal cancers, particularly colorectal cancer (CRC) and gastric cancer (GC), pose a significant global health challenge[1]. In the US, 2024 projections estimate 152,810 new CRC diagnoses (81.540 in males, 71.270 in females) and 53.010 deaths (28.700 men, 24.310 women)[2-4]. CRC incidence and mortality exhibit racial/ethnic disparities, with Black Americans experiencing the highest rates, followed by Native Americans. Hispanic and Asian American/Pacific Islander populations show lower incidence, with Hispanic populations having better outcomes than non-Hispanic Whites[5-7]. These disparities stem from complex interactions of genetics, environment, and social determinants. GC has a distinct profile, with approximately 26,890 new cases (16.160 in males, 10.730 in females) and 10.880 deaths projected for 2024. Unlike CRC, GC incidence is higher among the Asian and Hispanic populations, as well as non-Hispanic Black Americans[8-10]. GC etiology includes Helicobacter pylori infection, tobacco use, diet, and familial predisposition, highlighting gene-environment interactions[11,12]. Understanding these patterns is crucial for investigating genetic polymorphisms influencing cancer risk[13].

Poly (ADP-ribose) polymerase-1 (*PARP-1*), also known as ADPRT, PARP, and NAD(+)-glycohydrolase, is crucial in the DNA damage response and repair via poly (ADP-ribosyl)ation[14,15]. In CRC, PARP-1 has a dual role, inhibiting tumor initiation via its interaction with the DNA repair protein O6-methylguanine-DNA methyltransferase, and promoting tumor progression influenced by genetic and environmental factors[16,17]. Increased *PARP-1* mRNA and protein levels are associated with worse outcomes in CRC, particularly in tumors with mutated p53[18-20]. *PARP-1* might also contribute to the cancer stem cell phenotype, essential for tumor aggressiveness and recurrence[18,21]. In GC, high *PARP-1* expression is associated with aggressive tumor traits such as invasion and metastasis, with specific single nucleotide polymorphisms of the *PARP-1* gene linked to increased susceptibility and lymph node metastasis[22]. *PARP-1* activation is involved in GC pathogenesis, especially through its interaction with Helicobacter pylori, which can stimulate *PARP-1* activity and promote inflammatory responses that encourage tumor development[23,24]. Consequently, the use of PARP inhibitors as potential treatments for GC has become more appealing, as they may improve the efficacy of standard chemotherapies like cisplatin[25].

The *PARP-1* gene, located on chromosome 1q41-42 and containing 23 exons, features the extensively studied SNP rs1136410 (Val762Ala)[13,26]. This SNP, resulting from a single nucleotide change that potentially alters *PARP-1*'s role in DNA repair and cancer-related processes, has been linked to cancer risk, particularly colorectal and GCs. Meta-analyses suggest a significant association between the rs1136410 C > T polymorphism and increased cancer susceptibility, especially in GC. The C allele is associated with increased risk for thyroid and cervical cancers, and decreased risk for brain cancer[27]. The association with GC is particularly strong in East Asian populations[27,28]. However, conflicting results suggest that the rs1136410 polymorphism may be protective or exhibit no correlation in some demographics or cancer types[29], highlighting the complexity of genetic influences on cancer. These inconsistencies necessitate comprehensive meta-analyses to clarify genetic associations. This meta-analysis investigates the relationship between *PARP-1* rs1136410 and CRC/GC risk, aiming to provide insights for patient risk assessment and management, especially in population-specific genetic counseling and precision medicine, and to establish evidence-based recommendations for incorporating genetic polymorphism data into clinical decision-making for gastrointestinal cancer prevention and early detection.

MATERIALS and METHODS

Literature Search and Database Selection

This systematic review and meta-analysis did not require ethical approval because it did not involve primary data collection from human subjects. A comprehensive literature search was conducted across

multiple electronic databases through February 1, 2025, to ensure complete and up-to-date coverage of relevant studies examining the association between the *PARP-1* rs1136410 polymorphism and CRC or GC risk, including both English and non-English publications. The databases searched included PubMed/MEDLINE, Scopus, EMBASE, Web of Science, Cochrane Library, BIOSIS Citation Index, LILACS, the Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, ProQuest Dissertations and Theses, Google Scholar, OpenGrey, and region-specific sources such as the China National Knowledge Infrastructure, Chinese Biomedical Database, Wan Fang Database, and VIP Information/Chinese Science and Technology Journal Database (VIP). The search strategy utilized a combination of Medical Subject Headings terms and free-text keywords, with cancer-related terms such as "colorectal cancer," "gastric cancer," "stomach cancer," "colon cancer," "rectal cancer," "digestive tract cancer," "gastrointestinal carcinoma," "gastric neoplasm," and "digestive system neoplasms"; *PARP-1* related terms including "poly (ADP-ribose) polymerase 1," "*PARP-1*," "NAD+ ADP-ribosyltransferase 1," "poly (ADP-ribose) synthase 1," and "DNA repair enzyme"; and polymorphism-specific terms such as "rs1136410," "Val762Ala," "V762A," "C>T," "single nucleotide polymorphism," "SNP," "genotype," "allele," "mutation," "variant," and "genetic susceptibility." Boolean operators (AND, OR, NOT), truncation, and proximity operators were applied to optimize retrieval. Reference lists of identified articles, relevant meta-analyses, and review papers were manually screened for additional studies. Ethical approval was not required for this systematic review and meta-analysis, as no primary data collection from human subjects was involved.

Study Selection Criteria

Studies were independently screened by two investigators using predefined inclusion and exclusion criteria. Inclusion criteria required: (1) case-control or cohort study design examining human subjects; (2) investigation of *PARP-1* rs1136410 polymorphism association with CRC or GC risk; (3) availability of sufficient genotype frequency data to calculate odds ratios (ORs) and 95% confidence intervals (CIs); (4) clearly defined case and control populations with appropriate diagnostic criteria. Exclusion criteria eliminated: (1) animal studies, in vitro experiments, or cell line investigations; (2) studies lacking complete genotype frequency data; (3) family-based or linkage studies involving related individuals; (4) abstracts, case reports, editorials, reviews, conference proceedings, or meta-analyses; (5) duplicate publications or overlapping study populations. When multiple publications reported on the same study population, only the most recent or largest study was included to prevent data duplication.

Data Extraction and Management

Two independent reviewers extracted data using standardized forms, with disagreements resolved through discussion or consultation with a third reviewer when necessary. Extracted variables included first author name and publication year; study design and geographic location; participant ethnicity categorized as Asian, Caucasian, African, Hispanic, or mixed populations; total sample sizes for cases and controls; genotype frequencies for *PARP-1* rs1136410 polymorphism in both cases and controls; genotyping methodology employed; Hardy-Weinberg equilibrium (HWE) test results in control groups; and minor allele frequencies in control groups. When data were unclear or missing, original study authors were contacted via email for clarification.

Quality Assessment

Study quality was evaluated using the Newcastle-Ottawa Scale (NOS), a validated tool for assessing the quality of non-randomized studies in meta-analyses[30,31]. The NOS evaluates three domains: selection of study groups (4 criteria), comparability of groups (1 criterion with 2 subcategories), and ascertainment of exposure or outcome (3 criteria). Each criterion awards one star except comparability, which can award up to two stars, resulting in a maximum score of nine stars. Studies scoring seven or more stars were classified as high quality, while those scoring five to six stars were considered of moderate quality and remained eligible for inclusion.

Statistical Analysis Methods

Meta-analysis was performed using Comprehensive Meta-Analysis software version 2.0. The association between *PARP-1* rs1136410 polymorphism and cancer risk was assessed using ORs with 95% CIs under five genetic inheritance models: allele comparison (C vs. T), homozygous comparison (CC vs. TT), heterozygous comparison (CT vs. TT), dominant model (CC+CT vs. TT), and recessive model (CC vs. CT+TT). HWE in control groups was evaluated using Fisher's exact test, with p-values <0.05 indicating deviation[32]. Between-study heterogeneity was assessed using Cochran's Q statistic and quantified using the I^2 statistic, which describes the percentage of total variation across studies due to heterogeneity rather than chance. I^2 values of 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively.

When significant heterogeneity was detected (P<0.10 for Q statistic or I^2>50%), a random-effects model using the DerSimonian-Laird method was employed; otherwise, a fixed-effects model using the Mantel-Haenszel method was applied[33-35]. Predefined subgroup analyses were conducted by ethnicity, geographic region, control source (population-based vs. hospital-based), and genotyping method to explore potential sources of heterogeneity[36]. Sensitivity analyses were performed by sequentially excluding individual studies to assess the robustness of pooled estimates. Publication bias was evaluated using Begg's funnel plots and Egger's linear regression test, with p<0.05 indicating significant bias. When publication bias was detected, the trim-and-fill method was applied to adjust pooled estimates. All statistical tests were two-sided with significance set at p<0.05.

RESULTS

Study Selection and Characteristics

As shown in Figure 1, the initial literature search identified 420 articles, which were reduced to 203 unique articles after removing duplicates. Title and abstract screening excluded 113 studies, leaving 90 for full-text review. Ultimately, 13 case-control studies met all eligibility criteria, comprising 3,591 cancer patients and 5,433 healthy controls. These included five CRC studies (1.807 cases, 2.912 controls) [37-41] and eight GC studies (1.784 cases, 2.521 controls)[42-48]. Table 1 shows the characteristics of selected studies. Published between 2004 and 2023, the studies represented global geographic distribution, including the United States, Singapore, China, Saudi Arabia, South Korea, and Brazil, and consisted predominantly of Asian (10 studies), with Caucasian (3 studies) and mixed ethnicity representation, reflecting

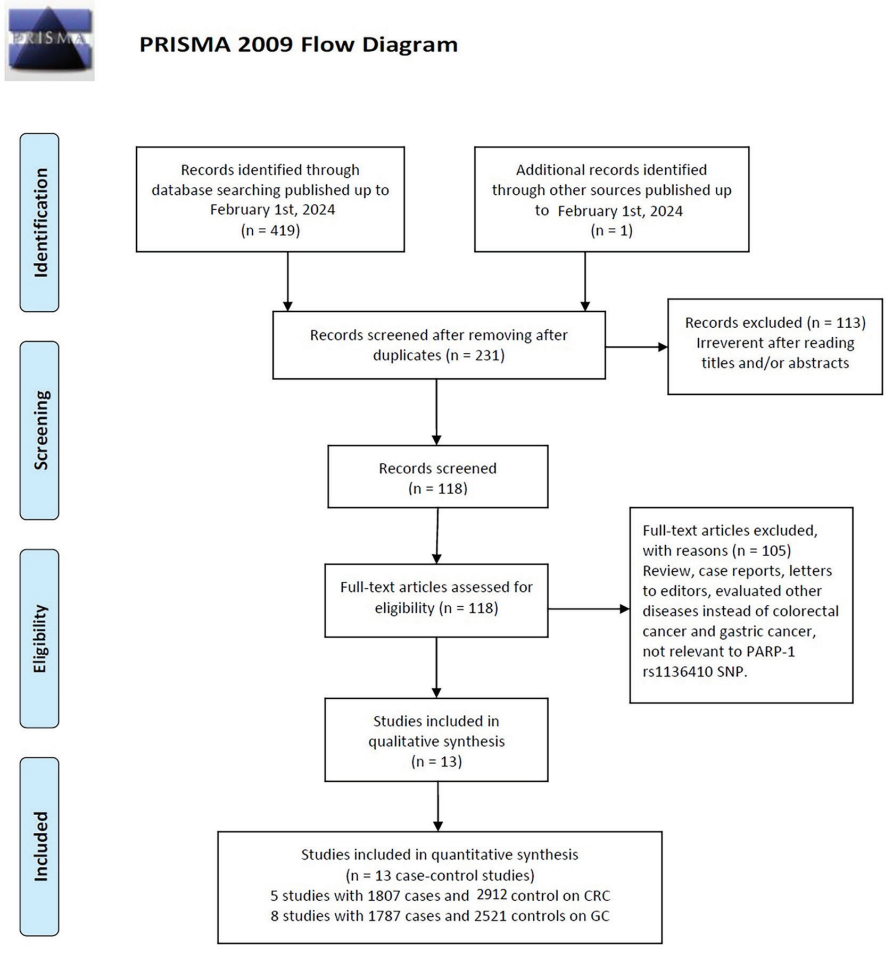

Figure 1. Flowchart depicting the selection process for eligible studies.

Illustrates the stepwise process used to identify and select studies meeting the inclusion criteria for this meta-analysis. It details the number of studies screened, assessed for eligibility, and included in the final analysis.

Table 1. Key features of the studies included in the meta-analysis.

Author/Year	Cancer type	Country (Ethnicity)	SOC	Genotype method	Case/ Control	Case					Control					HWE	MAF	NOS
						TT	TC	CC	T	C	TT	TC	CC	T	C			
Berndt[37]	CRC	USA(Caucasian)	PB	TaqMan	691/702	492	179	20	1163	219	488	183	31	1159	245	0.116	0.175	10
Stern[38]	CRC	Singapore(Asian)	PB	TaqMan	307/1173	93	150	64	336	278	381	564	228	1326	1020	0.456	0.435	11
Brevik[39]	CRC	USA(Caucasian)	FB	TaqMan	308/361	196	100	12	492	124	239	110	12	588	134	0.879	0.186	8
Li[40]	CRC	China(Asian)	HB	PCR-RFLP	451/626	134	228	89	496	406	222	319	85	763	489	0.078	0.391	8
Alshammari[41]	CRC	KSA(Asian)	HB	Sequence	50/50	47	2	1	96	4	49	1	0	99	1	0.943	0.010	8
Miao[42]	GC	China(Asian)	HB	PCR-RFLP	500/1000	150	257	93	557	443	396	492	112	1284	716	0.026	0.358	10
Zhang[47]	GC	China(Asian)	HB	PCR-RFLP	138/110	85	37	16	207	69	80	25	5	185	35	0.114	0.159	5
Zhang[43]	GC	China(Asian)	HB	PCR-RFLP	236/320	113	83	40	309	163	181	106	33	468	172	0.005	0.269	10
Kang[44]	GC	China(Asian)	PB	SNaPshot	150/152	70	67	13	207	93	88	50	14	226	78	0.089	0.257	5
Kim[45]	GC	Korea(Asian)	HB	GoldenGate	151/320	42	70	39	154	148	102	161	57	365	275	0.635	0.430	5
Wen[46]	GC	China(Asian)	HB	MassARRAY	307/307	96	154	57	346	268	105	132	70	342	272	0.024	0.443	5
Tang[25]	GC	China(Asian)	PB	PCR-RFLP	200/210	122	56	22	300	100	162	40	8	364	56	0.011	0.133	5
Dantas[48]	GC	Brazil(Mixed)	HB	AS-PCR	102/102	86	16	0	188	16	87	15	0	189	15	0.422	0.074	7

CRC: Colorectal cancer; GC: Gastric cancer; SOC: Source of controls, HB: Hospital based; PB, Population based, PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism, AS: Allele-specific polymerase chain reaction, HWE, Hardy-Weinberg equilibrium, MAF: Minor allele frequency; NOS: Newcastle-Ottawa score

cancer prevalence and enabling ethnic subgroup analyses. State-of-the-art genotyping methodologies were employed, including restriction fragment length polymorphism (RFLP)- polymerase chain reaction (PCR), TaqMan assays, SNaPshot sequencing, Illumina GoldenGate assays, Sequenom MassARRAY, and AS-PCR. The NOS indicated generally high study quality, but four GC studies (Miao[42], Zhang[43], Wen[46], Tang[25]) exhibited deviations from HWE ($p<0.05$), potentially impacting those analyses.

Quantitative Synthesis of Genetic Associations

Colorectal Cancer Risk Analysis

A meta-analysis of five studies investigating CRC risk found no significant overall association between the PARP-1 rs1136410 polymorphism and CRC risk across all genetic models (Table 2). However, ethnic stratification revealed significant population-specific differences. In Caucasian populations, the *PARP-1* rs1136410 C allele demonstrated a significant protective effect against CRC development. The allele comparison model (C vs. T) showed a substantial risk reduction (OR=0.487, 95% CI 0.299-0.794, p=0.004), indicating that individuals carrying the C allele have approximately 51% lower odds of developing CRC compared to those with the T allele. This protective effect was consistent across multiple genetic models. Conversely, Asian populations exhibited a significantly increased CRC risk associated with the C allele. The allele comparison revealed a substantial risk elevation (C vs. T: OR=5.785, 95% CI 4.481-7.467, p≤0.001), indicating nearly six-fold increased odds of CRC development. The homozygous comparison (CC vs. TT: OR=1.413, 95% CI 1.094-1.824, p=0.008) and recessive model (CC vs. CT+TT: OR=1.302, 95% CI 1.040-1.629, p=0.021) further supported this increased risk pattern in Asian populations.

Gastric Cancer Risk Analysis

The meta-analysis of eight GC studies demonstrated consistent and statistically significant associations between the *PARP-1* rs1136410 polymorphism and increased GC risk across multiple genetic inheritance models, providing robust evidence for genetic susceptibility to GC. The allele comparison model (C vs. T) showed a significant association with GC risk (OR=2.012, 95% CI 1.035-3.911, p=0.039), indicating that the C allele approximately doubles the odds of GC development (Figure 2A). The homozygous comparison (CC vs. TT) revealed an even stronger association (OR=1.766, 95% CI 1.239-2.515, p=0.002), suggesting that individuals homozygous for the C allele face nearly 77% increased odds of developing

GC (Figure 2B). Heterozygous comparison (CT vs. TT: OR=1.359, 95% CI 1.180-1.565, p≤0.001) demonstrated significant risk elevation even in carriers of a single C allele (Figure 2C). The dominant model (CC+CT vs. TT: OR=0.649, 95% CI 0.455-0.927, p=0.017) (Figure 2D) and recessive model (CC vs. CT+TT: OR=1.541, 95% CI 1.079-2.200, p=0.017) (Figure 2E) provided additional evidence for the association. Ethnic subgroup analysis revealed that the association between *PARP-1* rs1136410 polymorphism and GC risk was particularly pronounced in Asian populations, where the majority of included studies were conducted. This finding aligns with the known higher prevalence of GC in East Asian countries and suggests potential gene-environment interactions specific to these populations.

Heterogeneity Test

Heterogeneity analysis of the PARP-1 rs1136410 polymorphism showed variable inconsistency across genetic models and cancer types. In CRC, the C versus T genotype comparison exhibited high heterogeneity (I^2=98.36%, pH ≤0.001), indicating significant variability in study outcomes. Moderate heterogeneity was observed for CC versus TT and CC+CT versus TT (I^2 = 54.43% and

Table 2. Results of the meta-analysis on the PARP-1 rs1136410 polymorphism regarding the risk of colorectal and gastric cancer.

	Genetic model	Type of model	Heterogeneity		Odds ratio (OR)				Publication bias	
			I^2 (%)	P_H	OR	95% CI	Z_{OR}	P_{OR}	P_{Beggs}	P_{Eggers}
Colorectal Cancer										
Overall	C vs. T	Random	98.36	≤0.001	1..660	0.435-6.331	0.742	0.458	0.462	0.595
	CC vs. TT	Fixed	54.43	0.067	1.239	0.990-1.551	1.869	0.062	0.806	0.865
	CT vs. TT	Fixed	0.00	0.827	1.077	0.937-1.237	1.043	0.297	0.462	0.331
	CC+CT vs. TT	Fixed	48.29	0.102	0.844	0.689-1.033	-1.644	0.100	0.806	0.852
	CC vs. CT+TT	Fixed	48.29	0.102	1.185	0.968-1.451	0.713	0.476	0.806	0.852
Ethnicity										
Caucasians	C vs. T	Random	82.14	0.018	0.487	0.299-0.794	-2.883	0.004	NA	NA
	CC vs. TT	Fixed	36.90	0.208	0.791	0.494-1.268	-0.973	0.331	NA	NA
	CT vs. TT	Fixed	0.00	0.523	1.016	0.836-1.235	1.235	0.162	NA	NA
	CC+CT vs. TT	Fixed	28.99	0.235	1.270	0.795-2.029	1.001	0.317	NA	NA
	CC vs. CT+TT	Fixed	28.99	0.235	0.787	0.493-1.258	-1.001	0.317	NA	NA
Asians	C vs. T	Fixed	0.00	0.448	5.785	4.481-7.467	13.474	≤0.001	0.296	0.138
	CC vs. TT	Fixed	26.10	0.258	1.413	1.094-1.824	2.652	0.008	1.000	0.798
	CT vs. TT	Fixed	0.00	0.817	1.143	0.938-1.394	1.323	0.186	1.000	0.474
	CC+CT vs. TT	Fixed	26.67	0.256	0.768	0.614-0.962	-2.302	0.021	1.000	0.772
	CC vs. CT+TT	Fixed	26.67	0.256	1.302	1.040-1.629	2.302	0.021	1.000	0.772
Gastric Cancer										
Overall	C vs. T	Random	93.67	≤0.001	2.012	1.035-3.911	2.063	0.039	0.173	0.472
	CC vs. TT	Random	61.65	0.016	1.766	1.239-2.515	3.149	0.002	0.763	0.752
	CT vs. TT	Fixed	0.00	0.742	1.359	1.180-1.565	4.266	≤0.001	0.710	0.944
	CC+CT vs. TT	Random	67.94	0.007	0.649	0.455-0.927	-2.380	0.017	0.548	0.667
	CC vs. CT+TT	Random	67.94	0.005	1.541	1.079-2.200	2.380	0.017	0.548	0.667
Ethnicity										
Asians	C vs. T	Random	93.36	≤0.001	2.494	1.290-4.819	2.718	0.007	0.367	0.827
	CC vs. TT	Random	61.65	0.016	1.766	1.239-2.515	3.149	0.002	0.763	0.752
	CT vs. TT	Fixed	0.00	0.682	1.370	1.187-1.581	4.303	≤0.001	1.000	0.715
	CC+CT vs. TT	Random	67.94	0.005	0.649	0.455-0.927	-2.380	0.017	0.548	0.667
	CC vs. CT+TT	Random	67.94	0.005	1.541	1.079-2.200	2.380	0.017	0.548	0.667

NA: Not applicable, H: Heterogeneity, Beggs: Begg's test, Eggers: Egger's test

48.29%, respectively), but the p-values did not indicate significant heterogeneity. Conversely, in the CT versus TT and CC versus CT+TT models, no heterogeneity was observed (I^2=0.00%). In GC, substantial heterogeneity was also found for C versus T (I^2=93.67%, p≤0.001) and CC versus TT (I^2=61.65%, p=0.016), while CT versus TT showed no variability (I^2=0.00%). Random effects models for specific comparisons in both CRC and GC revealed significant heterogeneity, especially among Asian populations, suggesting potential genetic or environmental influences on cancer risk across ethnicities. Overall, these heterogeneity findings highlight the complexity of interpreting associations between the *PARP-1* rs1136410 polymorphism and cancer risk.

Sensitivity Analyses

The sensitivity analysis, conducted to evaluate the influence of individual studies on the meta-analysis of the *PARP-1* rs1136410 polymorphism, demonstrated that no single study significantly altered the overall ORs across different genetic models, confirming the robustness of the findings. Exclusion of four studies deviating from HWE (Miao[42], Zhang[43], Wen[46], and Tang[25]) did not substantially change the pooled OR estimates. Specifically, for GC, after excluding the HWE-violating studies, significant associations were observed in the allele model (C vs. T: OR=1.89, 95% CI 1.12-3.21, p=0.018), homozygous model (CC vs. TT: OR=1.68, 95% CI 1.15-2.46, p=0.007), heterozygous model (CT vs. TT: OR=1.31, 95% CI 1.09-1.58, p=0.004), and recessive model (CC vs. CT+TT: OR=1.39,

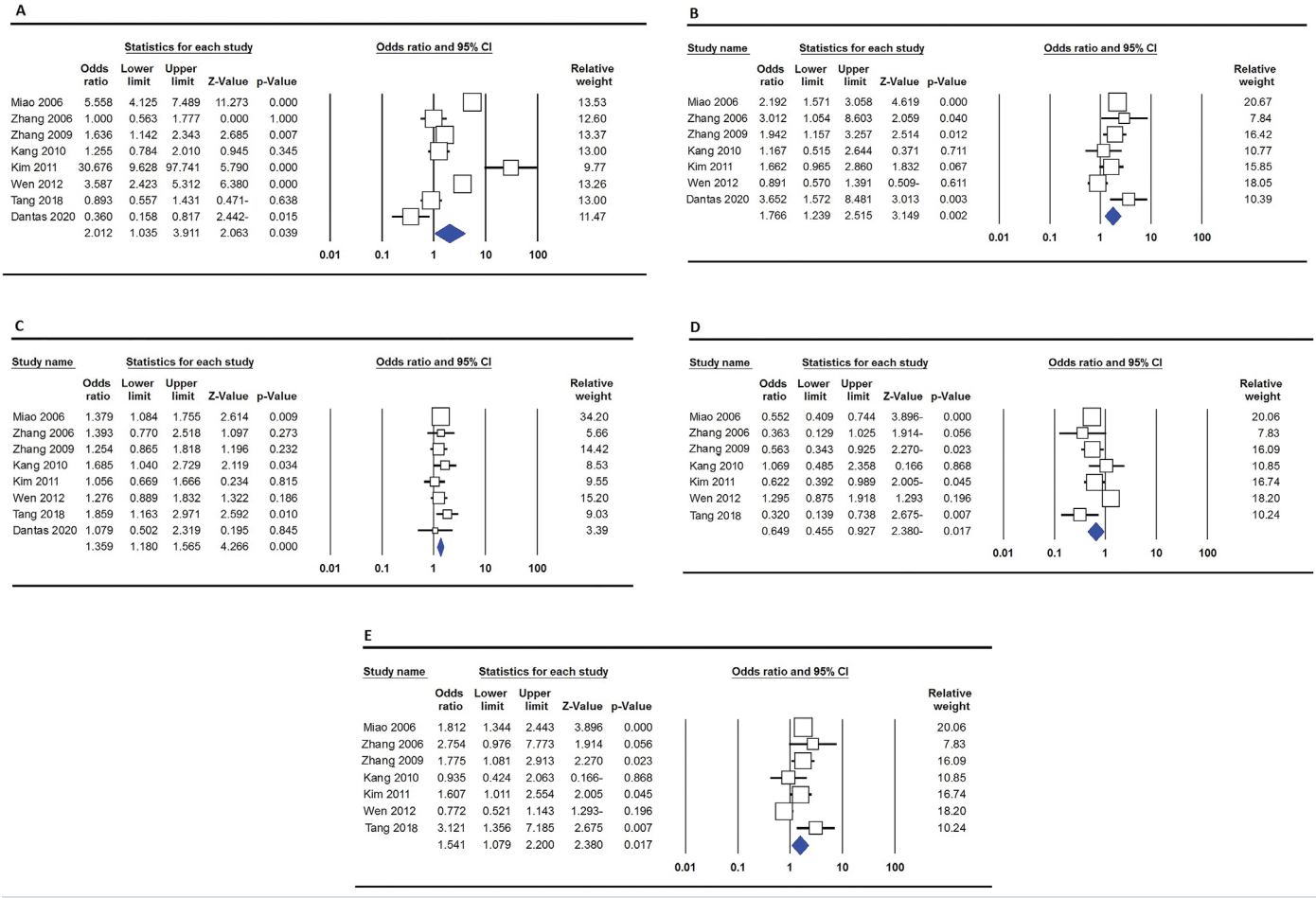

Figure 2. Forest plot illustrating the association between the *PARP-1* rs1136410 polymorphism and GC risk. **A)** allele (C vs. T). **B)** homozygote (CC vs. TT. **C)** heterozygote (CT vs. TT). **D)** dominant (CC+CT vs. TT). **E)** recessive (CC vs. CT+TT).

Presents a forest plot summarizing the meta-analysis results for the association between the *PARP-1* rs1136410 polymorphism and GC risk. Sub-figures A-E correspond to different genetic models (allele, homozygote, heterozygote, dominant, and recessive). The plot shows effect sizes (odds ratios) and 95% confidence intervals for individual studies and the overall pooled estimate.

95% CI 1.01-1.91, p=0.043), while the dominant model showed a borderline association (CC+CT vs. TT: OR=0.72, 95% CI 0.51-1.01, p=0.058). For CRC, all genetic models remained non-significant with effect sizes comparable to the main analysis, though ethnic-specific patterns -protective in Caucasians and risk-enhancing in Asians- persisted. Overall, these results reinforce the reliability of the meta-analysis conclusions and strengthen confidence in the observed association between the *PARP-1* rs1136410 polymorphism and cancer susceptibility, thereby enhancing the credibility of the study.

Publication Bias

The assessment of publication bias for the *PARP-1* rs1136410 polymorphism in relation to CRC and GC revealed varying results across different genetic models and ethnicities. Funnel plots (Figure 3A-E) indicate publication bias in the association between the PARP-1 rs1136410 polymorphism and the risks of CRC and GC. Begg's and Egger's tests indicated no significant publication bias for most genetic comparisons in CRC (p>0.05), including C vs. T, CC vs. TT, CT vs. TT, and CC+CT vs. TT models, and CC vs. CT+TT models (Begg's p=0.806;

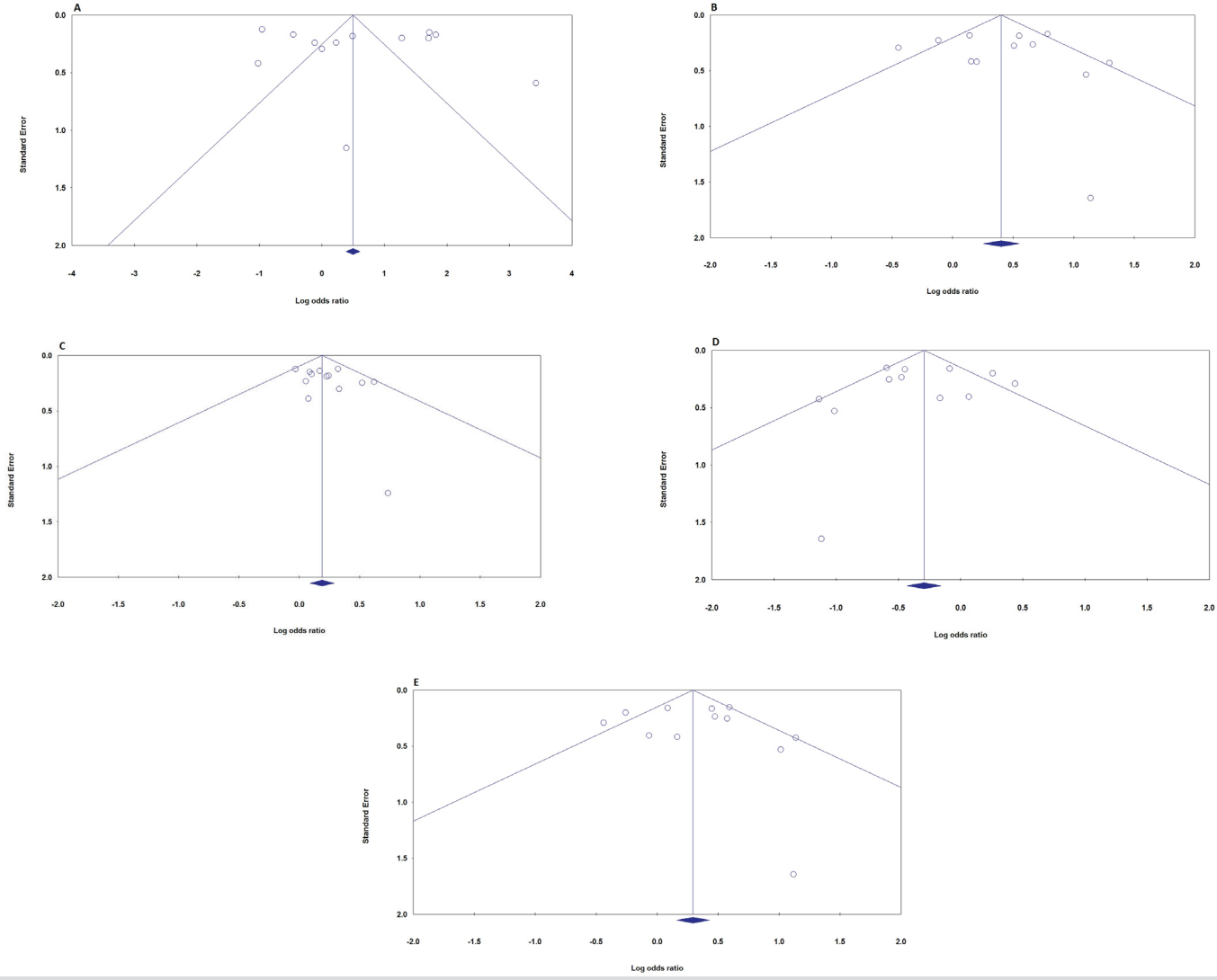

Figure 3. Funnel plots assessing publication bias for the association between the *PARP-1* rs1136410 polymorphism and CRC and GC risk. A) allele model (C vs. T). B) homozygote model (CC vs. TT). C) heterozygote model (CT vs. TT). D) dominant model (CC+CT vs. TT). E) recessive model (CC vs. CT+TT).

Displays funnel plots evaluating publication bias in the meta-analysis of PARP-1 rs1136410 and CRC/GC risk. Sub-figures A-E represent the same genetic models as in Figure 2. The funnel plot shapes indicate potential presence or absence of publication bias.

Egger's p=0.852). Similarly, among Asians, the C vs. T model showed no evidence of publication bias (Begg's p=0.296; Egger's p=0.138), while other comparisons exhibited limited variation, mostly indicating no bias (p≥0.050). For GC, assessment of publication bias also showed consistent results, with most comparisons displaying no significant bias, (C vs. T: Begg's p=0.173; Egger's p=0.472; CC vs. TT: Begg's p=0.763; Egger's p=0.752). These findings suggest a general absence of publication bias across the analyzed genetic models, reaffirming the robustness of the examined associations between the *PARP-1* rs1136410 polymorphism and cancer risk.

DISCUSSION

This meta-analysis provides strong evidence for a link between the *PARP-1* rs1136410 polymorphism and increased GC risk. This aligns with *PARP-1*'s role in gastric carcinogenesis. The C allele is associated with an approximate two-fold increase in GC odds (OR=2.012, 95% CI 1.035-3.911, p=0.039). Furthermore, individuals with two copies of the risk allele (CC vs. TT: OR=1.766, 95% CI 1.239-2.515, p=0.002) show a substantially elevated cancer susceptibility, suggesting a gene-dosage effect. Even a single copy of the variant allele (CT vs. TT: OR=1.359, 95% CI 1.180-1.565, p≤0.001) confers a measurable increased risk, indicating a dose-dependent influence on cancer development. The Val762Ala amino acid substitution encoded by rs1136410, located within the *PARP-1* catalytic domain, may affect enzyme activity and DNA repair. In GC, where *Helicobacter pylori* infection causes chronic inflammation and DNA damage, altered *PARP-1* function could impair cellular responses to genotoxic stress. Previous meta-analyses support this association, with Li et al.[26] suggesting a borderline significant increase in cancer risk associated with the C allele, particularly for GC. Qin et al.[28] found elevated risk for GC among Asian populations (OR=1.17, 95% CI 1.09-1.25). Hu et al.[29] also reported increased susceptibility to gastrointestinal cancers, especially within Asian populations.

In contrast to the GC findings, no overall association was found between the *PARP-1* rs1136410 polymorphism and CRC risk. This difference may reflect fundamental distinctions in the molecular pathways driving colorectal versus gastric carcinogenesis. Factors like distinct embryological origins, tissue microenvironments, and mutational signatures could lead to varying selective pressures on DNA repair mechanisms. The multistep nature of colorectal carcinogenesis, involving specific genetic alterations such as *Adenomatous Polyposis Coli* mutations and microsatellite or chromosomal instability, might overshadow the impact of *PARP-1* variants. Different roles of environmental factors in gastric and colorectal carcinogenesis may also modulate the penetrance of genetic susceptibility variants. The meta-analysis, encompassing five CRC studies (807 cases and 2.912 controls), provides sufficient statistical power to suggest that any association is likely small and clinically insignificant at the population level.

Ethnic Stratification and Population-Specific Risk Assessment

Ethnic stratification analysis reveals opposing effects of the *PARP-1* rs1136410 polymorphism on CRC risk in Caucasian and Asian populations. In Caucasians, the C allele demonstrates a protective effect, with approximately 51% risk reduction (OR=0.487, 95% CI 0.299-0.794, p=0.004), while in Asians, it is associated with a six-fold increase in CRC odds, (OR=5.785, 95% CI 4.481-7.467, p≤0.001). These differences likely reflect complex interactions between the rs1136410 variant and population-specific genetic backgrounds, including linkage disequilibrium patterns, modifier gene allele frequencies, and distinct evolutionary histories. The magnitude of these ethnic differences necessitates a fundamental reconsideration of how genetic risk factors are incorporated into clinical practice and public health strategies. For Caucasian populations, individuals carrying the C allele of rs1136410 may warrant less intensive CRC screening, while Asian individuals carrying this variant may benefit from enhanced surveillance. However, the implementation of such ethnicity-specific recommendations requires careful consideration of factors such as the accuracy of self-reported ethnicity, the increasing prevalence of mixed-ancestry individuals, and the potential to exacerbate healthcare disparities. Future research should prioritize identifying causal variants and developing polygenic risk scores that account for population-specific effect sizes and allele frequencies.

Methodological and Geographic Considerations

This meta-analysis identified HWE deviations in nine of the included studies (p<0.05), indicating potential issues in study design, population stratification, or genotyping quality. While excluding these studies in sensitivity analyses did not significantly change the overall findings, the frequency of HWE deviations suggests a need for more rigorous quality control in future research. Significant heterogeneity across studies, addressed using random-effects models, reflects the complexity of genetic associations across diverse populations, study designs, and environmental contexts. Quality assessment using the NOS indicated predominantly high-quality

studies,majority ≥7 stars, supporting the validity of the included research despite these challenges. Moreover, the global distribution of studies, including data from the United States, Singapore, China, Saudi Arabia, South Korea, and Brazil, strengthens the analysis by allowing for assessment of population-specific effects. However, the predominance of Asian studies (10 out of 13) may limit the generalizability of the findings and underscores the need for more research in underrepresented populations, particularly African and Hispanic populations. The temporal span of included studies (2004-2023) reflects advancements in genotyping technology, from RFLP-PCR to high-throughput platforms like TaqMan assays, Illumina GoldenGate, and Sequenom MassARRAY. The consistency of results across this technological timeline suggests that the observed associations represent genuine biological phenomena rather than platform-specific artifacts, although technological evolution likely contributes to some observed heterogeneity.

Clinical Implications and Translational Potential

This meta-analysis suggests that incorporating *PARP-1* rs1136410 genotyping into personalized gastrointestinal cancer risk assessment is warranted. In GC, the consistent association with the C allele across multiple genetic models indicates its potential contribution to polygenic risk scores for stratified screening, particularly in high-risk individuals or those with additional risk factors. The observed two-fold increased risk with the C allele is clinically relevant and could influence screening recommendations. For CRC, ethnic-specific effects (protective in Caucasians, increased risk in Asians) offer opportunities for ancestry-specific risk stratification, but require careful implementation in diverse healthcare systems. Future research should focus on clinical decision support tools integrating genetic information, traditional risk factors, and ancestry-specific effect sizes. The association between *PARP-1* rs1136410 and GC risk also has implications for PARP inhibitor therapy. Individuals with risk variants may exhibit differential responses due to altered baseline *PARP-1* activity or dependencies on PARP-mediated DNA repair. Genotype-associated tumor characteristics may guide patient selection for PARP inhibitor monotherapy or combination therapy with chemotherapeutics like cisplatin. Understanding the functional consequences of the Val762Ala substitution may reveal mechanisms of PARP inhibitor resistance and sensitivity, informing the development of improved PARP inhibitors. Ethnic-specific effects suggest potential pharmacogenomic considerations for optimizing PARP inhibitor dosing across diverse populations.

Limitations and Future Research Directions

Despite the rigorous methodology applied in this meta-analysis, several limitations should be acknowledged. These include reliance on retrospective case-control studies that limit causal inference and introduce potential selection bias, and significant heterogeneity across included studies likely reflects unmeasured confounders. Frequent deviations from HWE in control groups, particularly in GC studies, may suggest underlying biases, while the ethnic imbalance caused by the predominance of Asian populations constrains the generalizability of the findings. Additionally, variability in genotyping methods and incomplete adjustment for environmental exposures may have further influenced the results. Looking forward, future research should prioritize prospective cohort studies with robust phenotyping and environmental exposure assessment, alongside large-scale, multi-ethnic genome-wide association studies and polygenic risk scoring to improve predictive accuracy. Functional investigations are essential to clarify the biological consequences of the rs1136410 (Val762Ala) variant and its role in DNA repair, while Mendelian randomization could help establish causality. Integrating multi-omics approaches would provide a more comprehensive understanding of how *PARP-1* variants contribute to disease susceptibility, while pharmacogenomic studies linking rs1136410 genotype with PARP inhibitor response in clinical trials could yield immediate translational relevance. Ultimately, the development of ancestry-informed genetic risk calculators incorporating multiple variants alongside clinical and environmental risk factors represents an important step toward advancing precision medicine.

CONCLUSION

This meta-analysis provides compelling evidence for cancer-type and ethnicity-specific associations between *PARP-1* rs1136410 polymorphism and gastrointestinal cancer risk, with significant implications for personalized medicine approaches in oncology. The consistent association with increased GC risk across multiple genetic models, combined with the striking ethnic differences in CRC susceptibility, underscores the complexity of genetic influences on cancer development and the necessity of population-specific risk assessment strategies. These findings advance our understanding of the genetic architecture of gastrointestinal cancers and provide a foundation for developing more precise, ancestry-aware approaches to cancer screening, prevention, and treatment. Future research should focus on mechanistic studies, multi-ethnic validation, and

clinical implementation strategies to translate these genetic insights into improved patient outcomes and population health benefits.

Ethics

Ethics Committee Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Footnotes

Author Contributions

Surgical and Medical Practices: A.N., Concept: M.H.A., A.N., S.M.H., H.N., Design: S.S., R.N., Data Collection and/or Processing: A.N., M.K.-M., B.M., M.V.I-O.,Analysis or Interpretation: A.S.-D., H.N., Literature Search: S.S., R.N., A.S.-D.,Writing: M.V.I-O., H.N.

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Original Article

Clinical Impact of Cerebrospinal Fluid Multiplex Polymerase Chain Reaction (PCR) Testing in Children with Suspected Central Nervous System Infection

Merkezi Sinir Sistemi Enfeksiyonu Şüphesi Olan Çocuklarda Serebrospinal Sıvı Multipleks Polimeraz Zincir Reaksiyonu (PCR) Testinin Hasta Yönetimine Etkisi

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ABSTRACT

Objective: Central nervous system (CNS) infections remain a significant cause of morbidity and mortality in children. This study aimed to evaluate the impact of cerebrospinal fluid (CSF), multiplex polymerase chain reaction (PCR) panel results on clinical decision-making and patient management in children who underwent lumbar puncture (LP) with a preliminary diagnosis of meningitis/meningoencephalitis.

Methods: Patients aged 1 month to 18 years who underwent LP for suspected CNS infection in our pediatric emergency or intensive care units between 2018 and 2023, and who had a CSF multiplex PCR meningitis/encephalitis panel performed, were retrospectively evaluated in terms of demographics, clinical presentation, laboratory parameters, and treatments. Patients younger than 1 month or older than 18 years, those who underwent LP for non-infectious indications, and those with ventriculoperitoneal shunts were excluded. Data were analyzed using SPSS version 24.

Results: The median age of the 144 patients was 2.7 (6.7) years, and 93 (64.6%) were male. At least one pathogen was detected by multiplex PCR in 35 patients (24.3%). Of these, 22 had viral agents (enterovirus in 9, HSV-1 in 4, HHV-8 in 2, HHV-7 in 2, VZV in 2, CMV in 2, and HHV-6 in 1), 11 had bacterial agents [*Streptococcus pneumoniae (S. pneumoniae)* in 7, *Neisseria meningitidis* in 3, and *Haemophilus influenzae type b (Hib) in 1*], and 2 had multiple agents (*S. pneumoniae + Hib* + HHV-6 in one case; enterovirus + HHV-6 in one case). No significant clinical differences were observed between viral and bacterial infections. In 51 patients (35.4%), treatment was modified based on PCR results, most often by discontinuing acyclovir (22.1%), antibiotics (7.6%), or both (3.5%).

ÖZ

Amaç: Merkezi sinir sistemi enfeksiyonları çocuklarda hala önemli mortalite ve morbidite nedenleri arasındadır. Bu çalışmanın amacı, menenjit/meningoensefalit ön tanısı ile lomber ponksiyon (LP) yapılmış olan hastalarda bakılan beyin omurilik sıvısı (BOS) multipleks polimeraz zincir reaksiyonu (PCR) paneli sonuçlarının hasta yönetimi üzerine etkisinin değerlendirilmesidir.

Yöntemler: 2018-2023 yılları arasında çocuk acil ve çocuk yoğun bakım servislerimizde santral sinir sistemi enfeksiyonu şüphesiyle LP yapılmış ve BOS multipleks PCR yöntemiyle menenjit/ensefalit paneli gönderilmiş olan 1 ay-18 yaş arasındaki hastalar demografik, klinik, laboratuvar ve tedavi yöntemleri açısından retrospektif olarak değerlendirildi. Çalışmamıza <1 ay, >18 yaş, enfeksiyonla ilgili olmayan durumlar için LP yapılmış olan hastalar, Ventrikülperitoneal şantı olan hastalar dahil edilmedi. Verilerin istatistiksel analizleri için SPSS 24 programı kullanıldı.

Bulgular: Çalışmaya dahil edilen 144 hastanın medyan yaşı 2,7 (6,7) yıldı ve 93'ü (%64,6) erkekti. Toplam 144 hastanın 35'inde (%24,3) multipleks PCR yöntemiyle patojen saptandı. Bunların 22' sinde virüs (9 enterovirüs, 4 HSV-1, 2 HHV-8, 2 HHV-7, 2 VZV, 2 CMV, 1 HHV-6), 11'inde bakteri [7 pnömokok, 3 meningokok, 1 *Haemophilus influenzae* tip b (Hib)], 2' sinde çoklu pozitiflik (pnömokok + *Hib* + HHV-6, enterovirüs + HHV-6) vardı. Bakteri ve viral etken tespit edilen 35 olgu klinik bulgular açısından karşılaştırıldığında olgular arasında klinik açıdan anlamlı farklılık saptanmadı. Toplam 144 hastanın 51'sinde (%35,4) BOS-PCR sonucuna göre tedavi değişikliği yapıldı. En sık yapılan tedavi değişiklikleri sırasıyla asiklovir tedavisinin kesilmesi (%22,1), antibiyotik tedavisinin kesilmesi (%7,6), asiklovir ve antibiyotik tedavilerinin kesilmesi (%3,5) idi.

Sonuçlar: Çalışmamızda, BOS-PCR sonuçlarına göre hastaların yaklaşık üçte birinde gereksiz antiviral ya da antibiyotik tedaviler kesilmiş ve bu

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Conclusions: In approximately one-third of cases, unnecessary antiviral or antibiotic treatments were discontinued based on PCR results, demonstrating the utility of molecular diagnostics in guiding clinical management. Especially in patients who had received antibiotics prior to LP, early pathogen detection via PCR may help reduce treatment costs, complications, and length of hospital stay.

Keywords: Central nervous system infections, cerebrospinal fluid, polymerase chain reaction (PCR)

moleküler testin hasta yönetimine yön verdiği gösterilmiştir. Özellikle LP öncesi antibiyotik tedavisi almış hastalarda, kültürde üretilemeyen bakterilerin PCR ile erken tespit edilebilmesi ve HSV dışındaki selim seyirli viral enfeksiyonlarda gereksiz antimikrobiyal tedavinin sonlandırılabilmesi için alınan BOS örneklerinden multipleks PCR paneli çalışılması gereksiz tedavi maliyetlerini ve komplikasyonları önleyip, hastane yatış sürelerinin azalmasına katkı sağlayabilir.

Anahtar kelimeler: Merkezi sinir sistemi enfeksiyonları, beyin omurilik sıvısı, polimeraz zincir reaksiyonu (PCR)

INTRODUCTION

Central nervous system (CNS) infections are defined as inflammation of the meninges or brain parenchyma due to infectious causes, and they remain among the leading causes of morbidity and mortality in children. With the inclusion of conjugated *Streptococcus pneumoniae* and *Haemophilus influenzae* type B (*Hib*) vaccines in routine immunization programs, viruses have become the most common cause of meningitis worldwide, including in Türkiye[1]. Most cases of viral meningitis except those caused by herpes simplex virus (HSV) generally follow a benign clinical course and are treated symptomatically. Although the incidence of bacterial meningitis has decreased, it remains a serious disease because of the risk of irreversible neurological damage or death if not promptly treated, making it a medical emergency.

It is often difficult to clinically distinguish between viral and bacterial CNS infections at presentation. Both types of meningitis can manifest with similar symptoms in children, such as fever, headache, photophobia, and neck stiffness. In neonates and infants, the typical signs of meningitis may be absent. Furthermore, commonly used diagnostic parameters like cerebrospinal fluid (CSF) pleocytosis and acute-phase reactants [e.g., C-reactive protein (CRP) and white blood cell count] are insufficient to reliably distinguish viral from bacterial meningitis[2]. The inability to determine the etiology early often leads to prolonged and potentially unnecessary use of antibiotics and antivirals, as well as extended hospital stays.

Molecular diagnostic tests based on polymerase chain reaction (PCR) -particularly multiplex PCR panels- allow rapid and reliable identification of multiple neurotropic pathogens in CSF samples. These tests enable timely and accurate pathogen identification and initiation of appropriate treatment. Moreover, if a benign viral agent such as enterovirus (EV) is identified, PCR can inform early clinical decision-making and potentially prevent unnecessary antimicrobial use [2-5].

The primary objective of this study was to evaluate the impact of CSF-PCR panel results on clinical decision-making and patient management in children who underwent lumbar puncture (LP) in pediatric emergency and intensive care units with a preliminary diagnosis of meningitis/meningoencephalitis. The secondary objective was to determine the frequency of viral and bacterial agents detected by the CSF-PCR panel and to assess whether clinical or laboratory features could predict the likely pathogen.

MATERIALS and METHODS

This retrospective study included patients aged one month to 18 years who underwent LP for suspected CNS infection in our pediatric emergency or intensive care units between 2018 and 2023, and had a CSF multiplex PCR Meningitis/Encephalitis panel (Bio-Speedy Meningitis/Encephalitis RT-PCR MX-17, Bioeksen, Istanbul, Türkiye) performed. We recorded each patient's demographic and clinical characteristics, laboratory findings, and treatment modalities.

Patients younger than one month or older than 18 years, those who underwent LP for non-infectious reasons (e.g., suspected intracranial hypertension, evaluation of seizures, metabolic workup, Guillain-Barré syndrome, autoimmune or vasculitic disease, malignancy, subarachnoid hemorrhage), and those with ventriculoperitoneal shunts were excluded.

Patients were classified into two groups based on the CSF-PCR results: viral or bacterial. Patients with mixed pathogens were assigned to the group that best matched their clinical presentation, as determined by the treating physician. The two groups were then compared in terms of clinical and laboratory characteristics. For acute-phase reactants, we defined the cut-off values as procalcitonin (PCT) >0.5 ng/ml and CRP >5 mg/L. For CSF analysis, pleocytosis was defined as >10 leukocytes/mm^3 for infants aged 1-3 months and >5 leukocytes/mm^3 for children older than 3 months. Elevated CSF protein was defined as >75 mg/dL for infants aged 1-3 months and >45 mg/dL for children older than 3 months. Low CSF glucose was defined as <40 mg/dL (or a CSF/serum glucose ratio <0.6) for infants aged 1-3 months, and <50 mg/dL (or a ratio <0.5) for children older than 3 months[6].

Children who have received at least two doses of conjugated Streptococcus pneumoniae vaccine and three doses of Haemophilus influenzae type B vaccine are considered 'fully vaccinated'.

Statistical Analysis

Statistical analyses were performed using SPSS version 24.0 (IBM, Chicago, IL, USA). Categorical variables were compared using the chi-square test. Continuous variables were compared using the Student's t-test or the Mann-Whitney U-test, depending on the distribution. A p-value <0.05 was considered statistically significant.

Ethics Committee Approval: This study was approved by the Istanbul Medeniyet University Goztepe Suleyman Yalcin City Hospital Clinical Research Ethics Committee (decision no: 2023/0604, date: 20.09.2023).

RESULTS

The median age of 144 patients was 2.7 years, with a standard deviation of 6.7 years, and 64.6% were male. Fourteen patients (9.8%) were incompletely vaccinated or had unknown vaccination status. At least one pathogen was detected by the multiplex PCR panel in 35 patients (24.3%). The demographic and clinical characteristics of patients with a detected pathogen are shown in Table 1. Of these, 22 had viral agents (EV in 9, HSV-1 in 4, Human herpes virus (HHV-8 in 2, HHV-7 in 2, Varicella zoster virus (VZV) in 2, Cytomegalovirus (CMV) in 2, and HHV-6 in 1), 11 had bacterial agents (*S. pneumoniae* in 7, *Neisseria meningitidis* in 3, and *Hib* in 1), and 2 had multiple agents (*S. pneumoniae* + *Hib* + HHV-6 in one case; EV + HHV-6 in one case) (Figure 1). Of the two patients with mixed infections, one was categorized in the viral group and the other in the bacterial group based on clinical presentation. The median turnaround time for CSF-PCR results was 24 hours (range 3-118 hours).

Among the patients with an identified pathogen (n=35), fever was present in 91.4%, vomiting in 57.1%, seizures in 34.3%, altered consciousness in 31.4%, signs of meningeal irritation in 28.6%, and headache in 25.7%. There were no statistically significant differences in presenting symptoms between the viral and bacterial cases (Table 1).

Table 1. Comparison of demographic and clinical characteristics and outcomes of bacterial and viral meningitis.

	Total (n=35)	Bacterium (n=12)	Virus (n=23)	p-value
Demographical features				
Gender, male†	20 (57,1)	6 (50)	14 (60,9)	0,537
Age, year§	5,5 (7,9)	2,1 (4,9)	1,3 (6,6)	0,614
Missing/unknown vaccination†	5 (14,3)	3 (25)	2 (8,6)	0,415
Immunosuppression†	0 (0)	0 (0)	0 (0)	
Antibiotic use before LP†	10 (28,6)	7 (58,3)	3 (13)	**0,015**
Clinical findings†				
Fever	32 (91,4)	12 (100)	20 (87)	0,536
Rash	3 (8,6)	1 (8,3)	2 (8,7)	1
Respiratory symptoms	5 (14,3)	1 (8,3)	4 (17,4)	0,64
Diarrhea	3 (8,6)	1 (8,3)	2 (8,7)	1
Headache	9 (25,7)	3 (25)	6 (26,1)	1
Vomiting	20 (57,1)	9 (75)	11 (47,8)	0,163
Change of consciousness	11 (31,4)	4 (33,3)	7 (30,4)	1
Convulsion	12 (34,3)	4 (33,3)	8 (34,8)	1
Meningeal irritation sign	10 (28,6)	4 (33,3)	6 (26,1)	0,706
Focal neurological deficit	2 (5,7)	0 (0)	2 (8,7)	0,536
Prognosis†				
Neurological sequela	4 (11,4)	2 (16,7)	2 (8,7)	0,594
Death	0 (0)	0 (0)	0 (0)	

†number (%), §median (interquartile range)

Laboratory comparisons revealed that patients in the bacterial group had significantly higher leukocyte counts, CRP, and PCT levels than those in the viral group (p=0.001, p<0.001, and p<0.001, respectively). In the CSF analysis, pleocytosis, elevated protein, and low glucose levels were significantly more frequent in the bacterial group compared to the viral group (p=0.027, 0.001, and 0.011, respectively) (Table 2).

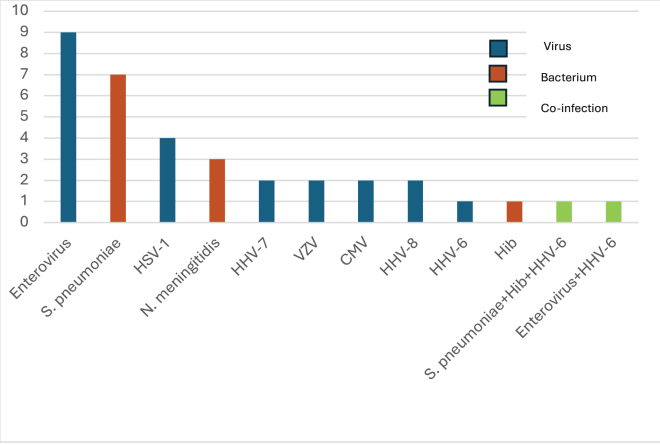

Figure 1. Distribution of microorganisms according to PCR results.

S.pneumoniae: Streptococcus pneumoniae, HSV-1:Herpes simplex virus type 1, *N. meningitidis: Neisseria meningitidis*, HHV-7: Human herpes virus 7, VZV: Varicella zoster virus, CMV: Cytomegalovirus, HHV-8: Human herpes virus 8, Hib: Haemophilus influenzae type B, HHV-6: Human herpes virus-6

Among 12 patients in the bacterial pathogen group, 9 (75%) were fully vaccinated and 7 (58.3%) had received antibiotics prior to LP. Only 2 (16.7%) of these 12 patients had positive CSF and/or blood culture results (Table 3). None of the PCR-negative patients had a positive culture.

In 51 of 144 patients (35.4%), the CSF-PCR results led to a change in clinical management. The most frequent modifications were discontinuation of acyclovir (22%), discontinuation of antibiotics (8%), and discontinuation of both (3%) (Figure 2). Among the 35 patients with a detected pathogen, 20 (57.1%) had their treatment altered based on the PCR findings. In terms of treatment duration, cases in the viral group had a significantly shorter antibiotic course, with a median of 5 days compared to 10 days in the bacterial group. Meanwhile, cases in the bacterial group had a significantly shorter antiviral (acyclovir) course, with a median of 2 days compared to 14 days in the viral group (p<0.001 for both) (Table 4).

DISCUSSION

In this study of 144 pediatric patients who underwent LP for suspected CNS infection, a multiplex PCR panel identified a viral or bacterial pathogen in approximately one-quarter of cases. Based on the CSF-PCR results, empiric treatments were modified in about one-third of all patients and in nearly half of those in whom a pathogen was detected. These modifications led to shorter antibiotic courses in viral meningitis cases and reduced

Table 2. Comparison of laboratory features of bacterial and viral meningitis.

	Total (n=35)	Bacteria (n=12)	Virus (n=23)	p-value
Acute phase reactants				
Leukocyte count[$], mm^3	11600 (9000)	18400 (14850)	10600 (5100)	**0,001**
Leukocytosis[†]	18 (51,4)	9 (75,0)	9 (39,1)	**0,044**
CRP, mg/L[$]	5 (22)	102 (116)	3 (16)	**<0,001**
Elevated CRP[†]	22 (62,9)	12 (100)	10 (43,5)	**0,001**
Procalcitonin, ng/ml[$]	0,2 (1,6)	30,7 (34,0)	0,2 (0,3)	**<0,001**
Elevated Procalcitonin[†]	11 (31,4)	8 (66,7)	3 (13)	**0,002**
CSF test findings[†]				
Pleocytosis	23 (65,7)	11 (91,7)	12 (52,2)	**0,027**
Elevated protein	16 (45,7)	10 (83,3)	6 (26,1)	**0,001**
Decreased glucose	8 (22,9)	6 (50)	2 (8,7)	**0,011**

[†]number (%), [$]median (interquartile range)
CSF: Cerebrospinal fluid, CRP: C-reaktive protein

Table 3. Characteristics of patients diagnosed with bacterial meningitis.

Patient number	Age, months	Gender	Vaccination status	Antibiotic use before LP	CSF culture growth	Blood culture growth	CSF Multiplex PCR
1	1	Boy	Fully vaccinated	No	Negative	Negative	*Streptococcus pneumoniae*
2	5	Girl	Fully vaccinated	Yes	Negative	Negative	*Streptococcus pneumoniae*
3	6	Girl	Unknown	Yes	Negative	Negative	*Streptococcus pneumoniae*
4	33	Boy	Fully vaccinated	No	Negative	Negative	*Streptococcus Pneumoniae*
5	60	Boy	Fully vaccinated	Yes	Negative	Negative	*Streptococcus pneumoniae*
6	84	Boy	Fully vaccinated	Yes	*Streptococcus pneumoniae*	Negative	*Streptococcus pneumoniae*
7	132	Boy	Fully vaccinated	Yes	Negative	Negative	*Streptococcus pneumoniae*
8	6	Girl	Unknown	No	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus Pneumoniae* + *Haemophilus influenza* type B+ Human herpes virus-6
9	7	Girl	Fully vaccinated	No	Negative	Negative	*Neisseria meningitidis*
10	36	Boy	Fully vaccinated	Yes	Negative	Negative	*Neisseria meningitidis*
11	67	Girl	Fully vaccinated	Yes	Negative	Negative	*Neisseria meningitidis*
12	19	Girl	Incompletely vaccinated	No	Negative	Negative	*Haemophilus influenza* type B

LP: Lomber puncture; CSF: Cerebrospinal fluid, PCR: Polymerase chain reaction

Table 4. Impact of positive PCR results on patient management.

	Total (n=35)	Bacterium (n=12)	Virus (n=23)	p-value
Treatment change according to PCR†	20 (57,1)	7 (58,3)	13 (56,5)	0,918
Duration of antibiotic treatment, day§	10 (7)	10 (44)	5 (7)	**<0,001****
Duration of acyclovir treatment, day§	5 (12)	2 (2)	14 (7)	**<0,001****
Duration of hospitalization, day§	14 (16)	10 (50)	14 (15)	0,31

†number (%), § median (interquartile range)
*p<0,05, **p<0,01, PCR: Polymerase chain reaction

antiviral (acyclovir) use in bacterial meningitis cases. Our findings thus demonstrate the clinical utility of multiplex CSF-PCR panels in guiding patient management.

Identifying the causative agent in CNS infections is critical for determining appropriate treatment and predicting prognosis. However, clinical features often overlap between viral and bacterial infections, and the sensitivity and specificity of individual acute-phase reactants (e.g., CRP, PCT) are low in identifying specific pathogens. Therefore, it is recommended to interpret these markers alongside clinical scoring systems[7,8]. In our study, we found no significant differences in clinical

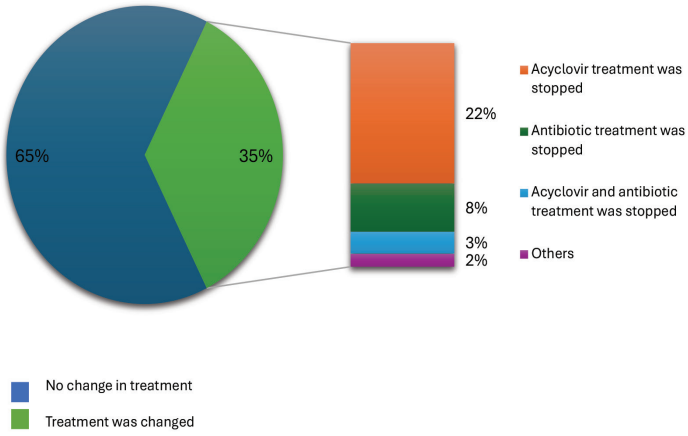

Figure 2. Treatment changes based on PCR results.

presentation between viral and bacterial cases, but laboratory markers such as CRP, PCT, and leukocyte count were significantly higher in the bacterial group ($p<0.001$ for all). Similarly, CSF analysis showed that pleocytosis, elevated protein, and decreased glucose were significantly more common in bacterial meningoencephalitis than in viral cases ($p=0.027$, 0.001, 0.011). While these findings suggest that laboratory parameters may aid in the differential diagnosis, the gold standard remains the identification of the specific pathogen via molecular methods or culture.

Recently developed multiplex PCR panels provide rapid, highly sensitive, and specific identification of causative microorganisms. For example, a multicenter evaluation of the FilmArray ME panel reported 100% sensitivity for most bacterial pathogens and over 99% specificity[9]. Published positivity rates for CSF multiplex PCR range from 18.8% to 32.8%[10-12], aligning with our positivity rate of 24.3%. Differences in detection rates may be due to variability in clinical indications for LP and differences in the PCR panels used.

Viruses are currently the leading cause of meningitis worldwide. In pediatric studies using PCR for the etiologic diagnosis of meningoencephalitis, viral pathogens were detected in 79.7% of cases in the study by Ayhan et al.[12], 84.5% in the study by Mizuno et al.[13], and 53.7% in the study by Bal et al.[14] In our study, viral pathogens accounted for 65.7% of PCR-positive cases, which is consistent with these reports. Globally, EVs are responsible for nearly 85% of viral meningoencephalitis cases[15]. EVs generally cause a mild clinical course and result in aseptic meningitis[3,4,16]. Although HSV-1 is encountered less frequently, it is the most common cause of sporadic necrotizing encephalitis[17].

Findings from different regions show some variations in the prevalent viral agents. In a multicenter pediatric study from Japan, parechovirus (45%) and EV (43%) were the most common causes of viral CNS infections, whereas a study from Türkiye found EV (23.5%), adenovirus (22%), and HHV-6 (22%) to be the most common,[13,18]; HSV-1 positivity in the latter study was 5.9%. In our study, EV was detected in 43.5% of viral cases, followed by HSV-1 in 17.4%, a finding which aligns with the literature. Differences between studies may result from variations in seasonality, socioeconomic status, indications for LP, patient age, and geographic location.

Although the prevalence of bacterial meningitis has declined in countries with routine *S. pneumoniae* and *Hib* vaccination programs, it remains a major concern due to its high morbidity and mortality[19]. While CSF culture is the gold standard for diagnosing bacterial meningitis, prior antibiotic use often decreases culture yield. Thus, multiplex PCR testing has become increasingly important. Studies from various countries report bacterial pathogen detection rates via multiplex PCR ranging from 15.5% to 23% [13,20]. In Türkiye, Ayhan et al.[12] reported a bacterial detection rate of 20.6%, and Bal et al.[14] reported a rate of 36.3%. Our study found a rate of 34.3%. The higher rates in studies from Türkiye compared to other regions might be attributable to differences in vaccination coverage, population characteristics, or study periods.

In patients with suspected meningitis, antibiotic treatment is often initiated before LP is performed, complicating culture-based diagnosis. Additionally, performing LP in children can be technically challenging, and the CSF volume obtained may be insufficient for culture. In a multicenter Turkish study by Ceyhan et al.[19], among 645 patients with bacterial pathogens detected by PCR, 74% had received antibiotics before LP, and the culture positivity rate was only 16.2%. Similarly, in our study, of 12 patients with bacterial pathogens detected by PCR, 7 (58.3%) had received antibiotics before LP, and only one had a positive culture. Overall, bacterial growth in CSF and/or blood cultures was identified in just 2 cases (16.7%). These findings highlight that multiplex PCR panels can detect bacterial meningitis pathogens even in patients who have already received antibiotics, allowing for earlier targeted treatment and potentially reducing morbidity and mortality. However, PCR results should always be interpreted in the context of the clinical presentation, as some pathogens may not be included in the panel and false-positive or false-negative results, though rare, can occur.

As a general principle, children with suspected CNS infection should be treated empirically for bacterial meningitis or HSV encephalitis until those diagnoses are excluded. This approach can lead to unnecessary prolonged use of antibiotics or acyclovir, longer hospital stays, and increased healthcare costs, especially in cases that ultimately prove to be benign viral infections[4]. Previous studies have shown that rapid PCR testing of CSF can reduce unnecessary antimicrobial use and shorten hospital stays[1,4]. For example, in pediatric patients with a positive CSF EV PCR result, the duration of IV antibiotic therapy was shortened by a median of 1.5 days, hospital stay decreased from 71.5 to 42 hours, and discharge occurred about 5 hours after the result became available. In contrast, PCR-negative or untested patients often received longer empirical treatment[4]. In our study, therapeutic modifications were made in 51 patients (35.4%) based on the PCR results. The most common change was discontinuation of acyclovir (22.1% of patients), followed by antibiotics (7.6%) and both (3.5%). These changes significantly shortened the duration of antibiotic therapy in viral cases and the duration of antiviral (acyclovir) therapy in bacterial cases ($p<0.001$ for both). The widespread use of multiplex PCR panels in emergency settings may help identify benign viral cases early and allow prompt discontinuation of unnecessary treatments, thereby reducing hospital stays and healthcare costs.

The impact of PCR results on clinical decision-making also depends on the test turnaround time. When results are available within 24 hours, studies report that antibiotic use can be reduced by approximately 20%, yielding significant cost savings[3]. In our study, the median PCR turnaround time was 24 hours (range 3-118 hours), which is relatively long. While this turnaround did not facilitate early discharge from the emergency department, it did allow for shorter durations of unnecessary antimicrobial use. In the future, the use of faster molecular diagnostic methods may enable earlier discontinuation of empirical treatments and safe discharge from the emergency department[3,4].

Study Limitations

This study has some limitations. It was a single-center retrospective study with a relatively small sample size, and we did not perform confirmatory testing (e.g., sequencing or separate PCR assays) for the pathogens detected by the panel. Prospective studies with larger cohorts and broader testing panels are needed to further evaluate the clinical and cost-effectiveness of multiplex PCR testing in CNS infections.

CONCLUSION

CSF multiplex PCR testing is a valuable diagnostic tool for children with suspected CNS infections, as it can rapidly identify causative pathogens and guide early therapeutic decisions. Our findings indicate that PCR results enabled the discontinuation of unnecessary antimicrobial treatments in many cases, thereby shortening treatment durations and potentially reducing hospital stays and healthcare costs. Wider implementation of rapid multiplex PCR panels in clinical practice, along with further large-scale studies, may help improve the management and outcomes of pediatric CNS infections.

Ethics

Ethics Committee Approval: This study was approved by the Istanbul Medeniyet University Goztepe Suleyman Yalcin City Hospital Clinical Research Ethics Committee (decision no: 2023/0604, date: 20.09.2023).

Informed Consent: This is a retrospective study.

Footnotes

Author Contributions

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

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

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Original Article

Short- and Long-Term Statin Persistence and Determinants in Patients Initiating Statin Therapy

Statin Başlanan Hastalarda Kısa ve Uzun Dönem Statin Persistansı ve Belirleyicileri

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ABSTRACT

Objective: Despite the established efficacy of statins in reducing major cardiovascular events and mortality, rates of statin persistence remain low. This study aimed to assess short- and long-term statin persistence rates and identify factors influencing persistence in patients initiating statin therapy.

Methods: A retrospective, observational, clinical study was conducted, enrolling a consecutive total of 903 patients aged 18 years and older (692 female, 211 male, mean age: 60.74 ± 11.70 years) who had initiated statin therapy between January 1, 2016, and January 1, 2017. Short-term (2018) and long-term (2023) statin persistence statuses were determined. Groups persisting and non-persisting with statin therapy were compared for demographic characteristics; presence of cardiometabolic diseases such as diabetes mellitus (DM), coronary artery disease (CAD), and hypertension (HT); statin therapy intensities; and indications for statin initiation (primary or secondary prevention) for both time periods.

Results: The study included 903 patients with a mean age of 60.7±11.7 years and a female predominance of 76.6%. In 2018, 498 (55.1%) patients continued statin therapy, while 405 (44.9%) discontinued. In 2023, excluding 36 cases with death (18 cases were among those continuing statin treatment, and 18 cases were who did not continue). Four hundred and forty-eight (51.7%) patients persisted with statin therapy, while 419 (48.3%) discontinued. Statin non-persistence was more frequent in patients initiated on statins for primary prevention ($p<0.01$) and more frequent in those under 45 years old ($p=0.028$ and $p=0.036$, respectively), while it was less common in patients with HT, DM, and CAD (all $p<0.01$).

Conclusions: The study reveals the low and declining rates of statin persistence in patients initiating statin therapy, both in the short and long term. Furthermore, persistence rates are lower in younger patients and those initiated on statins for primary prevention compared to those with established cardiovascular risk factors

Keywords: Statin persistance, cardiometabolic disease, statin intensity

ÖZ

Amaç: Randomize kontrollü çalışmalarda statinlerin majör kardiyovasküler olay riskini ve tüm nedenlere bağlı mortaliteyi azalttığı gösterilmiş olmasına ve güncel lipid kılavuzlarının etkin kolesterol tedavi önerilerine rağmen statine devam etme (statin persistansı) oranlarının düşük olduğu gözlenmektedir. Bu çalışmanın amacı statin başlanan hastalarda kısa ve uzun dönem statin persitansı oranlarının ve bunun üzerine etkili faktörlerin belirlenmesidir.

Yöntemler: Retrospektif, gözlemsel, klinik çalışmaya 01.01.2016-01.01.2017 tarihleri arasında statin tedavisi başlanmış 18 yaş ve üzeri toplam 903 hasta (692 kadın, 211 erkek, ortalama yaş: 60.74±11,70 yıl) ardışık olarak alındı. Kısa (2018) ve uzun dönem (2023) statin persistans durumları belirlendi. Her iki dönem için statin tedavisine devam eden ve etmeyen hasta grupları demografik özelliklerine, diabetes mellitus (DM), koroner arter hastalığı (KAH) ve hipertansiyon (HT) gibi kardiyometabolik hastalık varlıklarına, statin tedavi yoğunluklarına (*low-intensity, moderate-intensity, high-intensity*) ve statin başlama endikasyonlarına (primer veya sekonder koruma) göre karşılaştırıldı.

Bulgular: 2018 yılı kontrollerinde; 903 olgudan statin tedavisine devam eden 498 (%55.1), devam etmeyen 405 (%44.9), 2023 yılı kontrollerinde; eksitus olan 36 olgu (statine devam edenlerde 18, etmeyenlerde 18 olgu) hariç tutulduğunda 867 olgudan statine devam eden 448 (%51.7), devam etmeyen 419 (%48.3) olgu vardı. Her iki kontrolde de statine devam etmeyenlerin sıklığı primer koruma amaçlı statin başlananlarda (ikisi için de $p<0.01$) ve <45 yaş olanlarda (sırasıyla $p=0.028$ ve $p=0.036$) yüksek, HT, DM ve KAH olanlarda düşüktü (tümü için $p<0.01$).

Sonuçlar: Bu çalışma statin başlanan hastalarda statin persistansının hem kısa hem de uzun dönem takiplerde düşük ve düşme eğiliminde olduğunu ve genç yaştaki hastalar ile kardiyovasküler riski yüksek olmayan ve primer koruma amaçlı statin başlanan hastalarda persistansın diğer hastalara göre daha düşük olduğunu göstermiştir.

Anahtar kelimeler: Statin devamlılığı, kardiyometabolik hastalık, statin yoğunluğu

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INTRODUCTION

Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), commonly used in the treatment of hypercholesterolemia, have been shown to reduce the risk of major cardiovascular events and all-cause mortality in both primary and secondary prevention studies[1-3]. Despite the effective cholesterol treatment recommendations outlined in current lipid guidelines, low rates of statin persistence and adherence are observed in many countries[4-6]. Factors contributing to this phenomenon include low disease awareness among patients, fear of side effects, biases regarding treatment efficacy, negative experiences with previously used medications, and inadequate disclosure of the benefits and side effects of treatment by healthcare providers[7-9]. Exaggerated negative media coverage of statin side effects in both visual and written media has also been shown to adversely affect treatment continuation and adherence among both patients initiating statin therapy and those already using statins[10,11]. Especially in patients at high cardiovascular risk, premature discontinuation of statin therapy has been shown to increase the risk of death from atherosclerotic cardiovascular diseases[12].

The aim of this study was to determine the proportion of patients who continued statin therapy in the short and long term, the changes in this proportion over the years, and the factors thought to influence statin persistence, such as age, sex, cardiometabolic diseases, statin indications, and intensity of statin therapy.

MATERIALS and METHODS

A retrospective, observational, clinical study was conducted at Istanbul Medeniyet University Goztepe Prof. Dr. Suleyman Yalcın City Hospital, Internal Medicine Outpatient Clinics, including consecutive patients aged 18 and above who initiated statin therapy between January 1, 2016, and January 1, 2017, and had regular medical records available. Patients receiving non-statin lipid-lowering therapy and those with missing medical records were excluded from the study. The flow of the study is depicted in Figure 1. Informed consent was obtained from the patients participating in the studies. Local Ethics Committe of Istanbul Medeniyet University approval was obtained for the study (reference number: 2023/0714, date: 25.10.2023), and the study adhered to the principles of the Helsinki Declaration throughout its duration.

The primary endpoint of the study was to evaluate the proportion of patients continuing statin therapy among those who initiated it, and the changes in this proportion over the years.

The secondary endpoint was aimed at determining whether there were differences in demographic and cardiometabolic disease characteristics, statin intensity, and indications for statin initiation between patients continuing and discontinuing statin therapy.

Study Design

Patients' baseline age, gender, presence of cardiometabolic diseases such as diabetes mellitus (DM), coronary artery disease (CAD), and hypertension (HT), as well as indications for statin initiation (primary or secondary prevention), and statin classification (low-intensity, moderate-intensity, high-intensity) were determined from their medical records. Short-term (2018) and long-term (2023) statin persistence statuses were also determined from the medical records as part of the study flow process. Patient groups persisting, and non-persisting with statin therapy were compared for demographic characteristics, presence of cardiometabolic diseases, statin intensity, and indications for statin initiation for both time periods.

Definitions

Cardiometabolic diseases and mortality

Patients' diagnoses of HT, DM, and CAD were determined from their medical records using International Classification of Diseases (ICD-10) 10th revision ICD-10 codes. Mortality records were obtained from the National Death Notification System.

Statin indication and persistence

Patients who had not experienced a cardiovascular event before starting statin therapy were classified as receiving statin therapy for primary prevention, while those who had experienced an atherosclerotic cardiovascular event were classified as receiving statin therapy for secondary prevention. Among patients initiated on statins for primary prevention, those who experienced an atherosclerotic cardiovascular event between 2018 and 2023 were additionally classified as receiving statin therapy for primary and secondary prevention.

Statin discontinuation was defined as a period of ≥90 days without statin therapy.

Statin Intensity

Low-intensity statin therapy was defined as fluvastatin 20-40 mg, lovastatin 20 mg, simvastatin 10 mg, pitavastatin 1 mg, and pravastatin 10-20 mg. Moderate-

intensity statin therapy was defined as atorvastatin 10-20 mg, fluvastatin twice daily 40 mg or once daily 80 mg (extended-release formulation), lovastatin 40 mg, pitavastatin 2-4 mg, pravastatin 40-80 mg, rosuvastatin 5-10 mg, and simvastatin 20-40 mg. High-intensity statin therapy was defined as atorvastatin 40-80 mg or rosuvastatin 20-40 mg.

Statistical Analysis

SPSS 26 (Statistical Package for the Social Sciences) software was used for data analysis. Descriptive statistical methods, such as mean, standard deviation, median, minimum, and maximum values, were used to evaluate quantitative variables, while qualitative variables were presented as frequencies and percentages. The normal distribution of data was assessed using the Shapiro-Wilk test, and box plots. The chi-square test and McNemar test were used to compare qualitative data. Results were evaluated at a significance level of $p<0.05$ with a 95% confidence interval.

RESULTS

A total of 903 patients, with a mean age of 60.7±11.7 years and a female predominance (76.6%), were included in the study. Of these, 574 (63.6%) were initiated on atorvastatin, 284 (31.5%) on rosuvastatin, 41 (4.5%) on pitavastatin, and 5 (0.5%) on simvastatin.

The majority of patients (90.8%) were prescribed moderate-intensity statin therapy, while 9.2% received high-intensity statin therapy; no patients received low-intensity statin therapy. Among the patients, 88.4% were initiated on statin therapy for primary prevention, while 11.6% were initiated on statin therapy for secondary prevention. HT was the most common comorbid cardiometabolic disease (43.3%). The majority of patients (86.3%) were between the ages of 45 to 79 years (Table 1).

The frequencies of patients continuing and discontinuing statin therapy in the 2018 and 2023 assessments are shown in Table 2. In the 2018 assessment, out of 903 patients, 498 (55.1%) continued statin therapy, while 405 (44.9%) discontinued. In the 2023 assessment (excluding those who were deceased), out of 867 patients, 448 (51.7%) continued statin therapy, while 419 (48.3%) discontinued. Between 2018 and 2023, it was observed that 36 patients (3.9%) died. The mortality rate was 3.6% (n=18) among those continuing statin therapy and 4.4% (n=18) among those discontinuing statin therapy (p=0.526).

In the 2018 assessment, out of 480 patients continuing statin therapy, 325 (67.7%) continued in the 2023 assessment, while 155 (32.3%) discontinued. Among the 387 patients who discontinued statin therapy in the 2018 assessment, 123 (31.8%) restarted statin therapy in the 2023 assessment, while 264 (68.2%) remained discontinued.

In the 2018 assessment, the frequency of statin non-persistence was higher in patients who were initiated on statins for primary prevention compared to those who were initiated for secondary prevention (p=0.001). Patients with HT, DM, and CAD, were observed to have a higher rate of statin persistence compared to those without these comorbidities (all p=0.001). In terms of age groups, patients aged <45 years, had a higher frequency of statin non-persistence compared to other age groups (p=0.028) (Table 3).

Table 1. Baseline characteristics of the patients.

Age (year)	Mean±Sd	60.74±11.70
	Median (min-max)	61 (19-95)
Age groups (n,%)	<45	79 (8.7)
	45-64	485 (53.7)
	65-79	294 (32.6)
	≥80	45 (5.0)
Gender (n, %)	Female	692 (76.6)
	Male	211 (23.4)
Statin intensity (n, %)	Moderate-intensity	820 (90.8)
	High-intensity	83 (9.2)
Statin indication (n, %)	Primary prevention	798 (88.4)
	Secondary preventiom	105 (11.6)
Cardiometabolic diseases (n, %)	Hypertension	391 (43.3)
	Diabetes mellitus	213 (23.6)
	Coronary artery disease	100 (11.1)

Sd: Standard deviation

Table 2. Change in statin persistence over the years.

	Total	Continuing statin therapy	Discontinuing statin therapy
Patient data for the year 2018 (n,%)	903	498 (55.1)	405 (44.9)
Those who exited between 2018 and 2023 (n,%)	36	18 (3.6)	18 (4.4)
Patient data for the year 2023 (n,%)	867	448 (51.7)	419 (48.3)

In the 2023 assessment, patients using moderate-intensity and primary prevention statins had a higher frequency of statin non-persistence compared to those continuing statin therapy (both p=0.001). Meanwhile, patients using high-intensity and secondary prevention statins had a higher frequency of statin persistence compared to those discontinuing statin therapy (both p=0.001). Similar to the 2018 assessment, patients with HT, DM, and CAD had a higher rate of statin persistence compared to those without these comorbidities (all p=0.001). Patients aged <45 years had a higher frequency of statin non-persistence (p=0.036) (Table 4).

Table 3. Clinical characteristics of patients continuing and discontinuing statin therapy based on 2018 data.

		Continuing statin therapy (n=498)	Discontinuing statin therapy (n=405)	p
Gender (n, %)	Female	125 (25.1)	86 (21.2)	0.172
	Male	373 (74.9)	319 (78.8)	
Statin intensity (n, %)	Moderate-intensity	452 (90.8)	368 (90.9)	0.958
	High-intensity	46 (9.2)	37 (9.1)	
Statin indication (n, %)	Primary prevention	416 (83.5)	382 (94.3)	**0.001**
	Secondary prevention	82 (16.5)	23 (5.7)	
Cardiometabolic disease (n, %)	Hipertansiyon	252 (50.6)	139 (34.3)	**0.001**
	Diabetes mellitus	147 (29.5)	66 (16.3)	**0.001**
	Coronary artery disease	79 (15.9)	21 (5.2)	**0.001**
Age (n, %)	<45	32 (6.4)	47 (11.6)	**0.028**
	45-64	265 (53.2)	220 (54.3)	
	65-79	174 (34.9)	120 (29.6)	
	≥80	27 (5.4)	18 (4.4)	

Table 4. Clinical characteristics of patients continuing and discontinuing statin therapy based on 2023 data.

		Continuing statin therapy (n=448)	Discontinuing statin therapy (n=419)	p
Gender (n, %)	Female	343 (76.6)	330 (78.8)	0.438
	Male	105 (23.4)	89 (21.2)	
Statin intensity (n, %)	Moderate-intensity	393 (87.7)	397 (94.7)	0.001
	High-intensity	55 (12.3)	22 (5.3)	
Statin indication (n, %)	Primary prevention	348 (77.7)	373 (89.0)	0.001
	Secondary prevention	68 (15.2)	27 (6.4)	
	Primary-secondary prevention	32 (7.1)	19 (4.5)	
Cardiometabolic disease (n, %)	Hypertension	215 (48)	150 (35.8)	0.001
	Diabetes mellitus	128 (28.6)	70 (16.7)	0.001
	Coronary artery disease	65 (14.5)	25 (6)	0.001
Age (n, %)	<45	29 (6.5)	50 (11.9)	0.036
	45-64	250 (55.8)	229 (54.7)	
	65-79	150 (33.5)	126 (30.1)	
	≥80	19 (4.2)	14 (3.3)	

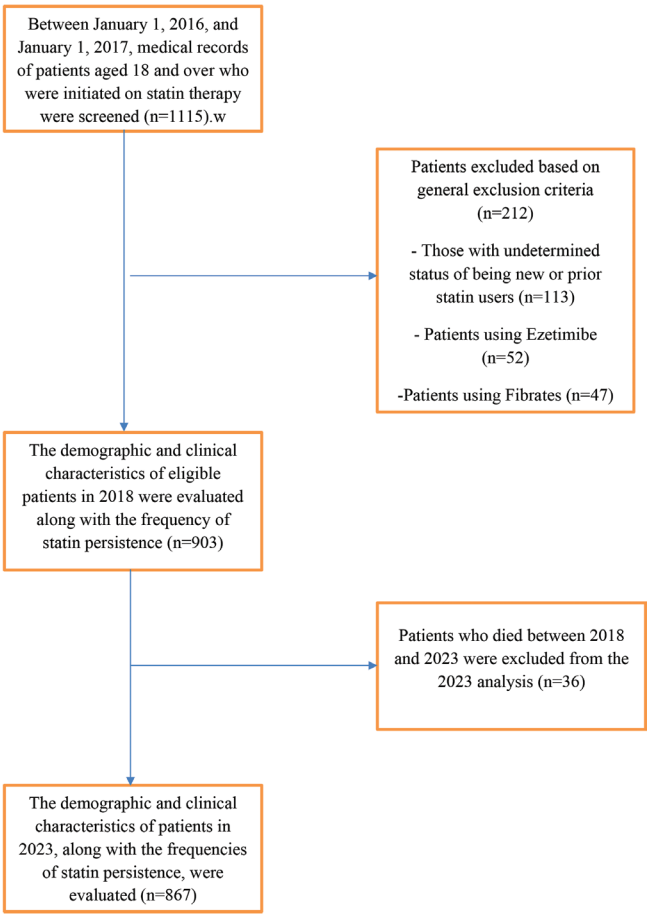

Figure 1. Study flow process.

DISCUSSION

The results of this study have demonstrated that statin persistence is low in both short and long-term follow-ups of patients who initiate statin therapy, with no improvement over time. Particularly, lower statin persistence has been observed in young patients (<45 years), patients with low cardiovascular risk, and patients initiated on statins for primary prevention.

In our cohort, approximately 90% of patients were initiated on statins for primary prevention. This relatively low rate of secondary prevention can be explained by the specific patient profile and departmental context of our study. In our internal medicine clinic, the majority of our patients had comorbidities such as DM and HT, for whom statin therapy was initiated primarily for primary prevention. In contrast, patients with established cardiovascular diseases-such as myocardial infarction, stroke, or peripheral artery disease-are often managed by cardiology, neurology, or cardiovascular surgery departments, where statin initiation typically occurs. Moreover, real-world studies have also shown that the number of patients initiated on statins for primary prevention is substantially higher than the number of those receiving statins for secondary prevention. This disparity in statin prescriptions appears consistent with the findings of our study[13].

It has been reported that among therapies aimed at controlling cardiometabolic diseases such as dyslipidemia, DM, and HT, patients using statins exhibit the lowest adherence and persistence rates[14]. Similar findings are observed among different lipid-lowering drugs such as statins, ezetimibe, and anti-proprotein convertase subtilisin/kexin type 9 monoclonal antibodies, where statin initiators demonstrate the lowest persistence and adherence rates[15]. Cohort studies have reported a decreasing trend in both short and long-term statin persistence and adherence rates[16,17]. In patients aged 45 and above initiated on statins for DM and/or cardiovascular disease, the likelihood of continuing statin therapy decreases to 47% after one year and 19% after five years; with statin persistence falling below 25% regardless of the presence of revascularization, heart failure, peripheral artery disease, or renal disease at the outset of a 5-year follow-up[7].

In our study, it was observed that 44.9% of patients initiated on statins, did not continue statin therapy at the first-year evaluation, and this rate increased to 48.3% at the sixth-year evaluation. This finding is in line with existing literature, indicating that nearly half of patients initiated on statins discontinue treatment over time. It can be interpreted as a promising improvement that 31.8% of patients who did not continue statin therapy in the first year resumed statin therapy at the sixth-year evaluation. However, although this is not sufficient.

Various demographic factors such as age and gender, as well as drug-related characteristics such as the indication for statin initiation (primary or secondary prevention), statin intensity, and statin type, are known to play a role in statin persistence and adherence. Clinical trials have reported lower statin persistence and adherence, especially in the youngest and oldest individuals and those without cardiovascular disease or risk factors. In contrast, higher persistence and adherence are observed in patients initiated on statins for secondary prevention, and with high-intensity statins, although the effect of gender remains controversial[13,17,18]. In our study, it was observed that both in the first and sixth-year evaluations, patients initiated on statins for primary prevention and those under 45 years of age were less likely to continue statin therapy. In contrast, patients initiated on statins for secondary prevention and those

with HT, DM, or CAD were more likely to continue statin therapy. The lower statin persistence in patients under 45 years of age and those initiated on statins for primary prevention can be explained by these individuals being mostly asymptomatic and having a lower awareness of cardiovascular risk due to their relatively good health status and lower prevalence of cardiovascular events.

No significant difference in statin persistence was found in patients initiated on moderate or high-intensity statins at the first-year evaluations. However, at the sixth-year evaluations, statin persistence was lower in patients using moderate-intensity statins compared to those using high-intensity statins. The higher persistence rates observed in patients using high-intensity statins for secondary prevention appear to be consistent with the notion that individuals at high cardiovascular risk are more adherent and responsive to statin therapy. No gender difference in statin persistence was observed in our study.

An increase in exaggerated media reports about statin side effects has been observed to have a negative impact on the statin persistence and adherence[19]. Early discontinuation of statins has been reported to increase the risk of myocardial infarction and cardiovascular mortality in these patients. Although the effect of media reports on statin persistence was not evaluated in our study, the fact that approximately one-third of patients discontinued or resumed treatment at the first and sixth follow-ups suggests that patients are still influenced by them.

Study Limitations

Retrospective design is one of the limitations in this study. Firstly, the reasons for discontinuation of statins by patients, whether due to drug characteristics, physician or media influence, or patient factors, were not evaluated. Secondly, due to the intervening COVID-19 pandemic, the relationship between lipid control and statin persistence could not be established because of the lack of regular lipid monitoring in patients. Thirdly, since it was not clearly determined whether mortality causes were cardiovascular, or not, a cause-effect relationship between statin persistence and cardiovascular events and mortality could not be established. Fourth, due to the absence of patients initiated on low-intensity statins, the effect of statin intensity on statin persistence could be limited. Lastly, due to the asymmetric distribution of initiated statin types (mostly atorvastatin), a comparable statistical analysis could not be performed for the relationship between statin type and statin persistence.

CONCLUSION

In conclusion, the results of this study have shown that statin persistence is low for both short- and long-term follow-ups, regardless of the reason for initiation. Although there is no simple way to increase statin adherence, increasing patient awareness and consciousness of cardiovascular risk through listening to patient concerns about treatment, informing about potential side effects, engaging in discussions, and involving patients in the decision-making process for treatment should be the primary approach to improve statin persistence and adherence.

Ethics

Ethics Committee Approval: Local Ethics Committe of Istanbul Medeniyet University approval was obtained for the study (reference number: 2023/0714, date: 25.10.2023).

Informed Consent: This is a retrospective study.

Footnotes

Author Contributions

Surgical and Medical Pratices: M.U., E.E., L.C., Concept: M.U., C.T., G.A.U., Design: M.U., C.T., E.E., Data Collection and/or Processing: M.U., C.T., E.E., G.A.U., L.C., Analysis or Interpretation: M.U., C.T., E.E., Literature Search: M.U., C.T., E.E.,Writing:. M.U., C.T., E.E.

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Original Article

Epidemiological Insights into HACEK Bacteria: A Seven-year Retrospective Analysis at a Tertiary Care Center in Istanbul

HACEK Bakterilerine Yönelik Epidemiyolojik Bulgular: Istanbul'daki Bir Üçüncü Basamak Merkezde Yedi Yıllık Retrospektif Analiz

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ABSTRACT

Objective: HACEK bacteria (*Haemophilus parainfluenzae, Aggregatibacter* spp., *Cardiobacterium hominis, Eikenella corrodens,* and *Kingella kingae*) represent a group of fastidious organisms implicated in endocarditis and a range of opportunistic infections. Despite their clinical importance, epidemiological data on HACEK infections remain limited, particularly in Türkiye.

Methods: This retrospective analysis investigated 30 cases of HACEK infections diagnosed at a tertiary care hospital in Istanbul over a seven-year period (2017-2023). Data were collected from electronic medical records and laboratory databases.

Results: Patients ranged in age from 0 to 76 years, with isolates derived from a variety of clinical specimens. *Cardiobacterium hominis* was notably absent among the identified species. Polymicrobial growth was documented in 20 cases, predominantly involving Gram-positive cocci, particularly *Streptococcus* spp. Antimicrobial susceptibility testing was performed for four isolates, revealing significant challenges in interpretation due to the absence of standardized guidelines for HACEK pathogens. None of the cases received pathogen-specific therapy; all were managed with empirical antimicrobial regimens. Clinical outcomes were favorable in all but one patient, who succumbed to complications of coronavirus disease-2019. No cases of recurrent HACEK infection or infective endocarditis were observed during follow-up.

Conclusions: These findings underscore the diagnostic challenges associated with HACEK infections and the potential underestimation of their prevalence. Prospective, multicenter studies are needed to clarify the epidemiological and clinical significance of these organisms. Moreover, the development of standardized antimicrobial susceptibility testing protocols and evidence-based therapeutic strategies is essential to optimize patient management and improve clinical outcomes.

Keywords: HACEK group, infectious disease epidemiology, opportunistic infections, polymicrobial infections, microbial sensitivity tests

ÖZ

Amaç: HACEK grubu bakteriler (*Haemophilus parainfluenzae, Aggregatibacter* spp., *Cardiobacterium hominis, Eikenella corrodens* ve *Kingella kingae*), endokardit ve çeşitli fırsatçı enfeksiyonlara neden olabilen, zor üreyen mikroorganizmalardır. Klinik önemlerine rağmen, Türkiye'de HACEK enfeksiyonlarına ilişkin epidemiyolojik veriler oldukça sınırlıdır.

Yöntemler: Bu retrospektif çalışmada, Istanbul'daki bir üçüncü basamak hastanede 2017-2023 yılları arasında tanı konan 30 HACEK enfeksiyonu olgusunu incelemiştir. Veriler elektronik hasta kayıtları ve mikrobiyoloji laboratuvarı veritabanlarından elde edilmiştir.

Bulgular: Hastaların yaşları 0 ile 76 arasında değişmekte olup, izolatlar çeşitli klinik örneklerden elde edilmiştir. Tanımlanan türler arasında *Cardiobacterium hominis* saptanmamıştır. Yirmi olguda çoklu mikroorganizma üremesi görülmüş, en sık eşlik eden bakteriler Gram-pozitif koklar, özellikle *Streptococcus* türleri olmuştur. Antimikrobiyal duyarlılık testi yalnızca dört izolat için uygulanabilmiş, HACEK patojenlerine yönelik standart test kılavuzlarının bulunmaması yorumlamayı güçleştirmiştir. Tüm olgular ampirik antimikrobiyal rejimlerle tedavi edilmiş, hiçbir hasta özel olarak HACEK hedefli tedavi almamıştır. Takip sürecinde sadece bir hasta koronavirüs hastalığı-2019 komplikasyonları nedeniyle yaşamını yitirmiş, diğer hastalarda klinik seyir olumlu olmuştur. Nüks HACEK enfeksiyonu ya da enfektif endokardit olgusu gözlenmemiştir.

Sonuçlar: Bu bulgular, HACEK enfeksiyonlarının tanısal zorluklarını ve gerçek prevalanslarının muhtemel olarak düşük tahmin edildiğini göstermektedir. Bu mikroorganizmaların epidemiyolojik ve klinik etkilerini daha iyi anlayabilmek için çok merkezli prospektif çalışmalara ihtiyaç vardır. Ayrıca, standardize edilmiş antimikrobiyal duyarlılık test protokolleri ve kanıta dayalı tedavi yaklaşımlarının geliştirilmesi, hasta yönetiminin optimize edilmesi ve klinik sonuçların iyileştirilmesi açısından büyük önem taşımaktadır.

Anahtar kelimeler: HACEK grubu, enfeksiyon hastalıkları epidemiyolojisi, fırsatçı enfeksiyonlar, polimikrobiyal enfeksiyonlar, mikrobiyal duyarlılık testleri

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INTRODUCTION

The HACEK group, comprising *Haemophilus parainfluenzae, Aggregatibacter* spp.*, Cardiobacterium hominis, Eikenella corrodens (E. corrodens),* and *Kingella kingae (K. kingae),* is recognized for its role in infective endocarditis and various opportunistic infections, such as septic arthritis, osteomyelitis, and abscesses[1,2]. Predisposing factors for infections caused by these organisms include immunosuppression due to neutropenia, malignancies, and cancer chemotherapy[2].

These organisms are part of the normal flora in the oropharynx, respiratory tract, and gastrointestinal system, but often evade detection in routine microbiological workflows due to their fastidious growth requirements and extended incubation periods[3]. Beyond infective endocarditis, isolating HACEK bacteria from other clinical specimens presents challenges in distinguishing true pathogens from contamination.

While traditional culture methods for HACEK organisms yield low success rates, advancements in Matrix-assisted laser desorption/Ionization-time of flight (MALDI-TOF) mass spectrometry (MS) have significantly improved the rapidity and accuracy of bacterial identification; MALDI-TOF MS achieves species-level identification success rates of 66% with Microflex LT (Bruker) and 93% with Vitek MS (bioMérieux), and genus-level success rates of 88% and 95%, respectively[4]. Additionally, molecular diagnostic methods, such as polymerase chain reaction (PCR), enable the detection of *K. kingae*, a notoriously challenging bacterium to culture, using in-house developed protocols[5]. However, the clinical application of molecular tests for HACEK bacteria remains underdefined, emphasizing the need for clinical suspicion to guide testing.

While the pathogenicity of HACEK bacteria is typically limited, they are responsible for 1-3% of infective endocarditis cases. Notably, *K. kingae* is a leading cause of septic arthritis and osteomyelitis in children under three years of age[4-6]. Mortality rates for infective endocarditis caused by HACEK organisms vary by species, ranging from 5% to 18%[4]. However, data on the clinical outcomes of other HACEK-related infections are limited.

Diagnostic challenges associated with HACEK bacteria are further exacerbated by the absence of robust guidelines for antimicrobial susceptibility testing (AST). The clinical and laboratory standards institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide limited recommendations for HACEK pathogens, resulting in a significant gap in standardized testing protocols and complicating treatment decision-making[7,8].

In Türkiye, the literature on HACEK infections is scarce, primarily consisting of isolated case reports. Some abscess infections caused by *E. corrodens* have been documented[9-11]. A four-year multicenter study identified *Aggregatibacter actinomycetemcomitans* as the causative agent in one of 50 infective endocarditis cases[12]. Another multicenter study of 75 children with septic arthritis found *K. kingae* to be PCR-positive in three cases[13]. Beyond these studies, comprehensive epidemiological data on HACEK infections in Türkiye remain limited. This lack of information hampers a thorough understanding of their epidemiological and clinical impact in the region.

This study aims to fill the knowledge gap by retrospectively analyzing cases of HACEK infections diagnosed at a tertiary care center in Istanbul over a seven-year period (2017-2023). The findings are intended to provide valuable epidemiological data and offer insights into the clinical and microbiological characteristics of HACEK infections in the region.

MATERIALS and METHODS

A total of 30 cases of HACEK infections diagnosed over a seven-year period (2017-2023) at a tertiary care hospital in Istanbul were retrospectively analyzed. Data were obtained from electronic medical records and microbiology laboratory databases. The study was conducted in accordance with the Declaration of Helsinki and ethics committee approval for the study was obtained Istanbul Medeniyet University, from the Non-Interventional Ethics Committee (reference number: 2025/04-36, date: 05.03.2025).

Clinical specimens collected between 2017 and 2023 were cultured on 5% sheep blood agar, chocolate agar, and chromogenic agar (bioMérieux, France), under appropriate aerobic or microaerophilic conditions. Specimens submitted via swabs following abscess drainage or surgical procedures were processed as wound cultures. Bacterial species identification was performed using MALDI-TOF MS (Vitek MS, bioMérieux, France), in accordance with the manufacturer's protocols.

AST was performed for only four isolates due to the fastidious nature of HACEK organisms and the absence of standardized testing protocols, which limited routine AST implementation in clinical practice during the study period. AST was conducted using E-test strips (bioMérieux, France). Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard

and inoculated onto Mueller-Hinton Fastidious (MH-F) agar (bioMérieux, France). Minimum inhibitory concentrations (MICs) were interpreted according to the applicable versions of the EUCAST Clinical Breakpoint Tables (versions 9.1-13.1, corresponding to the study years 2019-2023), using pharmacokinetic/pharmacodynamic non-species-related breakpoints.

Inclusion criteria consisted of patients with confirmed HACEK infections in which the organism was isolated and clinically determined to be the causative agent by the treating physicians. Exclusion criteria included isolates considered clinically insignificant or likely contaminants based on specimen type, clinical context, and physician assessment. In cases of polymicrobial infection, HACEK organisms were deemed causative only when supported by clinical correlation and judgment by attending clinicians. Only one isolate per patient was included in the analysis.

Statistical Analysis

Given the descriptive nature of the study, no advanced statistical analyses were performed. Descriptive statistics were used to summarize patient demographics, infection types, specimen sources, isolated HACEK species, and the proportion of polymicrobial infections with co-isolated microorganisms. Continuous variables were reported as means, median, and range, while categorical variables were expressed as frequencies and percentages. The interquartile range (IQR) was calculated as the difference between the 25th and 75th percentiles of the patient age distribution. All analyses were conducted using Microsoft Excel for Windows (Microsoft 365; Microsoft Corp., Redmond, WA, USA). The findings are descriptive and should be interpreted with caution due to the limited sample size.

RESULTS

Over the 7-year study period, 30 cases of HACEK infections were identified in patients. The isolates were obtained from various clinical specimens, reflecting the diverse clinical presentations of HACEK infections (Table 1).

Of the patients, 20 (66.7%) were male and 10 (33.3%) female, with a median age of 37 years (IQR: 18-59 years; range: 0-76 years). *E. corrodens* was the most frequently isolated species, identified in 25 cases (83.3%) and associated with the broadest spectrum of infections, including 12 wound cultures. Other isolates included *H. parainfluenzae* (n=2, 6.7%), *Aggregatibacter segnis* (n=1, 3.3%), *Aggregatibacter aphrophilus* (n=1, 3.3%), and *K. kingae* (n=1, 3.3%). No cases of *C. hominis* or *Aggregatibacter actinomycetemcomitans* were detected. Clinical specimens were most frequently obtained from wound cultures (n=13, 43.3%), followed by blood (n=5, 16.7%), tissue (n=4, 13.3%), peritoneal fluid (n=4, 13.3%), abscesses (n=2, 6.7%), sputum (n=1, 3.3%), and urine (n=1, 3.3%).

Polymicrobial growth was observed in 20 cases (66.7%), predominantly involving *Gram-positive bacteria* (Table 1). A total of 39 co-isolated non-HACEK organisms were identified, most frequently *Streptococcus species* (30.7%, n=12), followed by *anaerobes* (23%, n=9), including *Parvimonas micra* (n=3), *Prevotella* spp. (n=3), *Fusobacterium* spp. (n=1), and *Gemella* spp. (n=2), and then *Staphylococcus species* (15.3%, n=6).

Bacteremia was documented in five patients: *H. parainfluenzae* in a child with congenital dyserythropoietic anemia; *E. corrodens* in two patients with pulmonary infections and one with acute cholecystitis; and *K. kingae* in a 2-year-old with chronic diarrhea without additional comorbidities.

AST results were available for only four isolates (Table 2), reflecting the challenge posed by the lack of standardized guidelines for HACEK pathogens. None of the patients received targeted therapy for HACEK infections due to the absence of specific treatment protocols; instead, they were managed with empirical therapy containing cephalosporins or ciprofloxacin. Clinical outcomes were favorable in all but one patient, who died of COVID-19 related complications (*A. segnis* isolated from sputum). No mortality directly attributable to HACEK infections was observed. Follow-up cultures showed no recurrence of HACEK growth or subsequent infective endocarditis in any patient.

Table 1. Characteristics of HACEK group infections in patients.

Species	Specimen	Gender	Age	Comorbidity	Polymicrobial infections
Haemophilus parainfluenzae (n=2)	Abscess (n=1)	F	44	Crohn's abscess	*Candida albicans*
	Blood (n=1)	M	7	Congenital dyserythropoietic anemia	*Streptococcus mitis/oralis, Leuconostoc lactis*
Aggregatibacter segnis (n=1)	Sputum (n=1)	M	61	Lung cancer, COVID-19 pneumoniae	-
Aggregatibacter aphrophilus (n=1)	Wound (n=1)	F	8	-	*Staphylococcus epidermidis, Staphylococcus hominis, Streptococcus constellatus*
Eikenella corrodens (n=25)	Wound (n=12)	8 M, 4 F	7-72	• Stomach cancer • Crohn's abscess • Necrotizing fasciitis • Mandibular abscess (n=2) • Cervical lymphadenopathy after cat scratch • Cellulitis after paronychia • Furuncle/carbuncle • Pilonidal cyst abscess • Branchial cleft cyst • Laryngocutanous fistula • Liver abscess	Polymicrobial (n=6); detected with one or more of the following: *S. constellatus (n=3), Streptococcus sanguinis, Streptococcus parasanguinis, Parvimonas micra (n=2), Atopobium parvulum, Fusobacterium nucleatum, Prevotella nigrescens, Prevotella denticola, Klebsiella pneumoniae*
	Tissue (n=4)	2 M, 2 F	13-71	• Insulin-related cellulitis • Skin abscess • Paronychia abscess • Osteomyelitis	Polymicrobial (n=3); detected with one or more of the following: *Staphylococcus aureus, S. epidermidis, Staphylococcus haemolyticus, Streptococcus intermedius, S. anginosus, S. constellatus, Corynebacterium tuberculostearicum*
	Peritoneum (n=4)	3 M, 1 F	4-63	• Perforated appendicitis (n=3) • Colon cancer (n=1)	Polymicrobial (n=4); detected with one or more of the following: *Streptococcus pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Gemella haemolysans*
	Blood* (n=3)	3 M	44-76	• Bronchial cancer and lung abscess • Acute cholecystitis • Bronchiectasis, empyema	Polymicrobial (n=3); detected with one or more of the following: *S. aureus, S. anginosus, Gemella morbillorum, Prevotella* spp., *Actinomyces odontolyticus, Parvimonas micra, Citrobacter freundii, Klebsiella oxytoca*
	Abscess (n=1)	M	26	Submandibular sialadenitis	-
	Urine (n=1)	M	0	Hydronephrosis, nephrostomy	*Candida parapsilosis, Stenotrophomonas maltophilia*
Kingella kingae (n=1)	Blood (n=1)	F	2	Chronic diarrhea	-

*: In addition to blood samples, *Eikenella corrodens* was also identified in the pleural fluid of one patient and in bronchoalveolar lavage samples from another.

Data include species, specimen type, patient demographics, comorbidities, and co-isolated organisms in polymicrobial infections.

"Polymicrobial infections" indicate other microorganisms isolated from the same sample.

Age is given in years; M: Male, F: Female

Table 2. Antimicrobial susceptibility testing results for HACEK isolates.

Bacteria	Specimen	Year	Antimicrobial	Result*
Eikenella corrodens	Wound	2019	Ampicillin, ceftriaxone	Susceptible
Eikenella corrodens	Aspirate	2022	Penicillin, ampicillin	Susceptible
Eikenella corrodens	Wound	2023	Ampicillin, ceftazidime, ertapenem, imipenem, meropenem, ciprofloxacin, levofloxacin, linezolid	Susceptible
Haemophilus parainfluenzae	Blood	2023	Ceftriaxone, cefotaxime, cefuroxime, ertapenem, meropenem, imipenem, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole	Susceptible except trimethoprim-sulfamethoxazole

*Antimicrobial susceptibility testing was performed and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using pharmacokinetic/pharmacodynamic (PK/PD) breakpoints. Results are reported as "susceptible" for isolates showing *in vitro* sensitivity to the listed antibiotics. *H. parainfluenzae* isolate demonstrated resistance to trimethoprim-sulfamethoxazole.

DISCUSSION

C. hominis is a low-virulence organism primarily associated with infective endocarditis, and its isolation from specimens other than blood cultures is exceedingly rare[2]. Consistent with this characteristic, *C. hominis* was not reported in our study, further supporting the infrequent nature of infections caused by this organism in clinical settings.

K. kingae colonizes the upper respiratory tract of infants starting at 6 months, with peak colonization rates occurring between 6 months and 2 years of age[4]. It is a rare cause of bacteremia and endocarditis, particularly in children with underlying conditions, and has also been reported in immunocompetent adults following dental procedures[2].

In our study, we documented a case of *K. kingae* bacteremia in a 2-year-old child, with no identifiable underlying predisposing conditions. While the potential association between a recent dental procedure and the onset of infection remains speculative, it highlights the need for further research to better understand possible predisposing factors. Although *K. kingae* is recognized as a causative agent of septic arthritis in children, arthritis was not observed in this case. The role of *K. kingae* PCR testing in diagnosing arthritis and other infections remains uncertain, and there is no consensus on when to apply PCR in suspected infections. Moreover, the availability of PCR testing is not consistent across all centers, complicating its widespread use. In Türkiye, there is a lack of data regarding oropharyngeal carriage and infection rates of *K. kingae*. As surveillance data become more comprehensive, clearer diagnostic and therapeutic guidelines are expected to emerge. Additionally, the role of dental procedures in *K. kingae* bacteremia warrants further investigation, especially in immunocompetent individuals.

E. corrodens has been implicated in a wide array of infections, including head and neck infections such as ocular, mastoid, submandibular, and thyroid abscesses, as well as pleuropulmonary infections like lung abscesses and empyema. These infections are particularly common in individuals with immunosuppression, a predisposition for pulmonary aspiration, or underlying lung disease. *E. corrodens* has also been isolated in both pure and mixed cultures from various wound infections, including necrotizing fasciitis[2]. In our study, *E. corrodens* was the most frequently isolated species, associated with bacteremia in two patients with pulmonary infections and one with acute cholecystitis.

The ability of *E. corrodens* to cause a diverse range of abscesses, including polymicrobial infections, complicates the identification of the pathogen versus contamination, particularly in mixed cultures. This highlights the challenge of distinguishing between colonization and true infection. Additionally, the pathogenesis of *E. corrodens* remains poorly understood, and further research is needed to elucidate the mechanisms by which this organism contributes to infection. Given its potential as both a pathogen and part of the normal flora, it is clear that more studies are required to better define its role in infections and refine diagnostic criteria. The clinical relevance of polymicrobial infections involving *E. corrodens* further underscores the importance of thorough clinical evaluation and microbiological analysis when identifying the causative agents.

Due to the fastidious nature of HACEK organisms, AST is challenging and is not commonly performed in many centers. Consequently, treatment is often empirically guided by published reports and clinical guidelines. β-lactamase-producing strains may be encountered, and therefore, the use of penicillin or ampicillin is not recommended for invasive infections, such as infective endocarditis. HACEK bacteria are generally susceptible to third-generation cephalosporins and fluoroquinolones,

with ceftriaxone being the first-line treatment and ciprofloxacin serving as an alternative to β-lactams[3,4,14]. The CLSI and the EUCAST provide limited recommendations for HACEK pathogens, resulting in gaps in standardized testing protocols and complicating treatment strategies. Since 2017, EUCAST v.7.1 has included disk diffusion and MIC breakpoints for *K. kingae*, MH-F broth, and for agar. However, guidelines for *Haemophilus* species are restricted to *H. influenzae*[8]. The CLSI guideline M45-ED3 outlines MIC breakpoints for *Aggregatibacter, Cardiobacterium, E. corrodens*, and *Kingella,* using cation-adjusted mueller-hinton broth/laked-horse-blood[7]. Both guidelines cover susceptibility testing for β-lactams, macrolides, fluoroquinolones, tetracyclines, rifampin, and trimethoprim-sulfamethoxazole, and they include a recommendation for β-lactamase testing. Despite these frameworks, significant challenges persist in testing fastidious HACEK bacteria due to their stringent growth requirements and limited data, resulting in the unmet need for standardized protocols across all HACEK group members. Furthermore, the lack of robust resistance surveillance data hampers our ability to monitor trends in antimicrobial resistance, especially in regions where surveillance systems are underdeveloped.

In our data, MIC values were reported for three *E. corrodens* strains and one *H. parainfluenzae* isolate. All four samples were susceptible to β-lactams, while *H. parainfluenzae* exhibited resistance to trimethoprim-sulfamethoxazole. These findings highlight the need for further research into the resistance patterns of HACEK organisms, as both local and global surveillance data on antimicrobial resistance remain limited. Aside from β-lactamase production, the mechanisms underlying resistance to other antibiotic classes remain largely unknown. Despite this, the empirical use of cephalosporins remains a reasonable and effective alternative for treating infections caused by these organisms. The scarcity of comprehensive resistance surveillance data, coupled with the fastidious nature of HACEK bacteria, underscores the need for standardized testing protocols and broader research to better understand the evolving resistance landscape of this group. As more data become available, these findings will contribute to improving treatment strategies and guiding clinical decision-making for infections caused by HACEK organisms.

Study Limitations

This study has several limitations. Its retrospective and single-center design may limit the generalizability of the findings and carry an inherent risk of missing or incomplete data. The relatively small sample size, with only 30 culture-positive cases, restricted the ability to perform detailed subgroup comparisons and limited the statistical power. The absence of standardized AST guidelines for HACEK pathogens posed additional challenges in interpreting susceptibility data. Furthermore, the high proportion of polymicrobial infections complicated the assessment of the pathogenic role of HACEK organisms as the primary causative agents.

CONCLUSION

The prevalence of HACEK infections is likely underestimated due to their demanding growth requirements and limited research, particularly in Türkiye. Future research should prioritize prospective, multicenter studies to better define the epidemiology and clinical significance of the condition. The role of HACEK organisms in polymicrobial infections, especially in immunocompromised hosts, warrants further study to fully elucidate their pathogenic potential. Developing standardized AST protocols and evidence-based treatment recommendations will be crucial for improving patient care and outcomes in HACEK-related infections.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and ethics committee approval for the study was obtained Istanbul Medeniyet University, from the Non-Interventional Ethics Committee (reference number: 2025/04-36, date: 05.03.2025).

Informed Consent: This is a retrospective study.

Footnotes

Author Contributions

Concept: M.E.K., T.O., Design: M.E.K., T.O., Data Collection and/or Processing: T.D., Z.H., Analysis or Interpretation: T.D., Z.H., Literature Search: T.D., Writing: T.D.

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Original Article

Impact of Eosinophil Levels on Disease Progression and Clinical Outcomes in Chronic Obstructive Pulmonary Disease (COPD) Patients: A Retrospective Study

Kronik Obstrüktif Akciğer Hastalığı (KOAH) Hastalarında Eozinofil Düzeylerinin Hastalık İlerlemesi ve Klinik Sonuçlar Üzerindeki Etkisi: Retrospektif Bir Çalışma

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ABSTRACT

Objective:: The role of total eosinophil count (EOS) in chronic obstructive pulmonary disease (COPD) remains debated, with studies suggesting both positive and negative impacts on disease progression. This retrospective study aimed to investigate the relationship between stable-state blood EOS levels and clinical outcomes, including hospitalizations, emergency room (ER) visits, and pneumonia, in COPD patients.

Methods: Data from 398 COPD patients were analyzed, focusing on blood EOS counts and percentages acquired during stable periods. Patients were categorized based on EOS thresholds of 150 cells/μL and 2%. The number of hospitalizations, ER visits, and pneumonia diagnoses in the preceding year was retrieved from hospital records and patient reports.

Results: Patients with EOS levels below 150 cells/μL or 2% showed a significantly higher number of hospitalizations. Additionally, patients with EOS percentages below 2% had higher COPD Assessment Test and Modified Medical Research Council scores, indicating greater symptom burden and dyspnea. Logistic regression analysis confirmed that a lower EOS percentage was an independent predictor of increased hospitalizations, similar to its association with lower FEV1% and more than two ER visits.

Conclusions: This study suggests that low blood EOS counts are associated with increased hospitalizations and worse clinical outcomes in COPD patients. This finding highlights the importance of considering EOS levels as a potential biomarker for disease severity and may lead to personalized treatment strategies. Further prospective studies are needed to validate these findings and elucidate the underlying mechanisms.

Keywords: Eosinophils, forced expiratory volume, hospitalization, pulmonary disease, chronic obstructive

ÖZ

Amaç: Kronik Obstrüktif Akciğer Hastalığında (KOAH) eozinofillerin (EOS) rolü hala tartışılmaktadır ve çalışmalar hastalığın ilerlemesi üzerinde hem olumlu hem de olumsuz etkiler olduğunu ileri sürmektedir. Bu retrospektif çalışma, KOAH hastalarında stabil durumdaki kan EOS seviyeleri ile hastane yatışları, acil servis (AS) ziyaretleri ve pnömoni dahil klinik sonuçlar arasındaki ilişkiyi araştırmayı amaçlamaktadır.

Yöntemler: Üç yüz doksan sekiz KOAH hastasından alınan veriler, stabil dönemlerde elde edilen kan EOS sayıları ve yüzdelerine odaklanılarak analiz edildi. Hastalar 150 hücre/μL ve %2'lik EOS eşiklerine göre kategorize edildi. Önceki yıldaki hastane yatışları, AS ziyaretleri ve pnömoni tanıları hastane kayıtlarından ve hasta raporlarından alındı.

Bulgular: EOS seviyeleri 150 hücre/μL veya %2'nin altında olan hastalarda anlamlı sayıda daha fazla hastaneye yatış saptandı. Ek olarak, EOS yüzdeleri %2'nin altında olan hastalarda daha yüksek KOAH değerlendirme testi ve değiştirilmiş tıbbi araştırma konseyi skorları belirlendi ve daha fazla semptom yükü ve şiddetli dispne ile ilişkili olduğu görüldü. Lojistik regresyon analizi, daha düşük EOS yüzdesinin, daha düşük FEV1% ve ikiden fazla AS ziyaretine paralel olarak artan hastane yatışlarının bağımsız bir öngörücüsü olduğu gösterilmiştir.

Sonuçlar: Bu çalışma ile düşük kan EOS sayımlarının KOAH hastalarında artan hastane yatışları ve daha kötü klinik sonuçlarla ilişkili olduğu saptanmıştır. Bu bulgu, EOS düzeylerinin hastalık şiddeti için potansiyel bir biyobelirteç olarak dikkate alınmasının önemini vurgulamakta ve kişiselleştirilmiş tedavi stratejilerinin önünü açacaktır. Elde edilen verilerin ve altta yatan mekanizmaların doğrulanması için daha fazla prospektif çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Eozinofiller, görsel ekspiratuar bölüm, hastane yatışı, kronik obstrüktif akciğer hastalığı

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD), a preventable condition, presents a significant global health burden. It is the fourth leading cause of death worldwide, causing 3.5 million deaths in 2021. Nearly 90% of the deaths in individuals under 70 years of age occur in low and middle-income countries[1]. It is characterized by airflow limitation, respiratory symptoms, and exacerbations. The development of COPD results from a complex interaction between inherited predispositions, external influences, and inflammatory responses[2]. The focus on total eosinophil count (EOS) and its influence on COPD has grown substantially in the last few years. EOS are white blood cells that play a role in allergic inflammation and certain parasitic infections. EOS activity within COPD patients fluctuates based on specific disease presentations and how the condition progresses[3]. Although the role of EOS in COPD is not yet fully understood, existing evidence suggests that these cells play a significant role in airway inflammation and disease heterogeneity. Traditionally, COPD has been associated with neutrophilic inflammation. However, 37% of patients with COPD have EOS inflammation in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study[4]. This suggests the existence of different COPD phenotypes, each of which may require different treatment approaches[5]. The identification of EOSic COPD phenotypes has significant clinical implications. By monitoring blood EOS counts, clinicians can optimize corticosteroid regimens, potentially decreasing reliance on systemic corticosteroids and mitigating their associated side effects. EOS-guided therapy has been shown to be non-inferior to standard care in terms of days alive and out of hospital, while also reducing corticosteroid exposure in COPD exacerbations[6]. A positive correlation has been observed between increased EOS concentrations and enhanced short-term clinical outcomes in COPD patients, with a particular emphasis on those with a prior history of tobacco use. These patients tend to have shorter hospital stays and a better response to corticosteroid therapy compared to those with lower EOS counts[7].

Eosinopenia has also been associated with an increased risk of treatment failure in COPD exacerbations. Various thresholds for eosinopenia have been used in studies of patients with COPD exacerbations: <50 cells/μL, <150 cells/μL, <0.144 × 109/L, <2%[5,8,9]. Further research is needed on whether eosinopenia can be used as a marker of severity in COPD exacerbations and to determine an appropriate threshold value. The aim of this study was to investigate the relationship between blood EOS levels during the stable period and the number of hospitalizations, pneumonia, and emergency room (ER) admissions detected in the previous year.

MATERIALS and METHODS

The study included participants from two different study cohorts: Yazar et al's[10] and Gürel et al's[11]. In both studies, the relationship was evaluated between demographic characteristics such as age, gender, pulmonary function test values, EOS count and percentages in the blood measured during the stable period, history of hospitalizations due to COPD in the last year, number of ER visits, and history of pneumonia, in patients who have been under treatment and follow-up with a diagnosis of COPD for at least 1 year was evaluated. Both studies from which data were obtained received approval from the local Ethics Committee of Medeniyet University (decision number: 2018/24-05, date: 28.12.2018). First study from Biruni University, second study from Clinical Research Ethics Committee of Medeniyet University, Göztepe Training and Research Hospital. All patients included in the study were informed about the study, and written consent was obtained from them.

The inclusion criteria for patients were the following: 1. age ≥40 years; 2. routine baseline stable state peripheral blood test results before receiving any antibiotic or systemic corticosteroid therapy; 3. patients diagnosed with COPD according to the Global Initiative for Chronic Obstructive Lung Disease guideline with previous spirometry detected forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio of <0.70.

The exclusion criteria were 1. bronchial asthma, asthma-COPD overlap syndrome, parasites, or other allergic diseases associated with elevated EOS levels in peripheral blood; 3. patients with missing data; 4. Patients who do not consent to participate in the study

The number of hospitalizations due to COPD exacerbations, ER admissions, and pneumonia diagnoses in the previous year was retrieved from the hospital's database and patient's statements.

It has been observed that EOS counts measured during acute exacerbations are not associated with future rehospitalizations or long-term mortality[12]. To assess a patient's overall long-term risk profile and potential for future hospitalizations, EOS levels during the stable state provide a more reliable indicator, as this state is more consistent than the transient inflammatory status observed during an exacerbation. Therefore, blood samples from patients were collected during the stable state, defined as having no recent exacerbation or at least being four weeks after the last exacerbation.

The patients were divided into two groups based on their peripheral blood EOS count and percentage. Cutoff values are selected as 150 cells/mL and 2%.

Statistical Analysis

Continuous variables are expressed as mean values with standard deviation and compared with Student's t-test or Mann-Whitney test as appropriate, whereas categorical variables are expressed as percentages and compared by the chi-square test. Spearman's correlation was used to identify. Multiple logistic regression analyses were performed to assess possible associations between hospitalization and other variables. p-values less than 0.05 were deemed statistically significant.

RESULTS

This retrospective study included 398 patients with COPD. The study population had a mean age of 65.15 years, with a predominance of males (84.4%). A significant proportion of patients (46.3%) were current or former smokers, with a mean smoking history of 47.5 pack-years. The mean FEV1% value was 50.55±18.72, and the mean FVC % was 65.62±19.13 (Table 1). The mean EOS count was 221.67±150.75, and the EOS % was 2.51±1.71. In the year prior to the study, the mean number of hospitalizations was 0.17±0.58, ER admissions was 1.53±3.01, and the diagnosis of pneumonia was 0.79±1.57.

Patients were categorized based on blood EOS count (cutoff: 150 cells/μL) and EOS percentage (cutoff: 2%). Significant differences in the number of hospitalizations were observed between the groups defined by both EOS count and percentage (Table 2). Furthermore, when using the 2% EOS percentage cutoff, significant differences were also found in COPD Assessment Test (CAT) and modified Medical Research Council (mMRC) scores between the groups. Higher number of hospitalizations in the previous year (<2% vs. ≥2%; 0.27±0.74 vs. 0.09±0.43), higher number of patients with CAT score over 10 (<2% vs. ≥2%; %66.3 vs. % 56.0) and higher number of patients with mMRC score over 2 (<2% vs. ≥2%; %57.8 vs. %44.0) were detected in patients with EOS <2%.

The number of hospitalizations was correlated with FEV1% predicted (c: -0.206, p<0.001), ER admissions (c: 0.362, p<0.001), CAT (c: 0.183, p<0.001), and mMRC scores (c: 0.228, p<0.001). Correlations remained significant even when groups were separated (Table 3 and Figure 1).

Logistic regression analysis revealed that previous year's number of hospitalizations was significantly associated with lower predicted FEV1% [odds ratio (OR) 0.970, 95% CI 0.948-0.992, p: 0.008, EOS percentage less than 2% (OR: 2.505, 95% confidence interval (CI) 1.208-5.196, p: 0.014), and more than two ER admissions (OR: 6.361, 95% CI 2.865-14.123, p<0.001) (Table 4).

Table 1. Demographic characteristics of the study population.

Variable	n=398
Age (years)	65.15±8.50
Gender	
Male	336 (84.4)
Female	62 (15.6)
Smoking status	
Smoker	184 (46.30)
Exsmoker	214 (53.70)
Smoking package-year	47.50±27.64
FEV1 (L)	1.38±0.61
FEV1 % predicted	50.55±18.72
FVC (L)	2.25±0.83
FVC % predicted	65.62±19.13
FEV1/FVC	56.78±9.90
EOS count (cell/mL)	221.67±150.75
EOS %	2.51±1.71
Hospitalizations in the previous year	0.00 (0) (min-max 0.00-5.00)
ER admissions in the previous year	0.00 (2) (min-max 0.00-30.00)
Pneumonia diagnosis in the previous year	0.00 (1) (min-max 0.00-10.00)
CAT score	13.42±8.93
mMRC score	1.74±1.26

Data are presented as means±SD, median (Interquartile range and with minimum and maximum values) or number (%). FEV1: Forced expiratory volume in one second, FVC: Forced vital capacity, EOS: Total eosinophil count, ER: Emergency room, CAT: Chronic obstructive pulmonary disease assessment test, mMRC: modified medical research council, SD: Standard deviation

Table 2. Patients' characteristics regarding EOS count and %.						
Variables	**EOS <150 n=125**	**EOS ≥150 n=271**	**p-value**	**EOS <2% n=166**	**EOS ≥2% n=230**	**p-value**
FEV1% pred	51.02±19.02	50.33±18.62	0.733	49.87±18.53	51.03±18.89	0.543
FVC% pred	66.14±20.17	65.38±18.66	0.716	65.31±19.49	65.84±18.90	0.786
FEV1/FVC	56.89±9.38	56.73±10.15	0.886	56.39±9.75	57.06±10.02	0.509
Hospitalizations	0.27±0.76	0.12±0.47	0.039	0.27±0.74	0.09±0.43	**0.008**
ER	1.70±3.13	1.46±2.96	0.466	1.81±3.04	1.34±2.99	0.125
Pneumonia	0.82±1.79	0.78±1.47	0.851	0.92±1.77	0.70±1.41	0.189
CAT	12.98±8.69	13.63±9.04	0.506	14.02±8.89	13.00±8.95	0.258
CAT <10 CAT ≥10	48 (38.4) 77 (61.6)	110 (40.3) 163 (59.7)	0.720	56 (33.7) 110 (66.3)	102 (44.0) 130 (56.0)	**0.040**
mMRC	1.86±1.35	1.68±1.21	0.229	2.01±1.31	1.54±1.19	**<0.001**
mMRC <2 mMRC ≥2	59 (47.2) 66 (52.8)	141 (51.6) 132 (48.4)	0.410	70 (42.2) 96 (57.8)	130 (56.0) 102 (44.0)	**0.006**

EOS: Total eosinophil count, FEV1: Forced expiratory volume in one second, FVC: Forced vital capacity, ER: Emergency room, CAT: chronic obstructive pulmonary disease assessment test, mMRC: modified Medical Research Council

Table 3. Correlation table between hospitalisations, FEV1% predicted, ER admissions, CAT and mMRC score.				
	FEV1 % pred	**ER admission**	**CAT score**	**mMRC score**
Hospitalizations (All)	c: -0.206 p<0.001	c: 0.362 p<0.001	c: 0.183 p<0.001	c: 0.228 p<0.001
EOS < 150 cell/µL	c: -0.242 p: 0.006	c:0.384 p<0.001	c:0.210 p :0.019	c:0.225 p :0.012
EOS ≥ 150 cell/µL	c: -0.195 p: 0.001	c: 0.356 p<0.001	c:0.183 p:0.002	c:0.228 p<0.001
EOS < 2%	c: -0.212 p: 0.006	c: 0.356 p<0.001	c:0.213 p:0.006	c:0.223 p:0.004
EOS ≥ 2%	c: -0.211 p: 0.001	c: 0.381 p<0.001	c:0.148 p:0.025	c:0.199 p:0.002

FEV1 % pred: FEV1: Forced expiratory volume in one second predicted, ER: Emergency room admission, CAT: chronic obstructive pulmonary disease assessment test, mMRC: modified medical research council, EOS: Total eosinophil count,, p<0.05 considered significant, c: Pearson correlation coefficient

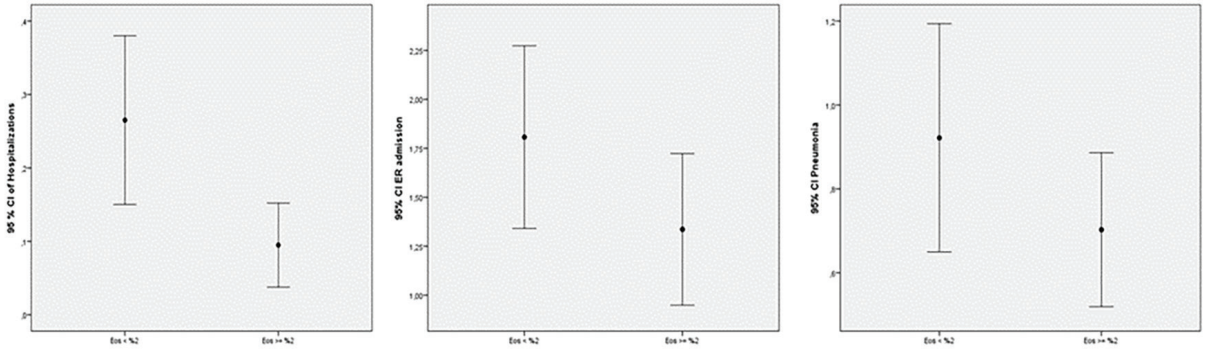

Figure 1. Errorbar graphics between blood EOS % and the number of hospitalizations, ER admissions and pneumonia diagnosis.

EOS: Total eosinophil count, ER: Emergency room, CI: Confidence interval

Table 4. Logistic regression analysis on factors affecting hospitalization.				
Model 1 Dependent variable: Hospitalisation in the previous year				
	B	**OR**	**95% CI**	**p-value**
FEV1% pred	-0.037	0.964	0.942-0.986	0.001
EOS %	-0.295	0.745	0.578-0.959	0.023
ER adm	0.202	1.224	1.107-1.353	<0.001
Model 2 Dependent variable: Hospitalisation in the previous year				
	B	**OR**	**95% CI**	**p-value**
FEV1% pred	-0.030	0.970	0.949-0.992	0.008
EOS ≥150 cell/μL	0.896	2.450	1.183-5.077	0.016
EOS ≥150 cell/μL	0.896	2.450	1.183-5.077	0.016

FEV1 % pred: Forced expiratory volume in one second % predicted, B: Regression coefficient, OR: Odds ratio, CI: Confidence interval, EOS %: Blood eosinophil count %, ER adm: Emergency room admission rate in the previous year, EOS ≥150: Total eosinophil count 150 cells/mL and above, ER adm adm ≥2: Emergency room admission rate 2 and above in the previous year

DISCUSSION

Our study demonstrated significant associations between the number of hospitalizations and predicted FEV1%, ER visits, CAT scores, and mMRC scores. These correlations remained statistically significant after stratifying by EOS count, supporting the hypothesis that low EOS counts may independently affect the clinical course and outcomes of COPD. In addition, this study identified a statistically significant association between low blood EOS counts (≤150 cell/μL or ≤2%) and an increased number of hospitalizations among patients with COPD during a stable phase. Despite ongoing debate regarding the precise mechanisms of EOS in COPD, studies have established a link between elevated EOS counts and exacerbations requiring hospital admission. Conversely, other research suggests that low EOS levels may indicate adverse clinical outcomes. Our findings are consistent with the "non-EOS COPD" phenotype as described in the literature, a distinct phenotype characterized by more severe airflow limitation, a higher frequency of exacerbations, diminished quality of life, and increased mortality risk[13].

The blood EOS level used in COPD phenotyping is related to disease prognosis and management. However, research findings conflict regarding the role of blood EOS levels in COPD exacerbations. Several studies indicate that increased EOS counts (at or above 2% or 300 cells/μL) correlate with improved responses to systemic corticosteroid therapy and reduced length of hospital stays[14,15]. We found that patients with EOS counts less than 150 cells/μL or 2% experienced a significantly higher number of hospital admissions, ER visits, and pneumonia compared to individuals with higher EOS levels.

Several potential mechanisms may explain the link between diminished EOS counts and heightened hospitalization risk. These include the activation of alternative inflammatory pathways, an elevated susceptibility to bacterial infections, a diminished responsiveness to corticosteroid treatment, and exacerbated systemic inflammation[9,14,16]. As EOS are immune cells normally found in the respiratory tract, providing defense against pathogens, their reduction may increase susceptibility to infections. Moreover, investigations have demonstrated that reduced EOS counts often coincide with elevated neutrophil counts, indicating a distinct inflammatory pattern[16]. Although our data did not include neutrophil counts, we observed a higher rate of pneumonia diagnoses in patients with low EOS levels, consistent with these findings.

As patients were categorized based on EOS levels, the distinction among their clinical parameters became particularly evident when a 2% threshold was applied to the EOS percentage in our study. We observed that patients with an EOS percentage below 2 had higher CAT and mMRC scores. This suggests that a low EOS percentage may correlate with the severity of symptoms and dyspnea, affecting patients beyond hospitalization. This finding supports the idea that EOS in COPD may have an impact on the long-term course of the disease and symptom management in addition to acute exacerbations. Similar to our findings, Lv et al.[17] reported significantly increased inflammation, reduced lung function, extended hospital stays, elevated mMRC and CAT scores, higher mortality, and greater utilization of non-invasive mechanical ventilation in patients with EOS counts below 2%. Furthermore, in two studies conducted by Ko et al.[12] and Greulich et al.[14], patients with low EOS

counts (<2% or <100 cells/μL) had consistently longer hospitalizations compared to patients with higher EOS levels.

While discrepancies exist across various studies, a general tendency suggests that elevated blood EOS counts in COPD patients might correlate with better, or at least comparable, lung function, however, the clinical significance of these improvements isn't always substantial. Despite this, high EOS counts have been linked to a rapid decline in lung function as indicated by published data[18-20]. Conversely, it's important to note that lower EOS counts don't consistently correspond to a slower decline. In our study, despite varying EOS thresholds (150 and 2%), no significant differences were observed between groups in baseline respiratory function tests, indicating that these specific EOS cut-offs may not be directly linked to the degree of pulmonary impairment at a stable state. Factors such as a history of smoking and patients' treatment differences are likely to have an influence on this complicated relationship[19,20]. Interestingly, despite the faster decline in FEV1, patients with consistently high EOS levels tend to have better survival rates and improved symptom control compared to those with lower EOS levels[16,21].

Study Limitations

Our investigation is subject to several limitations. A significant limitation is its single-center, retrospective design, which may inherently restrict the generalizability of our findings to broader populations. Furthermore, our analysis was confined to blood EOS counts, and we did not assess sputum EOS, other inflammatory markers such as interleukin-5, or comprehensive systemic inflammation markers. These factors introduce a degree of heterogeneity within our dataset, particularly regarding participants' inhaled corticosteroid use, diverse maintenance therapy regimens, and the presence of various comorbidities. The absence of uniformity in these aspects made the systematic inclusion of these critical variables in our logistic regression analysis infeasible. As a result, we could not precisely ascertain the independent contribution of these factors to the observed outcomes. While our findings offer valuable insights, their interpretation must consider the potential confounding effects of differing medication use and underlying health conditions that were not comprehensively addressed in the statistical models. Further investigations, ideally incorporating controlled, prospective, and multi-center methodologies, are essential to thoroughly decipher these complex interactions.

CONCLUSION

In conclusion, our findings suggest a potential association between low blood EOS counts and an elevated risk of hospitalization in patients with COPD. This finding highlights the heterogeneous nature of COPD and the clinical significance of different inflammatory phenotypes. Closer monitoring of COPD patients with low EOS counts and the development of personalised treatment strategies for this group are necessary. Further research is needed to investigate this area and improve the management of COPD.

Ethics

Ethics Committee Approval: Both studies from which data were obtained received approval from the local ethics committee. First study from Biruni University, second study from Clinical Research Ethics Committee of Istanbul Medeniyet University, Göztepe Training and Research Hospital. (decision number: 2018/24-05, date: 28.12.2018).

Informed Consent: All patients included in the study were informed about the study, and written consents were obtained from them.

Footnotes

Author Contributions

Surgical and Medical Practices: B.A.Y., E.E.Y., Concept: B.A.Y., E.E.Y., Design: B.A.Y., E.E.Y., Data Collection and/or Processing: B.A.Y., E.E.Y., E.H.K., E.Y.O.N., C.D., F.C.G., Analysis or Interpretation: B.A.Y., E.E.Y., E.H.K., E.Y.O.N., C.D., F.C.G., Literature Search: B.A.Y., E.E.Y., E.H.K., E.Y.O.N., C.D., F.C.G., Writing: B.A.Y., E.E.Y., E.H.K., E.Y.O.N., C.D., F.C.G.

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Original Article

Is Transient Tachypnea of the Newborn a Risk Factor for Bronchiolitis in the First Two Years of Life? A Population-Based Birth Cohort Study

Yenidoğanın Geçici Takipnesi Yaşamın İlk İki Yılında Bronşiyolit için Bir Risk Faktörü mü? Nüfus Temelli Doğum Kohort Çalışması

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ABSTRACT

Objective: Transient Tachypnea of the Newborn (TTN) is a common cause of respiratory distress in term infants, associated with delayed pulmonary fluid clearance resulting from dysfunction of the epithelial sodium channel. Although generally self-limiting, TTN may increase early childhood wheezing and asthma risk. This study aimed to assess the incidence and characteristics of acute bronchiolitis in TTN infants compared to those in healthy controls within a large birth cohort.

Methods: We conducted a population-based cohort study that included all live births in Istanbul from January 2016 to December 2018, utilizing the Turkish Ministry of Health's e-Nabız database. Infants diagnosed with isolated TTN (International Classification of Diseases [ICD]-10 P22.1) formed the study group. A randomly selected control group of healthy infants without respiratory diagnoses was included in the study. Both groups were followed for two years to identify episodes of acute bronchiolitis (ICD-10 J21*), excluding cases within the first month of life. The data collected included bronchiolitis incidence, recurrence, age at the time of the episode, and hospitalizations.

Results: Among 1,002,261 live births, 14,389 TTN infants and 14,500 controls were analyzed. Acute bronchiolitis occurred in 42.4% of TTN infants and 35.8% of controls (p<0.001). TTN infants had higher rates of single episodes, while controls experienced more recurrent episodes (p<0.001). Hospitalization was more frequent in the control group (p<0.001), with single hospitalizations predominating in the TTN group and multiple hospitalizations in the control group. The first episodes in TTN infants mainly occurred between 1-6 months, with controls showing more episodes early but fewer later (p<0.001). Recurrence was correlated with an earlier age of the first episode in both groups (p<0.001).

Conclusions: TTN infants experience more bronchiolitis episodes early in life, though recurrent episodes are more common among healthy controls.

ÖZ

Amaç: Yenidoğanın Geçici Takipnesi (YGT) zamanında doğan bebeklerde solunum sıkıntısının yaygın bir nedenidir ve epitelyal sodyum kanal disfonksiyonundan kaynaklanan gecikmiş pulmoner sıvı klirensi ile ilişkilidir. Genellikle kendi kendini sınırlasa da TTN erken çocukluk döneminde hışıltılı solunum ve astım riskini artırabilir. Bu çalışmada, geniş bir doğum kohortunda YGT öyküsü olan bebeklerde akut bronşiolit insidansı ve özellikleri, sağlıklı kontrollerle karşılaştırmalı olarak değerlendirildi.

Yöntemler: TC. Sağlık Bakanlığı e-Nabız veri tabanı kullanılarak, Ocak 2016-Aralık 2018 arasında Istanbul'da gerçekleşen tüm canlı doğumlar üzerinden toplum temelli bir kohort çalışması yürütüldü. İzole YGT (ICD-10: P22.1) tanısı konan bebekler çalışma grubunu oluşturdu. Solunum tanısı olmayan sağlıklı bebeklerden oluşan bir kontrol grubu ile eşleştirildi. Her iki grup, yaşamın ilk ayında görülen vakalar hariç tutularak, iki yıl boyunca akut bronşiolit (ICD-10: J21*) atakları açısından izlendi. Veriler arasında atak insidansı, nüks, atak sırasındaki yaş ve hastane yatışları yer almıştır.

Bulgular: 1.002.261 canlı doğumdan 14.389 YGT bebeği ve 14.500 kontrol grubu analiz edilmiştir. Bronşiolit, YGT grubunda %42,4; kontrol grubunda %35,8 oranında görülmüştür (p<0,001). YGT tanısı olan bebeklerde tek atak daha yüksek oranda görülürken, kontrol grubunda daha fazla tekrarlayan atak görülmüştür (p<0,001). Hastaneye yatış ise kontrol grubunda daha sıktır (p<0,001). İlk ataklar YGT grubunda 1-6 ayda yoğunlaşmıştı. Nüks, her iki grupta da erken ilk atak yaşı ile ilişkiliydi (p<0,001).

Sonuçlar: YGT'li bebeklerde yaşamın ilk iki yılında bronşiolit atağı geçirme sıklığı daha yüksek ancak tekrarlayan atak oranı sağlıklı bebeklerden daha düşüktür. Bu bulgular, YGT'nin ileride gelişebilecek astım ve hışıltılı solunumla ilişkisini araştıracak ileri çalışmalara temel oluşturabilir.

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Further studies are warranted to investigate the relationship between TTN and the development of wheezing and asthma.

Keywords: Transient tachypnea of the newborn, acute bronchiolitis, population-based cohort study

Anahtar kelimeler: Yenidoğanın geçici takipnesi, akut bronşiolit, toplum temelli kohort çalışması

INTRODUCTION

The presence of tachypnea defines the diagnosis of Transient Tachypnea of the Newborn (TTN) within the first 6 hours after birth. This includes the persistence of tachypnea for at least 12 hours and characteristic findings on at least one chest radiograph, such as prominent central vascular markings, thickened interlobar fissures, symmetric perihilar congestion, flattened diaphragmatic domes, or an increased anteroposterior diameter indicating hyperaeration, in the absence of other known causes of respiratory distress[1]. It is a common cause of respiratory distress in term newborns[2]. Respiratory distress occurs in approximately 1% of newborns, with TTN accounting for about 4.0% to 5.7% of these cases. The remaining cases are attributed to other causes such as meconium aspiration syndrome, pneumonia, sepsis, and pneumothorax[3]. Known risk factors for TTN include prematurity, cesarean delivery, multiple gestation, infants of diabetic mothers, macrosomia, maternal asthma, male sex, rapid or prolonged labor, delayed cord clamping, and low appearance, pulse, grimace response, activity, respiration scores[4].

The incidence of TTN ranges from 0.6% to 1.57%, depending on the presence of risk factors. Its frequency has been increasing mainly due to rising rates of elective cesarean deliveries[4,5,6]. However, the exact pathophysiology of TTN remains unclear. One of the leading hypotheses implicates impaired function of the epithelial sodium channels (ENaC) in the alveolar epithelium. Typically, ENaC expression increases near term to promote alveolar fluid clearance; however, in TTN, despite adequate surfactant levels, reduced ENaC activity leads to delayed fluid resorption and consequent respiratory distress after birth. Although TTN is considered a self-limiting condition, it may increase the incidence of wheezing respiratory episodes and asthma during early life[7,8]. Over the past three decades, randomized controlled trials and studies have investigated the pathogenesis and clinical manifestations of asthma related to ENaC and chloride ion channels[9]. Moreover, similarities in pathophysiology between TTN and viral bronchiolitis, particularly bronchiolitis caused by respiratory syncytial virus (RSV), have been proposed, with ENaC dysfunction playing a central role[10,11]. The relationship between TTN and RSV bronchiolitis has been extensively investigated. Among the most significant risk factors identified for hospitalizations due to viral bronchiolitis, particularly RSV, are prematurity, chronic pulmonary disease, and congenital heart disease[12]. However, most children hospitalized for RSV are previously healthy, leaving the reasons for increased disease severity in these patients unclear[10,11].

Wheezing is a symptom observed in both children with asthma and those with bronchiolitis. However, it can also indicate other conditions with different pathophysiology, clinical courses, and outcomes. When the characteristics, onset, progression, and associated illnesses of wheezing are considered together, it becomes evident that multiple pathologies can cause this symptom. Especially during the first two years of life, wheezing episodes may be due to viral bronchiolitis or early manifestations of childhood asthma. Given the challenges in accurately diagnosing asthma in preschool children, we investigated the relationship between TTN and recurrent bronchiolitis.

Our aim was to investigate the incidence and characteristics of bronchiolitis during the first two years of life in infants diagnosed with TTN, compared to a healthy control group, using a population-based birth cohort study conducted in the most populous and cosmopolitan province, which represents all regions of our country.

MATERIALS and METHODS

This study is a population-based cohort including all live births in Istanbul between January 1, 2016, and December 31, 2018. Data from the Ministry's National Health Information System (e-Nabız) were utilized in collaboration with the Ministry of Health of the Republic of Türkiye. In the initial phase, individuals to be included in the study, those to be excluded, or those assigned to the control group were identified based on the International Statistical Classification of Diseases and Related Health Problems (ICD) diagnostic codes within the online national database.

All live-born infants with the TTN ICD-10 diagnosis code (P22.1) and no additional diagnoses composed the main study group. Exclusion criteria for the study group included infants with specific ICD-10 diagnosis codes that

could cause diagnostic confusion or negatively affect the identification of risk factors. These comprised stillbirths, premature infants, and/or those weighing less than 500 grams at birth; infants with congenital malformations, including cardiac anomalies starting with the ICD-10 code Q; and infants diagnosed with congenital malformations, deformations, or chromosomal abnormalities coded as Z13.7 in the ICD-10 classification.

Selection of the Control Group and the Randomization Process

To enable comparative analysis, a control group was required, consisting of infants without a TTN diagnosis but with similar sociodemographic characteristics. For this purpose, infants born within the same timeframe and geographical region Istanbul as the TTN cases were identified using the Ministry of Health's National Health Information System (e-Nabız) database. These infants had no respiratory disease diagnoses during the first month of life and had only visited healthcare facilities for routine check-ups (ICD-10 codes: Z00.0, Z00.1, Z00.8). Following this initial selection, infants meeting the exclusion criteria-such as prematurity, congenital malformations, stillbirth, or any respiratory diagnosis-were excluded. From the remaining eligible control candidates, a total of 14,500 infants were randomly selected to match as closely as possible the number of TTN cases (n=14,234), approximating an approximate 1:1 sampling ratio. The randomization process was conducted as follows:

Creation of the Data Pool

A pool of infants was formed who met the exclusion criteria and had received healthcare services only with the diagnoses of Z00.0 (General medical examination), Z00.1 (Routine child health examination), or Z00.8 (Other general examinations), without any TTN or other respiratory diagnoses.

Random Number Assignment

Each individual in the pool was assigned a random number linked to their unique identification number. These random numbers were generated using the Excel RAND function, and the overall distribution of the generated numbers was examined.

Ranking and selection based on random numbers: All individuals were ranked according to their assigned random numbers, and the first 14,500 were automatically selected without any manual intervention to form the control group.

Bronchiolitis Diagnosis Status

Among the individuals initially included in the control group, those who had received a diagnosis of acute bronchiolitis or any respiratory condition other than TTN within the first 30 days of life were subsequently excluded. Therefore, the final sample was standardized as a control group with a "clean respiratory history" at baseline concerning bronchiolitis.

Using this method, an objective and representative control group was obtained, consisting of individuals born during the same period as those diagnosed with TTN and without a history of exclusionary conditions, thereby avoiding systematic error or selection bias during selection.

The randomization process was designed and implemented in accordance with classical epidemiological principles, aiming to create a comparison group with similar characteristics to the study group but without a TTN diagnosis. The selected method was based on the principle of simple random sampling and was carefully structured to minimize the potential impact of confounding factors.

In the second phase, follow-up records from January 1, 2018, to December 31, 2020, were reviewed for all infants in both the study and control groups who were born between January 1, 2016, and December 31, 2018. This allowed for the collection of complete data on each case up to two years of age. These records were linked using a unique personal identification number assigned to every individual in the country.

After the first postnatal month, infants with ICD-10 diagnosis codes J21 (Acute bronchiolitis), J21.0 (Acute bronchiolitis due to RSV), J21.8 (Acute bronchiolitis due to other specified organisms), or J21.9 (Acute bronchiolitis, unspecified) were identified as having experienced a bronchiolitis episode. Those diagnosed with bronchiolitis during the first month of life were excluded due to the potential difficulty in differential diagnosis. The age at the time of each bronchiolitis episode, the number of recurrent episodes, and any hospital admissions were recorded. The age at the time of bronchiolitis episodes was also categorized into groups for analysis (1-6 months, 7-12 months, 13-18 months, 19-24 months).

Maternal diabetes (P70.0, P70.1) and maternal asthma (J45) were identified through the database as potential risk factors for TTN in infants. However, due to the insufficient maternal diagnostic data available in the records, these variables could not be included in the final analysis. Similarly, an attempt was made to assess

the association between maternal asthma diagnosis and the number of acute bronchiolitis episodes in infants within the control group. Still, maternal asthma records were not available for evaluation.

The primary outcome was to compare the incidence of acute bronchiolitis episodes during the first two years of life between infants with TTN and healthy controls. Secondary outcomes were to compare the characteristics of bronchiolitis episodes (recurrence, age at first episode, and hospitalization rates) between the two groups, and to examine associations between age at first episode and recurrence.

Since this study was based on data from the national health records system and no direct contact was made with any individuals, obtaining ethical approval from participants was not required. However, ethical approval for conducting the study was obtained from the Ethics Committee of Istanbul Medeniyet University (approval number: 2020/0633, date: 05.03.2025).

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2016 software. Descriptive statistics were presented as frequencies and percentages for categorical variables, and as mean ± standard deviation or median (with minimum and maximum values) for continuous variables. The normality of the variables was assessed using the Kolmogorov-Smirnov test.

Pearson's chi-square test was used to compare categorical variables between groups. For multilevel categorical variables such as episode count, frequency of hospitalizations, and age distribution of episodes, statistical significance was assessed using Pearson's chi-square test in conjunction with cross-tabulation. Significant differences in pairwise comparisons were identified using post-hoc analysis methods with Bonferroni correction.

Column proportion comparison analyses were also performed to test the significance of differences in proportions between groups, with cells showing significant differences marked using the Bonferroni correction. Additionally, the distribution of acute bronchiolitis episodes was evaluated separately across subgroups defined by time intervals to assess the effect of age ranges on the frequency of bronchiolitis attacks.

A two-sided p-value of less than 0.05 was considered statistically significant in all analyses.

RESULTS

During the specified two-year period, the number of births in Istanbul was 1,008,655. Of these, 6,394 were stillbirths (0.63%). Among the 1,002,261 live-born infants, 14,389 (1.43%) had an isolated diagnosis of TTN and constituted the study group. The control group included 14,500 infants (Figure 1).

During the first two years of life, 42.4% of the TTN group experienced an acute bronchiolitis episode, compared to 35.8% in the control group ($p<0.001$).

Regarding the number of acute bronchiolitis episodes, 46.5% of patients in the TTN group experienced one episode, 22.7% had two episodes, and 30.9% had three or more episodes. In the control group, 23.6% of patients had one episode, 22.3% (n=1,160) had two episodes, and 54.1% experienced three or more episodes (Table 1).

The rate of experiencing a single episode was significantly higher in the TTN group. In contrast, the rate of having three or more episodes was considerably higher in the healthy control group ($p<0.001$).

Due to episodes of acute bronchiolitis, 13.4% of the TTN group and 15.6% of the control group were hospitalized for treatment ($p<0.001$). Among the TTN group, 71% were hospitalized once, 18.7% were hospitalized twice, and 10.2% were hospitalized three or more times between birth and two years of age.In the control group, among those who experienced acute bronchiolitis episodes, 61.1% were hospitalized once, 22.2% were hospitalized twice, and 16.7% were hospitalized three or more times for treatment (Table 1). When comparing the hospitalization frequency between the two groups, the rate of single hospitalizations was significantly higher in the TTN group. In contrast, multiple hospitalizations were more frequent in the control group ($p<0.001$).

In the TTN group, 44.0% experienced their first bronchiolitis episode between 1-6 months, 33.4% between 7-12 months, 14.4% between 13-18 months, and 8.1% between 19-24 months. In the control group, these rates were 47.8%, 33.1%, 12.5%, and 6.6% for the respective age intervals. Comparison of the groups revealed that the control group experienced a significantly higher rate of acute bronchiolitis episodes between 1 and 6 months, whereas the TTN group showed a significantly higher number of episodes during the 12-24 month period ($p<0.001$) (Table 1).

The age distribution of the first bronchiolitis episode was compared, based on the number of bronchiolitis attacks, between children with and without a history of

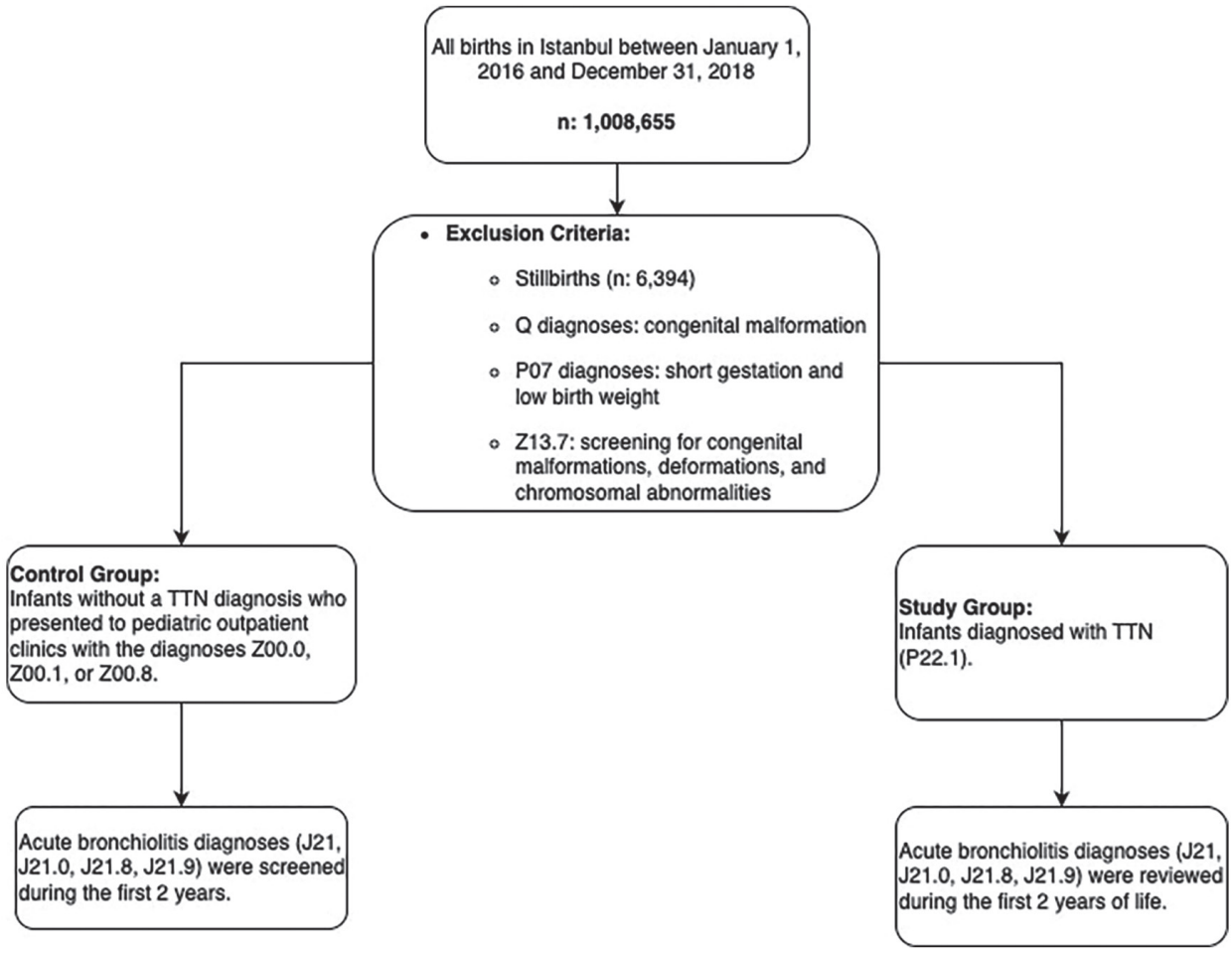

Figure 1. Flow diagram

TTN: Transient tachypnea of the newborn

TTN (Table 2). In the control group, 26.8% of those with a single bronchiolitis episode experienced their first attack between 1-6 months, increasing to 41.1% among those with two episodes and 59.7% among those with three or more episodes. Similarly, the proportions of first attacks occurring between 7 and 12 months were 32.2%, 37.9%, and 31.5%, respectively. A marked decrease in the incidence of first attacks was observed with increasing age: in the 13-18 month group, these rates were 22.9% (one episode), 14.4% (two episodes), and 7.3% (three or more episodes), while in the 19-24 month group, they declined to 18.2%, 6.6%, and 1.5%, respectively.

A similar pattern was observed in the TTN group, although the rates of bronchiolitis episodes between 1- to 6-months were higher across all groups. In this group, 32.7% of those with one episode, 44.7% of those with two episodes, and 60.6% of those with three or more episodes experienced their first attack within the first six months of life. The proportions of first attacks occurring between 7 and 12 months were 33.6%, 36.4%, and 31.1%, respectively. These rates declined markedly in older age groups: 20.0% (one episode), 13.0% (two episodes), and 7.1% (three or more episodes) in the 13- to 18- month group; and 13.7%, 5.9%, and 1.3% in the 19- to 24- month group.

Statistical analyses performed with Bonferroni correction for multiple comparisons revealed that, in both the TTN and control groups, an increasing number of bronchiolitis episodes was significantly associated with the first episode occurring earlier in life ($p<0.001$). Specifically, the proportion of children experiencing their first bronchiolitis episode within the first six months was 26.8% in the control group and 32.7% in the TTN group among those with a single episode. This rate increased to 59.7% in the control group and 60.6% in the TTN group among children with three or more episodes. Conversely,

Table 1. Frequency and clinical characteristics of acute bronchiolitis in children with a history of TTN compared to controls.

Variables	Category	Control group n (%)	TTN group n (%)	p-value
Acute bronchiolitis	No	9303 (64.20)	8293 (57.60)	**<0.001**
	Yes	5197 (35.80)	6096 (42.40)	
Hospitalization	No	12235 (84.40)	12462 (86.60)	**<0.001**
	Yes	2265 (15.60)	1927 (13.40)	
Number of hospitalization	1	1384 (61.10)	1369 (71.00)	**<0.001**
	2	502 (22.20)	361 (18.70)	
	≥3	379 (16.70)	197 (10.20)	
Number of bronchiolitis attack	1	1228 (23.60)	2834 (46.50)	**<0.001**
	2	1160 (22.30)	1381 (22.70)	
	≥3	2809 (54.10)	1881 (30.90)	
Age at bronchiolitis (months)	1-6	2481 (47.80)	2682 (44.00)	**<0.001**
	7-12	1720 (33.10)	2037 (33.40)	
	13-18	651 (12.50)	880 (14.40)	
	19-24	342 (6.60)	494 (8.10)	

TTN: Transient tachypnea of the newborn

Table 2. Comparison of the age and number of attacks in the TTN group and the control group.

	Number of bronchiolitis attacks) / Age at bronchiolitis (months)	1 n(%)	2 n(%)	≥3 n(%)	p-values
Control group	**0-6**	328 (26.8) A	477 (41.1) A B	1676 (59.7) B	**<0.001**
	7-12	394 (32.2) A C	440 (37.9) C	886 (31.5) A	
	13-18	280 (22.9) B C	167 (14.4) C	204 (7.3)	
	19-24	223 (18.2) B C	76 (6.6) C	43 (1.5)	
TTN group	**0-6**	926 (32.7) A	617 (44.7) A B	1139 (60.6) B	**<0.001**
	7-12	950 (33.6) A C	502 (36.4) C	585 (31.1) A	
	13-18	567 (20.0) B C	180 (13.0) C	133 (7.1)	
	19-24	388 (13.7) B C	82 (5.9) C	24 (1.3)	

*Based on column proportion comparisons with Bonferroni correction, comparison letters (A, B, C) were assigned to each cell. Cells bearing different letters within the same age row indicate statistically significant differences ($p<0.05$, Bonferroni correction applied).

TTN: Transient tachypnea of the newborn

the occurrence of first episodes between 13 and 24 months significantly decreased with increasing episode count in both groups.

DISCUSSION

We observed that children with a history of TTN experienced bronchiolitis episodes more frequently than healthy controls. In the TTN group, bronchiolitis episodes were predominantly observed within the first six months of life, whereas in the control group, they tended to occur between 12 and 24 months of age. Interestingly, hospitalisation rates and the occurrence of multiple admissions due to bronchiolitis were found to be higher among the healthy controls.

TTN, though generally benign and self-limiting, has been associated with increased long-term pulmonary morbidity. Several studies have suggested that infants diagnosed with TTN are at a higher risk of developing asthma and wheezing episodes during the preschool years[13]. RSV is a major contributor to viral bronchiolitis in infancy, with about two-thirds of affected infants developing wheezing within the first five months of

life[11,14]. Most infants hospitalized due to RSV bronchiolitis are previously healthy[6]. Therefore, the underlying reasons for increased disease severity in these cases remain unclear[10]. Studies in animal models and cell cultures have demonstrated that RSV increases alveolar fluid accumulation by inhibiting sodium-dependent pulmonary fluid clearance[9]. A similar pathophysiological mechanism has also been implicated in TTN.

Heinonen et al.[6] reported a novel association between TTN diagnosed at birth and the subsequent development of RSV bronchiolitis during the first year of life, proposing a potential mechanism involving ENaC within the alveolar epithelium. A birth cohort study in the literature reported higher rates of RSV-related hospitalizations among children with a history of TTN, while another study identified younger chronological age as an independent risk factor[6,14]. In contrast, our findings differed markedly; we observed a higher number of hospital admissions in the control group. However, it is essential to note that our study did not exclusively evaluate RSV bronchiolitis. Instead, it included all bronchiolitis episodes, which may account for this discrepancy. The studies mentioned above included only RSV- confirmed cases of bronchiolitis. In our study, however, we performed an analysis based on ICD-10 diagnoses from a birth cohort using a national health database, which included data from all levels of healthcare institutions-primary, secondary, and tertiary. As such, we recognize that in many of these settings, microbiological identification of the causative pathogen was not routinely performed. Therefore, the bronchiolitis cases without confirmed pathogen testing may also include a significant number of RSV-related episodes. We found that children with a history of TTN experienced more bronchiolitis episodes than their healthy counterparts. Among TTN infants, single bronchiolitis episodes were more common, whereas multiple episodes were more frequently observed in the control group and were also associated with a higher rate of hospitalization. Moreover, in TTN infants who had recurrent bronchiolitis, these episodes were predominantly concentrated within the first six months of life. From this perspective, it may be inferred that bronchiolitis was more severe in the TTN group. Given that RSV bronchiolitis typically peaks during the first six months of life, we speculate that the majority of early-life bronchiolitis episodes in TTN cases were likely attributable to RSV. Prospective studies involving larger patient populations with confirmed RSV diagnoses may clarify this relationship and potentially support including TTN in risk profiling for RSV- related hospitalizations.

In another retrospective study, 103 children with TTN and healthy controls were evaluated at two years of age through direct physical examination, review of medical history, and investigations. The study assessed factors such as the timing and frequency of wheezing episodes, as well as hospitalizations due to wheezing. The authors concluded that TTN is an independent risk factor for wheezing[5]. In this study, although the diagnosis of asthma in cases was considered more reliable than in questionnaire-based studies, the small sample size may limit the generalizability of the findings.

The relationship between TTN and wheezing or bronchiolitis episodes, as well as childhood asthma, has been extensively explored. We consider this inquiry natural, as diagnosing childhood asthma is challenging and requires careful differentiation of the causes of wheezing. Furthermore, severe bronchiolitis in early childhood-particularly following rhinovirus or RSV bronchiolitis-is known to be associated with an increased risk of developing asthma[15,16]. Additionally, recurrent wheezing episodes resembling bronchiolitis may represent early signs of asthma that develop later in life. The literature supports that having three or more wheezing attacks is associated with a higher likelihood of progression toward asthma[17]. A study aiming to identify potential risk factors for TTN and early childhood asthma selected infants with TTN and healthy births from a hospital database. Subsequently, families were contacted by phone and administered the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire to assess the presence of asthma. The association between TTN and asthma was found to be stronger than that of other factors, such as elective cesarean delivery and maternal asthma[18]. The ISAAC questionnaire used in this study assesses wheezing episodes, their characteristics, and other differential diagnostic factors. However, since the ISAAC questionnaire serves only as a screening tool for asthma, the results may differ from physician-confirmed diagnoses, which could potentially affect the reliability of the data. In another birth cohort study, based on hospital electronic medical records, infants with TTN and healthy controls were initially selected. Asthma diagnoses were then investigated using ICD codes recorded during the 2 to 5 years following birth at the same hospital[13]. TTN was found to be independently and significantly associated with a diagnosis of childhood asthma. The authors highlighted that TTN may serve as a marker of pulmonary dysfunction, reflecting a genetic predisposition to asthma[13]. Since we utilized the entire national database in our study, we were able to access all records, regardless of which hospital or district the cases were seen in. However, the referenced study relied solely on records from the hospital where the birth occurred. When interpreting their results, it is crucial to consider

that this approach may not fully capture the complete medical history of the cases.

In a study by Shohat et al.[19], 58 children aged 4 to 5 years with a history of TTN at birth were compared to age-matched controls without a TTN history. The TTN group showed significantly higher rates of atopic manifestations, a family history of atopy in first-degree relatives, more than two wheezing episodes, and a clinical diagnosis of childhood asthma compared to the control group[19].

In contrast to previous studies, our findings showed that the TTN group experienced more single episodes of bronchiolitis, while the frequency of three or more episodes was higher in the control group. Although recurrent bronchiolitis attacks can have multiple causes, they are often considered an early sign of asthma. Based on this information, our study suggests that progression to asthma was not more common in the TTN group compared to the control group.

The consistency of similar findings across various studies using different methods-such as surveys, clinical examinations, and ICD diagnosis codes-strengthens the association between wheezing/bronchiolitis episodes and TTN.

Strengths of the Study

This study has several important strengths that enhance its scientific validity and potential clinical contributions. Foremost, it is a population-based, large-scale birth cohort study conducted in Istanbul, the largest metropolitan area in Türkiye. The dataset, which covers over one million live births between 2016 and 2018, was obtained through the standardized electronic health record system (e-Nabız) of the Turkish Ministry of Health. This approach minimizes selection bias and ensures that the sample highly represents the general population.

Another significant strength of the study is its large sample size. Both the TTN and control groups included over 14,000 infants, which increased the statistical power and allowed the detection of small but clinically meaningful differences. Additionally, the carefully defined exclusion criteria eliminated the effects of congenital anomalies, severe prematurity, and other respiratory diseases, resulting in a homogeneous cohort with high internal validity.

A simple random sampling method was used to select the control group, which included only infants who had undergone healthy check-ups during the neonatal period. This approach ensured that the comparison group and the TTN group were similar in baseline characteristics, thereby minimizing the influence of confounding variables. Furthermore, the continuous follow-up of infants for 24 months from birth allowed detailed analysis of both the timing and frequency of acute bronchiolitis episodes.

The use of real-world data represents another key strength of this study, enhancing its applicability to health policy. By using nationally standardized health data, researchers ensured that the findings are not limited to Istanbul alone but can be generalized to broader populations with similar healthcare infrastructures. Moreover, these results may provide valuable insights for identifying target groups for preventive strategies against viral infections such as RSV.

Study Limitations

Limitations of the study include the fact that participants' health records encompassed all healthcare facilities, ranging from small health clinics to tertiary hospitals. In smaller centers, such as health clinics, specific ICD codes for bronchiolitis subtypes, like RSV bronchiolitis, may have been underreported, as confirmation of these specific diagnoses was not consistently possible at every facility.

CONCLUSION

Infants with TTN experience more bronchiolitis episodes during the first two years of life compared to healthy term infants. However, recurrent bronchiolitis attacks are less frequent in the TTN group than in healthy controls. Future studies employing new methodologies that retrospectively investigate the history of TTN in children with asthma and wheezing may provide further insights into this relationship.

Ethics

Ethics Committee Approval: Ethical approval for conducting the study was obtained from the Ethics Committee of Istanbul Medeniyet University (approval number: 2020/0633, date: 05.03.2025).

Informed Consent: Since this study was based on data from the national health records system and no direct contact was made with any individuals, obtaining ethical approval from participants was not required.

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We acknowledge that we employed ChatGPT 3.5 and 4 to assist us in refining the clarity of our writing while developing the draft of this case report. We always maintained continuous human oversight (editing-revising) and verified the artificial intelligence-generated output. We never used AI to find, locate, or review the literature or resources, summarize the articles, analyze the selected articles, or synthesize the findings. The authors completed all analyses with higher-level efforts.

Footnotes

Author Contributions

Surgical and Medical Practices: S.C.O., S.G., Concept: S.C.O., M.K.S., G.B., A.K.K., S.G., Design: S.C.O., M.K.S., D.M.T., Z.R.O., A.K.K., S.G., Data Collection and/or Processing: S.C.O., M.K.S., D.M.T., Y.A., S.G., Analysis and/or Interpretation: S.C.O., M.K.S., D.M.T., S.G., Literature Search: S.C.O., G.B., D.M.T., Z.R.O., Y.A., A.K.K., S.G., Writing: S.C.O., M.K.S., G.B., S.G.

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Original Article

Sex Estimation Using Patellar Morphometry: Evidence from a Late Roman Population in Anatolia

Patellar Morfometri Kullanarak Cinsiyet Tahmini: Anadolu'daki Geç Roma Dönemi Nüfusundan Kanıtlar

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ABSTRACT

Objective: Accurate sex estimation is a cornerstone in forensic and bioarchaeological investigations. While the pelvis and skull are traditionally used for this purpose, their absence due to taphonomic damage necessitates the use of alternative skeletal elements such as the patellar. This study evaluates the diagnostic potential of patellar morphometry for sex estimation in a Late Roman population.

Methods: The study analyzed 146 adult patellar (70 males, 76 females) recovered from the Karlığın Tepesi Necropolis 3rd-6th centuries AD in Malatya, Türkiye. Standard osteological methods were used to determine sex. Three patellar dimensions/length (PL), patellar width (PW), and patellar thickness (PT)-were measured. Intraobserver reliability was assessed via technical error of measurement (TEM), relative TEM (rTEM), and the reliability coefficient (R). Stepwise logistic regression and receiver operating characteristic analyses were conducted to identify the best predictors of sex. Area under the receiver operating characteristic (AUROC) values, cut-off thresholds, and effect sizes were reported.

Results: Statistically significant differences were found between males and females in PL (p=0.001), PW (p<0.001), and PT (p=0.003). The stepwise logistic regression model using PL and PT produced AUROC values of 0.906 in Step 1 and 0.920 in Step 2, with sensitivity and specificity ranging from 82.85% to 94.73%. All intraobserver reliability metrics (TEM, rTEM, R) indicated excellent measurement precision (R=1.000).

Conclusions: Patellar morphometry demonstrates high diagnostic accuracy for sex estimation, particularly when multivariate models are applied. Despite the moderate discriminative power of patella thickness alone, its combination with other parameters enhances overall performance. The study provides the first population-specific discriminant model for sex estimation using the patella in an Anatolian archaeological sample. However, the skewed sex distribution and the possibility of post-depositional changes in ancient skeletal remains should be considered when interpreting results. Additionally, the population-specific nature of the archaeological sample and the lack of external validation on independent datasets limit the generalizability of the model to other contexts.

Keywords: Patellae, sex determination by skeleton, receiver operating characteristic, logistic regression models, sexual dimorphism

ÖZ

Amaç: Cinsiyet tayini, adli tıp ve biyoarkeolojik araştırmalarda temel bir adımdır. Pelvis ve kafatası geleneksel olarak bu amaçla kullanılsa da bu kemiklerin yokluğu ya da tahrip olması durumunda alternatif kemik elemanlarına, örneğin patellaya başvurulması gerekebilir. Bu çalışma, Geç Roma Dönemi'ne ait bir Anadolu topluluğunda patella morfometrisinin cinsiyet tahminindeki tanısal değerini değerlendirmeyi amaçlamaktadır.

Yöntemler: Araştırma kapsamında, Türkiye'nin Malatya ilindeki Karlığın Tepesi nekropolünden çıkarılan 146 yetişkin patella (70 erkek, 76 kadın) incelenmiştir. Cinsiyet tayini standard osteolojik yöntemlerle gerçekleştirilmiştir. Patellanın uzunluk (PL), genişlik (PW) ve kalınlık (PT) ölçümleri alınmıştır. Tekrarlı ölçümlerin güvenilirliği teknik hata (TEM), göreli TEM (rTEM) ve güvenilirlik katsayısı (R) ile değerlendirilmiştir. Cinsiyet tahmini için aşamalı lojistik regresyon ve alıcı işletim karakteristiği eğrisi analizleri uygulanmıştır. Alıcıişletim karakteristiği eğrisi altında kalan (AUROC) değerleri, eşik noktaları ve etki büyüklükleri raporlanmıştır.

Bulgular: Erkek ve kadınlar arasında tüm patella ölçümlerinde anlamlı farklar bulunmuştur: PL (p=0,001), PW (p<0,001), PT (p=0,003). PL ve PT'yi içeren lojistik regresyon modeli Step 1'de AUROC =0.906, Step 2'de AUROC =0,920 elde etmiş, duyarlılık ve özgüllük %82,85 ila %94,73 arasında değişmiştir. Ölçüm güvenilirliği tüm parametrelerde mükemmel düzeydedir (R=1,000).

Sonuçlar: Patella morfometrisi özellikle çok değişkenli modellerle birleştirildiğinde, cinsiyet tahmini için yüksek tanısal doğruluk sağlamaktadır. Kalınlık ölçümünün tek başına ayırt edici gücü sınırlı olsa da diğer değişkenlerle birlikte kullanıldığında modelin performansını artırmaktadır. Bu çalışma Anadolu arkeolojik örnekleminde patella kullanılarak yapılmış ilk nüfus-özgül cinsiyet tahmin modeli olması açısından literatüre önemli katkı sunmaktadır. Ancak örneklemdeki cinsiyet dengesizliği ve iskelet kalıntılarında zamanla oluşabilecek bozulmalar dikkate alınmalıdır. Ayrıca arkeolojik örneklemin popülasyona özgü niteliği ve modelin bağımsız veri setlerinde test edilmemiş olması, sonuçların diğer bağlamlara genellenebilirliğini sınırlamaktadır.

Anahtar kelimeler: Patella, iskelet ile cinsiyet tahmini, ROC eğrisi, lojistik regresyon modelleri, cinsel dimorfizm

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INTRODUCTION

Sex determination of skeletal remains plays a vital role in the disciplines of forensic medicine, archaeology, anthropology, and anatomical education[1-3]. The human skeleton serves as a reliable source for determining sex. While the skull and pelvic bone are most commonly used for this purpose, their absence or fragmentation can necessitate the use of alternative skeletal elements, such as the patella[4]. In forensic contexts, accurate sex estimation can eliminate half of the possibilities in missing persons cases[5]. Although osteometric analyses have been shown to be effective in sex estimation, their results are population-specific and should be evaluated accordingly[6].

The patella, the largest sesamoid bone in the human body, has recently attracted significant attention due to its morphological differences between males and females. It is anatomically located within the quadriceps femoris tendon and articulates with the patellar surface of the femur[7,8]. Functionally, the patella enhances knee joint stability and quadriceps leverage during movement, and its cartilage covering helps reduce friction between articular surfaces[9,10]. Given its compact shape and frequent preservation in skeletal remains, the patella presents itself as a valuable alternative for sex estimation.

Recent forensic and anthropological studies have applied discriminant function analysis, logistic regression, and even artificial intelligence models to assess patellar dimensions such as length, width, and thickness for sex determination[11]. Although the accuracy of using the patella alone is generally lower than that of the more sexually dimorphic bones such as the pelvis or skull, combining patellar measurements with other skeletal metrics can significantly improve reliability[12].

Despite the growing body of research on patellar morphometry, there remains a significant gap in population-specific standards for sex estimation using the patella, particularly in archaeological populations from Anatolia. Most existing studies have focused on modern or clinical samples, which may not accurately reflect the morphological features of ancient populations. Our study addresses this gap by analyzing 144 adult patellae (44 males, 100 females) from the Late Roman necropolis of Karlığın Tepesi in Malatya, Türkiye. By applying logistic regression and receiver operating characteristic (ROC)-based modeling, this research aims to develop robust, population-specific discriminant values for sex estimation and to evaluate the diagnostic potential of the patella in cases where more traditional indicators, such as the pelvis or skull, are unavailable.

Therefore, this study aims to investigate the sexual dimorphism of the patella, evaluate its morphological differences between males and females, and determine the reliability and applicability of these differences in sex estimation within a bioarchaeological context.

MATERIALS and METHODS

This study has been approved by the Ordu University Non-Entrepreneurial Scientific Research Ethics Committee (approval no: 2025/181, date: 16.05.2025). The dry bones were obtained from the rescue excavations carried out by the Malatya Archaeological Museum in the Karlığın Tepesi Necropolis, in the Battalgazi district of the Malatya province, in 2020. According to the archaeological findings, there are underground rock grave chambers and boat-type graves carved into the rock in the necropolis dated to the 3rd to the 6th centuries *Anno Domini* (AD). During these excavations, 259 individuals were identified in 17 graves[13]. Our study was carried out on 146 adult dry patella bones of known sex (70 males, 76 females). The sex of the individuals was estimated based on morphologically dimorphic skeletal regions, particularly the pelvis and the cranium. In this process, the standard criteria described by Brothwell[14], Bass[15], İşcan and Steyn[16] and Olivier[17] were applied. Key pelvic traits, such as the morphology of the pubic symphysis, greater sciatic notch, and sacrum, as well as cranial features including glabellar prominence, mastoid process, mental eminence, and nuchal crest, were evaluated in accordance with established anthropological protocols. For each individual, multiple skeletal regions were assessed to ensure consistency and reliability in sex estimation[18-20]. Only those adult individuals whose sex could be confidently determined using these criteria were included in the patella analysis. Since our study was performed on dry bones, patient consent was not required. Patellae with any fracture deformity and paediatric patellae were not included in the study.

In our study, patellar length (PL), patellar width (PW), and patellar thickness (PT) were measured. Three morphological locations related to the patella were measured. Digital callipers with an accuracy of 0.01 millimetre (mm) were used for anthropometric measurements (Figure 1). Intraobserver reliability was assessed using technical error of measurement (TEM), relative TEM (rTEM), and the reliability coefficient (R), following Ulijaszek and Kerr[21] and applied in similar anthropometric research. To evaluate intraobserver reliability, a subset of patellae (n=30 for each sex) was re-measured by the same observer at a two-week interval. TEM, rTEM, and the R were calculated for length, width, and thickness of the patella. Among male individuals,

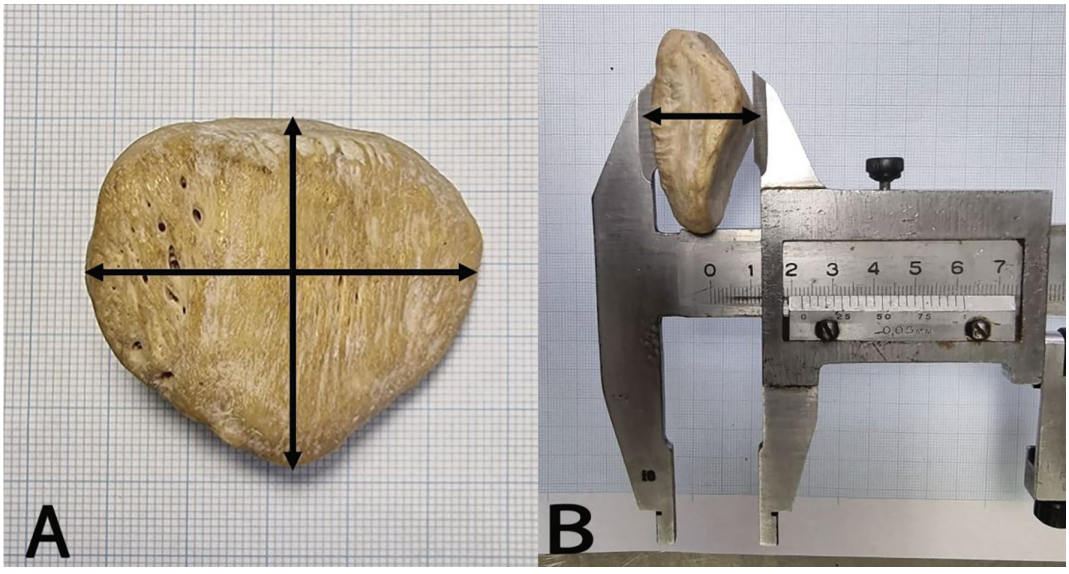

Figure 1. Morphometric landmarks and measurement directions of the patella: (A) length and width; (B) thickness.

TEM values ranged from 0.030 to 0.034 mm, rTEM values were below 0.24%, and the R were 1.000 for all measurements. Similarly, for female individuals, TEM values were consistently low, rTEM values ranged from 0.087% to 0.235%, and all R values were also 1.000. These results indicate excellent intraobserver precision and confirm the consistency of the anthropometric measurements used in this study.

Statistical Analysis

The fit of data to the normal distribution was assessed by histograms, q-q plots, and Shapiro-Wilk tests. The homogeneity of variance was examined by Levene's test. In binary comparisons, the independent two-sample t-test and Mann-Whitney U test were used for quantitative variables. To evaluate the predictive power of patellar dimensions on sex estimation, logistic regression analysis was performed using a stepwise (forward and backward) selection method to identify the most significant variables contributing to the model. This allowed for the construction of the most parsimonious model with optimal classification accuracy. ROC analysis was also conducted to determine cut-off values for sex estimation, and corresponding sensitivity and specificity values were calculated. Effect sizes (Cohen's d) were calculated for key comparisons to assess the magnitude of observed differences. Confidence intervals (95%) were provided for area under the receiver operating characteristic (AUROC) values and regression coefficients. No missing data were present in the analyzed dataset; all measurements were complete and suitable for statistical analysis. All statistical analyses were performed using R software version 3.2.2[1]. A p-value of <0.05 was considered statistically significant.

RESULTS

Descriptive statistics for PL, PW, and PT by sex are presented in Table 1. Males exhibited higher mean values across all dimensions. The mean PL was 39.9 mm [standard deviation (SD)=3.83] in males and 33.0 mm (SD=4.15) in females. PW averaged 41.7 mm (SD=3.75) in males and 36.2 mm (SD=4.53) in females. For patella thickness, the mean was 18.4 mm (SD=3.39) in males and 15.4 mm (SD=4.29) in females. All variables showed statistically significant differences between sexes: PL (p=0.001), PW (p<0.001), and PT (p=0.003).

Logistic regression analysis was conducted to assess the utility of patellar dimensions in sex estimation. In Step 1, using PL alone, the model achieved an AUROC of 0.906, yielding a sensitivity of 82.85% and specificity of 94.73% (p<0.001). In Step 2, the model included both PL and thickness, resulting in a higher AUROC of 0.920, sensitivity of 87.14%, and specificity of 86.84% (p<0.001), as shown in Table 2. Although PW was initially considered in the regression model, it was not statistically significant (p=0.266) and therefore excluded from the final equation. The multivariate model retained PL (p<0.001) and thickness (p<0.001) as the only significant predictors of sex.

According to the classification accuracy rates (Table 3), Step 1 achieved an overall classification accuracy of 84.9%

using PL, with 84.2% accuracy for females and 85.7% for males. Step 2, which incorporated both length and thickness, yielded an improved overall accuracy of 86.3%, with 85.5% for females and 87.1% for males. These results indicate that patellar morphometric measurements can provide high diagnostic accuracy for sex estimation in archaeological samples.

DISCUSSION

The patella (kneecap bone) is an important bone that plays a crucial role in knee joint function and stability[22]. The morphometry of the patella has been the subject of numerous studies. Significant differences in morphometric comparisons between the sexes have emerged[23,24].

In addition to its diagnostic value, the patella's compact morphology and central location within the extensor mechanism, make it less susceptible to postmortem fragmentation compared to long bones or cranial elements. This preservation advantage is particularly beneficial in archaeological contexts where taphonomic damage often limits the availability of complete skeletal elements. According to Tomaszewska et al. [4] the patella was among the most frequently preserved skeletal components in medieval burials in Poland, supporting its utility in sex estimation when more sexually dimorphic bones are missing or damaged. Similarly, in our sample, patellae were well-preserved, allowing for consistent measurements and reliable statistical modeling, which strengthens the forensic applicability of our findings. Only intact, fully measurable patellae were included in the analysis, and fragmented or heavily weathered bones were excluded. Moreover, all measurements were conducted shortly after excavation, minimizing the likelihood of postmortem alterations. Therefore, taphonomic processes are unlikely to have significantly impacted our results.

Moreover, the integration of patellar morphometry into sex estimation protocols holds promise in multidisciplinary contexts. In forensic casework, combining patellar data with available postcranial elements can improve identification rates in commingled or fragmentary remains. Recent advances in artificial intelligence and image-based modeling have further expanded the potential of morphometric data[25]. Future studies may consider employing three-dimensional imaging or machine learning algorithms to refine the discriminatory thresholds derived from patellar metrics. Additionally, comparing our findings with modern populations could help trace morphometric changes over time, which may reveal the influence of

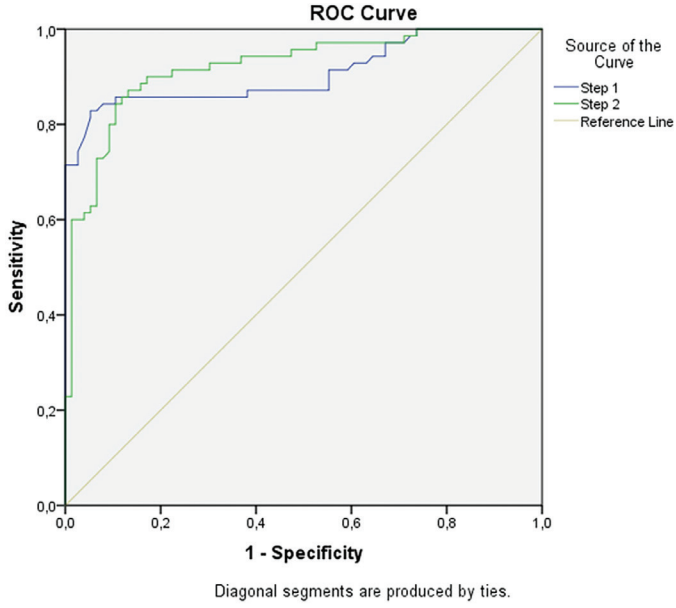

Figure 2. ROC curves showing the classification performance of Step 1 and Step 2 logistic regression models for sex estimation based on patellar measurements.

ROC: Receiver operating characteristic

Table 1. The measurements of patella.

	Sex	N	Mean	95% Confidence interval	SD	Cohen's d	Min	Max
PL	M	70	39.9	39-40.8	3.83	1.71	30	46.9
	F	76	33	32.1-34	4.15		22.7	38.8
PW	M	70	41.7	40.8-42.6	3.75	1.32	32.5	48
	F	76	36.2	35.1-37.2	4.53		26	45
PT	M	70	18.4	17.6-19.2	3.39	0.78	10.6	23
	F	76	15.4	14.4-16.4	4.29		6.7	21.9

Mean ±, minimum and maximum values (mm)

SD: Standard deviation, PL: Patella length, PW: Patella width, PK: Patella thickness, Min: Minimum, Max: Maximum

Table 2. ROC analysis results including AUC, cut-off probabilities, sensitivity, specificity, and significance for each model.

	AUROC (%)	Sensitivity (%)	Specificity (%)	p
Step 1	0.906	82.85	94.73	0.000
Step 2	0.920	87.14	86.84	0.000

ROC: Receiver operating characteristic, AUC: Area under the curve, AUROC: Area under the receiver operating characteristic

Table 3. Sex classification accuracy rates for each logistic regression step

	Female	Male	Overall percentage
Step 1. Patella length	84.2%	85.7%	84.9%
Step 2. Patella length patella thickness	85.5%	87.1%	86.3%

evolutionary, nutritional, or lifestyle-related factors on skeletal dimorphism[26].

In our study, all three patellar dimensions, length, width, and thickness showed statistically significant differences between males and females. The mean PL was measured as 39.9 mm in males and 33.0 mm in females, while the width was 41.7 mm in males and 36.2 mm in females. Similarly, patella thickness was 18.2 mm in males and 15.4 mm in females. Furthermore, the stepwise logistic regression model yielded an AUROC of 0.906 in Step 1 and 0.920 in Step 2, demonstrating excellent overall classification performance. These results indicate that the patella, especially when its dimensions are combined in a multivariate model, serves as a reliable skeletal element for sex estimation in bioarchaeological and forensic contexts. Although there was a slight imbalance in sex distribution (70 males vs. 76 females), this did not appear to affect the classification accuracy. However, the absence of age-at-death estimations and age stratification within the adult sample limits our ability to evaluate potential age-related effects on patellar morphology. Age estimation was not performed from the patella in this study, as the element is not a reliable indicator of chronological age. Our primary aim was to assess sexual dimorphism, and sex estimation was derived from pelvic and cranial morphology.

Although PW showed significant differences between sexes ($p<0.001$), it was not statistically significant as an independent predictor in the logistic regression analysis ($p=0.266$) and was thus excluded from the final model. In addition to statistical significance, effect size values (Cohen's d) indicated that PL and width differences between sexes were of very large magnitude ($d=1.71$ and $d=1.32$, respectively), while thickness differences were of a moderate-to-large magnitude ($d=0.78$). These values highlight the substantial practical relevance of patellar morphometry for sex estimation, particularly for length and width, which show strong discriminatory power beyond mere statistical significance. This indicates that multivariate analyses, through interactions among measurements, can yield different outcomes from univariate analyses. A possible reason for the diminished significance of PW in the regression model might be its high correlation with PL and thickness. Therefore, it is crucial to evaluate the independent contribution of each variable when developing multivariate models. Although intraobserver reliability was excellent, interobserver reliability was not assessed in this study. Nonetheless, standardized measurement protocols and consistent technique by a single trained observer ensured high precision. Future studies involving multiple observers could further assess reproducibility under broader research conditions.

The morphometry of the patella has been the subject of numerous studies, and significant differences in morphometric comparisons between the sexes have emerged. Studies generally show that male patellae are longer and wider than female patellae[27]. These values are broadly consistent with those reported by Kedia and Kadian[28], who found a mean PL of 42.21 mm in men and 36.07 mm in women, and a thickness of 19.3 mm in men and 17.7 mm in women. Koyuncu et al.[29] 2011 reported that the PL and thickness in men were greater than those in women, but there was no statistically significant difference, and anatomical variations may not always be associated with clinical implications. In our study, there was a significant difference in the mean length, width, and thickness of the patella between genders.

Sex-related morphometric differences in the patella have implications not only for forensic anthropology but also for understanding anatomical variation in historical populations. Previous studies have reported varying levels of accuracy for sex estimation using patellar measurements. For instance, Indra et al.[6] reported accuracy levels ranging from 62.8% to 83.8%, while Maio et al.[30] achieved up to 96% accuracy in a Portuguese sample. These values demonstrate the diagnostic potential of the patella, although differences in population, methodology, and sample size can affect comparability. Our study supports these findings, with an AUROC value of 0.920

in the multivariate model, indicating high diagnostic performance in a well-preserved archaeological sample from Late Roman Anatolia.

In forensic anthropology, diagnostic metrics such as accuracy, sensitivity, and specificity have direct implications for casework outcomes. False positive or false negative classifications in sex estimation can significantly affect the identification process in medico-legal investigations. In a bioarchaeological context, such misclassifications may alter demographic reconstructions and interpretations of past populations. Therefore, alongside reporting high diagnostic performance, it is essential to consider the potential consequences of classification errors in both forensic and archaeological applications.

Study Limitations

The sample consisted of 146 patellae from individuals excavated in a specific archaeological context in Malatya, Türkiye, dating to the 3rd-6th centuries AD. This limited geographical and temporal scope may reduce the generalizability of the results to other populations or modern individuals. Morphometric differences influenced by genetic, environmental, or cultural factors could affect the applicability of the established cut-off values. While the overall sample size was sufficient, a slight imbalance in sex distribution (70 males and 76 females) and the absence of age-group stratification, may have restricted the assessment of age-related variation in patellar morphology. External validity testing was not performed on an independent sample, and future studies should explore the reproducibility of the model across different populations. That the model was tested only on the present archaeological sample, represents a limitation regarding its applicability to different populations. Future studies should test the model's performance on independent datasets obtained from different geographical and chronological contexts, which will be critical for verifying its generalizability.

Although interobserver reliability was not assessed, intraobserver consistency was excellent, and measurements were conducted by a single trained observer using standardized protocols. As such, we consider the potential for measurement bias to be minimal. In addition, only well-preserved, intact patellae were included, and all bones were analyzed shortly after excavation, reducing the likelihood of postmortem alteration. Nevertheless, a systematic documentation of taphonomic changes could further strengthen data integrity in future research.

As this study focused exclusively on bone morphology, it does not consider the influence of surrounding soft tissues that might affect bone shape during life. This limits the applicability of the findings in living populations or in clinical contexts.

CONCLUSION

This study demonstrated that patellar dimensions, particularly length and thickness, are effective in sex estimation with high diagnostic accuracy when combined in a logistic regression model. Although patella width showed strong discriminatory power in univariate analysis, it was not statistically significant in multivariate modeling and was thus excluded. The model achieved an AUROC of 0.920 with high sensitivity and specificity, highlighting its utility in forensic anthropology and bioarchaeological investigations. These findings contribute valuable data from an ancient Anatolian sample, supporting the development of region-specific standards for osteometric sex estimation. However, limitations such as sample size imbalance and the lack of soft tissue evaluation should be considered in interpreting the results and designing future research.

Acknowledgement

Permission has been obtained from the Turkish Ministry of Culture and Tourism for the use of bone samples in this study.

Ethics

Ethics Committee Approval: This study has been approved by the Ordu University Non-Entrepreneurial Scientific Research Ethics Committee (approval no: 2025/181, date: 16.05.2025).

Footnotes

Author Contributions

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

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Original Article

Evaluation of Deaths in Malatya Due to the Earthquake Centered in Kahramanmaras on 6 February 2023

6 Şubat 2023'te Kahramanmaraş Merkezli Depremlerde Malatya'da Meydana Gelen Ölüm Olgularının Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to retrospectively examine the cases of individuals who lost their lives as a result of the Kahramanmaraş earthquakes on February 6, 2023, and were brought to the study area designated by the Malatya Forensic Medicine Group Presidency.

Methods: A total of 916 cases were referred for identification to the center designated as the study area by the Malatya Forensic Medicine Group Presidency following the earthquakes on February 6, 2023, and were examined retrospectively. The obtained data were coded and entered into IBM SPSS Version 22. Descriptive statistics and frequency tables were generated, and the data were analyzed.

Results: Among the 916 cases included in the study, 477 (52.1%) were male and 439 (47.9%) were female; 23.2% were under the age of 18, and 13.8% were over the age of 65. The majority (87.9%) were citizens of the Republic of Türkiye. Most injuries were localized to the head and neck region (37.5%). Body integrity was preserved in 88.3% of cases, and signs of decomposition were observed in 77.1%. Fast Technology for the Analysis of Nucleic Acids blood samples was obtained in 39.5% of cases. All bodies were identified through a combination of primary and secondary identification methods and subsequently delivered to their relatives.

Conclusions: In countries facing major disaster risks, forensic medicine units must always be prepared for such events. Postmortem examination and victim identification are multidisciplinary processes that require the coordinated efforts of experts from various fields.

Keywords: Earthquake, postmortem examination, identification

ÖZ

Amaç: Bu çalışmada 06.02.2023 tarihinde Kahramanmaraş depremleri sonucu hayatını kaybeden ve Malatya Adli Tıp Grup Başkanlığı tarafından belirlenen çalışma alanına getirilen olguların retrospektif olarak incelenmesi amaçlanmıştır.

Yöntemler: Çalışmamızda 6 Şubat 2023 tarihinde meydana gelen depremler sonucu Malatya Adli Tıp Grup Başkanlığı tarafından çalışma alanı olarak belirlenen merkeze kimlik tespiti amacıyla getirilen 916 olgu retrospektif olarak incelenmiştir. Elde edilen veriler kodlanarak IBM SPSS Version 22 programına girilmiştir. Verilerin tanımlayıcı istatistikleri ve frekans tabloları oluşturularak veriler analiz edilmiştir.

Bulgular: Çalışmamıza dahil edilen 916 olgunun 477'si (%52,1) erkek, 439'u (%47,9) kadın, %23,2'si 18 yaş altı, %13,8'i 65 yaş üstü, %87,9'u Türkiye Cumhuriyeti vatandaşı, olguların çoğunluğu baş-boyun bölgesinden yaralanmış (%37,5), %88,3'ü vücut bütünlüğünü korumuş, %77,1'inde ayrışma belirtileri var, %39,5'inden nükleik asitlerin analizi için Hızlı Teknoloji kartları-kan örnekleri alınmış ve tüm cesetler birincil ve ikincil kimliklendirme yöntemlerinin bir kombinasyonu kullanılarak eşleştirilerek yakınlarına teslim edilmiştir.

Sonuçlar: Büyük afet riskleriyle karşı karşıya olan ülkemizde, adli tıp birimleri her zaman afetlere hazır olmalıdır. Ceset muayenesi ve kimliklendirme, farklı alanlardaki birçok uzmanın koordineli çalışmasını gerektiren multidisipliner süreçlerdir.

Anahtar kelimeler: Deprem, ölü muayenesi, kimliklendirme

INTRODUCTION

Disaster is defined as a natural, technological, or human-induced event and its consequences which cause physical, economic, and social losses for the entire society or specific segments of it. Such events disrupt or halt normal life and human activities and exceed the capacity of the affected community to cope[1].

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Disaster management is a comprehensive management approach that requires the mobilization of all institutions, organizations, and societal resources toward a common purpose. It involves planning and implementing activities in the phases of mitigation, preparedness, response, and recovery, to prevent disasters and reduce their impact[2]. This approach encompasses technical, administrative, and legal measures to be undertaken before, during, and after a disaster. It ensures effective implementation during disasters and facilitates improvements to the system based on lessons learned from past events[3].

Following disasters that result in mass casualties, states have a duty to assess the scale of the disaster and ensure that the deceased are returned to their families. Historical experiences have demonstrated the necessity of establishing standardized procedures for victim identification[4].

One of the greatest challenges in disasters is the identification of the deceased. Identification refers to the process of determining an individual's identity by assessing characteristics. This process carries legal, sociological, humanitarian, and religious significance. Given these dimensions and the complexity of disaster settings, identification must be standardized, scientifically grounded, evidence-based, literature-supported, reliable, and feasible under field conditions[5].

A proper identification process requires the collection and correlation of antemortem and postmortem data. Antemortem data include an individual's physical characteristics, clothing, jewelry, personal belongings, and medical or dental records. Postmortem data are derived from external and internal examinations, autopsies, and laboratory analyses. Frequently used identification methods include external and internal examination findings, dental records, fingerprint data, DNA profiles, and visual recognition[6].

The Disaster Victim Identification Protocol, developed by Interpol, provides international guidelines for the identification of disaster victims. Within this framework, disasters are classified as open, closed, or hybrid based on the nature of antemortem and postmortem data available[7]. In Türkiye, the Türkiye Disaster Response Plan outlines the roles and responsibilities of coordination units and working groups in disaster response. It sets forth the fundamental principles of planning and implementation before, during, and after disaster events. Within this scope, the duties of the disaster identification and burial working group include identifying the deceased, recording and tracking bodies, forwarding death reports to the relevant authorities, and designating temporary morgue and mass grave sites to prevent decomposition[8].

On February 6, 2023, two major earthquakes struck southeastern Türkiye, affecting 11 provinces and resulting in 53,537 deaths[9]. In this study, we aimed to examine the precautions for identification processes to be taken before disasters, as well as the procedures required during and after disasters. For this purpose, we evaluated data from the Kahramanmaraş-centered earthquake of February 6, 2023, in Malatya City, including age groups, sex, nationality, timing of post-earthquake examinations, trauma localization, body integrity, late signs of death, and biological samples collected for forensic analysis.

MATERIALS and METHODS

Ethics Committee Approval:

Ethics committee approval for this study was obtained from the Inonu University Scientific Research and Publication Ethics Committee (Health Sciences Non-Interventional Clinical Research Ethics Committee) (decision no: 2023/4929, date: 26.12.2023).

This retrospective study included 916 cases that died in the Kahramanmaraş-centered earthquakes on February 6, 2023, and were referred to the designated working area of the Malatya Forensic Medicine Institute for postmortem examination, identification, and burial authorization. Based on the approval letter of the Forensic Medicine Institute (dated 20.09.2023, No: 2023/893), data were obtained from the Malatya Forensic Medicine Group Presidency Morgue Specialization Department. Sources included photographs of the deceased, postmortem examination forms, burial permits, and DNA reports of cases not identified through secondary methods.

Since the study was conducted on autopsied cadavers, informed consent was not required. All procedures were performed in accordance with ethical standards and the principles of the Declaration of Helsinki.

When evaluating trauma localization, each injury in cases with multiple affected regions was assessed separately. For genetic analysis, if more than one sample had been collected from the same case, each sample was evaluated individually Age classification was based on the Turkish Statistical Institute: 1-18 years (children), 18-65 years (adults), and ≥65 years (elderly)[10-12]. Nationality was determined by the presence of a Turkish Republic identity number, foreign identity number, or passport number, as recorded in death examination forms and burial permits.

Cases with insufficient data due to incomplete forms, missing information, or non-standard photographs were excluded from the study. Photographs taken during postmortem examinations were used to evaluate injury localization. As limitations, some injuries not directly contributing to death may not have been detected, and possible errors in assessing body integrity and putrefaction status were acknowledged.

Statistical Analysis:

All data were coded and entered into IBM SPSS Statistics Version 22. Descriptive statistics and frequency tables were generated. Inferential statistics were not used.

RESULTS

Of the 916 earthquake-related fatalities, referred to the study area designated by the Malatya Forensic Medicine Group Presidency, following the Kahramanmaraş-centered earthquakes on February 6, 2023, 477 (52.1%) were male and 439 (47.9%) were female (Figure 1).

Age distribution analysis revealed that 23.2% (n=213) of the cases were children (<18 years), 62.8% (n=576) were adults (18-65 years), and 13.8% (n=127) were elderly (>65 years) (Table 1). The majority of victims (87.9%, n=805) were citizens of the Republic of Türkiye, while 12.1% (n=111) were foreign nationals (Table 2).

The distribution of postmortem examinations by day is presented in Figure 2. On the day of the earthquake (06.02.2023), examinations were conducted on 152 cases.

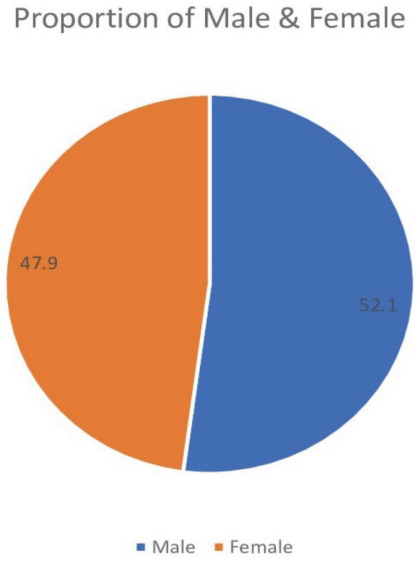

Figure 1. Sex of people who died in the earthquake

A further 82 cases were examined on the second day (07.02.2023), 171 cases on the third day, and 200 cases on the fourth day. No documentation regarding the examination date was available for 11 cases (Figure 2). Among Turkish citizens, 139 examinations were performed on the first day, 77 on the second day, 149 on the third day, and 188 on the fourth day, with the numbers fluctuating thereafter. Among foreign nationals, 13 were examined on the first day; 5 on the second; 22 on the third; and 12 on the fourth day (Table 3).

Examination of photographs, death reports, and burial permits demonstrated that the head and neck region was the most frequently affected site of injury (n=731). Thoracic and abdominal injuries were the next most common. No evidence of trauma was detected in 30 cases, and injury localization could not be determined in 34 cases due to insufficient data (Table 4).

Table 1. Age groups of the cases.

	n	%
<18 Years	213	23.25
18-65 Years	576	62.88
>65 Years	127	13.86
Total	916	100.0

Table 2. Nationality of the cases.

	n	%
Citizen of the Republic of Turkiye	805	87.9
Foreign National	111	12,1
Total	916	100.0

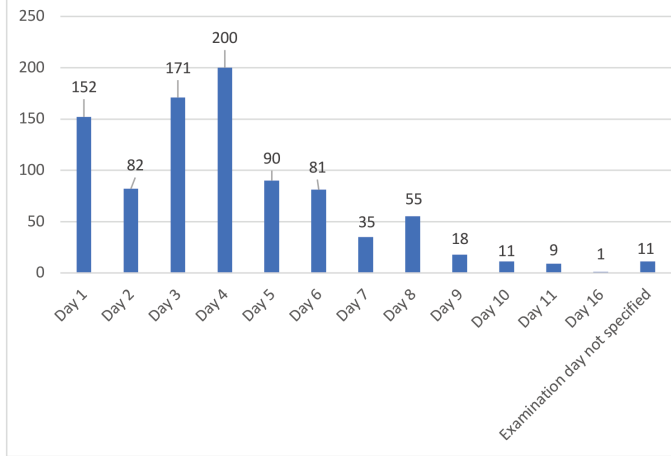

Figure 2. Examination Day/Number of Examinations

Table 3. Days on which postmortem examinations were conducted according to the nationality of the cases.				
		Citizen of the Republic of Turkiye	**Foreign national**	**Total**
Date of examination*	1. Day	139	13	152
	2. Day	77	5	82
	3. Day	149	22	171
	4. Day	188	12	200
	5. Day	67	23	90
	6. Day	74	7	81
	7. Day	27	8	35
	8. Day	46	9	55
	9. Day	12	6	18
	10. Day	9	2	11
	11. Day	9	0	9
	16. Day	1	0	1
	Eaxmination date not specified	7	4	11
Total		805	111	916
*The period from 06.02.2023, when the earthquake occurred, to 21.02.2023 was taken as basis				

Table 4. Injury areas of the cases.		
	n	**%**
Head-neck	731	37.5%
Thorax	532	27.3%
Abdomen	291	14.9%
Lower extremity	159	8.1%
Upper extremity	139	7.1%
Vertebra	35	1.8%
Nontraumatic	30	1.5%
Undetermined with available data	34	1.7%

Regarding body integrity, 88.3% (n=809) of the cases had preserved integrity, while 9.5% (n=87) demonstrated compromised integrity (Table 5). Signs of putrefaction were observed in 77.1% (n=706) of cases, while 20.5% (n=188) showed no evidence of putrefaction at the time of examination (Table 6).

Forensic sampling revealed that biological materials collected for potential genetic analysis included Fast Technology for the Analysis of Nucleic Acids (FTA) blood cards, oral swabs, hair roots, teeth, amputated tissue, and skin. The most frequently collected material was FTA blood samples (39%), followed by hair (35.5%), teeth (13.4%), and oral swabs (6.6%). More than one type of DNA sample was collected in several cases. In 42 cases, DNA sampling was documented, but the sample type was not specified, preventing further evaluation (Table 7).

Table 5. Body integrity of the cases.		
	n	**%**
Preserved	809	88.3
Impaired	87	9.5
Undetermined with available data	20	2.2
Total	916	100,0

Table 6. Putrefaction findings of the cases.		
	n	**%**
Exist	706	77.1
Absent	188	20.5
Undetermined with available data	22	2.4
Total	916	100,0

In addition, fingerprint samples were obtained from all cases. While FTA blood cards were the most commonly used sampling method during the initial days following the earthquake, their use decreased over time, with hair and dental sampling becoming more prevalent in subsequent days (Table 8).

DISCUSSION

In this study, 52.1% of the earthquake-related fatalities examined at the Malatya Forensic Medicine Institute Branch Directorate following the Kahramanmaraş-centered earthquakes of February 6, 2023, were male, and 47.9% were female. These findings are consistent

with the 2022 Gender Statistics data published by the Turkish Statistical Institute[13].

The death examination and identification processes, which began on the day of the first earthquake (February 6, 2023), showed a relative decrease on the second day, but increased again on the third and fourth days. The first earthquake occurred at 04:17 and the second major earthquake at 13:24 on the same day[14]. We consider that the slowdown of the second day, followed by an acceleration on subsequent days, was due to the disruptive impact of the second destructive earthquake and the subsequent disorganization and reorganization of operational processes.

Regarding injury distribution, the most commonly affected region was the head and neck (37.5%), followed by the thorax (27.3%) and abdomen (14.9%). In contrast, the literature frequently reports extremity and soft-tissue injuries as the most common earthquake-related trauma, while head, thoracic, and abdominal injuries are described less frequently[15-17]. We suggest that this discrepancy may be due to the relatively lower incidence, but higher lethality, of head, thoracic, and abdominal injuries[18,19]. It is also important to note that in tectonic disasters such as earthquakes, death may result not only from acute trauma but also from asphyxia, hypovolemia, hypothermia, hypoglycemia, or cardiac pathologies triggered by fear and panic[20,21]. We, therefore, consider that non-traumatic deaths in our series were more likely attributable to pre-existing chronic diseases, acute exacerbations, and environmental conditions rather than direct trauma.

Signs of putrefaction were identified in 77.1% of cases. The earthquakes occurred during severe winter conditions[22]. Although cold weather is generally expected to delay putrefactive changes[23], studies using thermal imaging have demonstrated that damaged or collapsed buildings retain and radiate thermal energy[24]. We believe that the retention of heat within collapsed structures contributed to accelerated decomposition processes despite the cold climate.

Forensic sampling for genetic identification included FTA blood cards, oral swabs, hair roots, teeth, amputated tissue, and skin. The most frequently collected samples were FTA blood cards (39.0%), followed by hair (35.5%), teeth (13.4%), and oral swabs (6.6%). During the initial days of the disaster, FTA blood cards were the preferred

Table 7. Samples taken from cases.

	n	%
FTA	378	39.0%
Oral swab	64	6.6%
Hair	344	35.5%
Tooth	130	13.4%
Bone	3	0.3%
Amputated limb	1	0.1%
Skin	6	0.6%
Unspecified sample	42	4.3%
Total*	957	100.0%

*More than one sampling method was used in some of the cases
FTA: Fast Technology for the Analysis of Nucleic Acids

Table 8. Samples taken and their numbers according to the date of examination.

		1. Day	2. Day	3. Day	4. Day	5. Day	6. Day	7. Day	8. Day	9. Day	10. Day	11. Day	16. Day	Examination date not specified	Total
Sample	**FTA**	111	69	74	69	28	14	0	1	7	0	1	0		378
	Oral swab	3	0	60	1	0	0	0	0	0	0	0	0		64
	Hair	32	7	29	133	66	39	12	10	8	1	1	0		344
	Tooth	2	0	1	1	2	31	25	43	8	8	7	1		130
	Bone	0	0	0	0	0	0	0	0	0	2	1	0		3
	Amputated limb	0	0	0	0	0	1	0	0	0	0	0	0		1
	Skin	1	3	0	0	2	0	0	0	0	0	0	0		6
	Unspecified sample	4	3	20	1	1	1	0	1	0	0	0	0	11	42
Total*		152	82	170	200	90	81	35	55	18	11	9	1		968

*Cases that cannot be evaluated are cases that were not recorded on the examination form even though DNA sampling was performed
FTA: Fast Technology for the Analysis of Nucleic Acids

sampling method. However, in subsequent days, their use declined, while hair and dental samples were collected more frequently. This shift was likely due to environmental conditions, bodies retrieved later were exposed to freezing temperatures, resulting in coagulated blood and making blood sampling technically challenging.

CONCLUSION

The identification methods employed in disaster settings vary according to the magnitude of the event, geographical and seasonal conditions, and socio-economic factors. The decisions made by forensic physicians in response to these conditions are therefore of critical importance. For instance, when seasonal factors reduce the reliability of a particular sampling method, alternative approaches must be applied.

DNA-based primary identification methods remain indispensable, particularly in cases where the body integrity is severely compromised and visual recognition is impossible. Although primary methods-especially DNA analysis- are considered the gold standard, in large-scale disasters, the reliance on secondary identification methods becomes increasingly significant. From both a time and cost perspective, the application of secondary methods in mass fatality incidents provides practical advantages by accelerating burial procedures, facilitating disaster management, and ensuring that future legal processes can proceed without interruption.

Ethics

Ethics Committee Approval: Ethics committee approval for this study was obtained from the Inonu University Scientific Research and Publication Ethics Committee (Health Sciences Non-Interventional Clinical Research Ethics Committee) (decision no: 2023/4929, date: 26.12.2023).

Informed Consent: This is a retrospective study.

Footnotes

Author Contributions

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

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Original Article

Anti-Nuclear Antibody Staining Patterns in Juvenile Idiopathic Arthritis: Association of AC-1 Pattern and Elevated Titers with Uveitis

Juvenil İdiopatik Artritte Anti-Nükleer Antikor Boyanma Paternleri: AC-1 Paterni ve Yüksek Titrelerin Üveit ile İlişkisi

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ABSTRACT

Objective: This study aimed to investigate antinuclear antibody (ANA) staining patterns and titers in patients with juvenile idiopathic arthritis (JIA)-associated uveitis, idiopathic uveitis, and JIA without uveitis, in order to identify serologic profiles that may contribute to disease pathogenesis and guide clinical decision-making.

Methods: We analyzed patients with JIA and/or uveitis at our tertiary center with ANA titers ≥1/100. Patients were grouped as JIA-associated uveitis, JIA without uveitis, and idiopathic uveitis. Diagnoses followed International League of Associations for Rheumatology and standardization of uveitis nomenclature criteria. ANA testing was performed by indirect immunofluorescence on *HEp-2* cells, and patterns and titers were evaluated per International Consensus on ANA Patterns guidelines. ANA profiles were compared across patient groups and JIA subtypes.

Results: Ninety-one patients were included: 21 (23%) with idiopathic uveitis, 12 (13.1%) with JIA-associated uveitis, and 58 (63.7%) with JIA without uveitis. The AC-1 pattern was present in all uveitis patients. The most common ANA patterns in JIA were AC-1 (65.7%), AC-4/5 (21.4%), and AC-2 (10%). ANA profiles differed across JIA subtypes ($p<0.001$), with AC-1 dominant in oligoarticular JIA (74.5%) and AC-4/5 in enthesitis-related arthritis (50%).

Conclusions: Our findings show that ANA pattern differences in JIA subtypes may provide significant clues regarding disease pathogenesis and clinical prediction. In particular, the prominence of the AC-1 pattern in JIA-associated uveitis may suggest a potential biomarker for the early identification of uveitis risk, which should be further explored in larger prospective studies

Keywords: Antinuclear antibodies, uveitis, juvenile idiopathic arthritis, staining patterns, titers

ÖZ

Amaç: Bu çalışma, juvenil idiyopatik artrit (JIA) ile ilişkili üveit, idiyopatik üveit ve üveitsiz JIA hastalarında antinükleer antikor (ANA) boyama paternlerini ve titrelerini araştırarak, hastalığın patogenezine katkıda bulunabilecek ve klinik karar verme sürecine rehberlik edebilecek serolojik profilleri belirlemeyi amaçlamıştır.

Yöntemler: Üçüncü basamak merkezimizde ANA titresi ≥1/100 olan JIA ve/veya üveitli hastaları analiz ettik. Hastalar JIA ile ilişkili üveit, üveitsiz JIA ve idiyopatik üveit olarak gruplandırıldı. Tanı, Uluslararası Romatoloji Dernekleri Birliği ve üveit terminolojisinin standartlaştırılması kriterlerine göre konuldu. ANA testi, *HEp-2* hücreleri üzerinde dolaylı immünofloresan ile yapıldı ve paternler ve titreler, ANA Paternleri Uluslararası Konsensüsü kılavuzlarına göre değerlendirildi. ANA profilleri, hasta grupları ve JIA alt tipleri arasında karşılaştırıldı.

Bulgular: Doksan bir hasta çalışmaya dahil edildi: 21 (%23) idiyopatik üveit, 12 (%13,1) JIA ile ilişkili üveit ve 58 (%63,7) üveitsiz JIA tanılı idi. AC-1 paterni tüm üveit hastalarında mevcuttu. JIA'da en sık görülen ANA paternleri AC-1 (%65,7), AC-4/5 (%21,4) ve AC-2 (%10) idi. ANA profilleri JIA alt tiplerine göre farklılık gösterdi ($p<0,001$), oligoartiküler JIA'da AC-1 (%74,5) ve entezit ilişkili artritte AC-4/5 (%50) baskındı.

Sonuçlar: Bulgularımız, JIA alt tiplerindeki ANA patern farklılıklarının, hastalığın patogenezi ve klinik öngörü ile ilgili önemli ipuçları sağlayabileceğini göstermektedir. Özellikle, JIA ile ilişkili üveitte AC-1 paterninin öne çıkması, üveit riskinin erken tanımlanması için potansiyel bir biyomarker olarak düşünülebilir. Ancak, bu sonuçları doğrulamak ve klinik uygulamaya entegre etmek için daha büyük, prospektif çalışmalar gereklidir.

Anahtar kelimeler: Antinükleer antikorlar, üveit, juvenil idiyopatik artrit, boyama desenleri, titreler

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of diseases characterized by chronic arthritis of unknown cause with onset before the age of 16[1,2]. Different clinical subtypes are defined by international classification criteria and antinuclear antibody (ANA) positivity stands out as a determinant serologic marker, especially in oligoarticular JIA[3]. Chronic anterior uveitis is a significant clinical manifestation of JIA, predominantly observed in the oligoarticular subtype, and in those with ANA positivity[4]. If not promptly identified and managed, this condition can result in irreversible vision impairment, thereby constituting a major health concern for individuals with JIA[4-7].

The ANA test is a widely used method in the serologic evaluation of autoimmune diseases[8,9]. This test, which is performed on *HEp-2* cells by indirect immunofluorescence (IIF) method, defines not only autoantibody positivity and titer level, but also staining patterns determined according to the target structures of autoantibodies[10]. These patterns are mainly categorized into nuclear, cytoplasmic, and mitotic groups and provide important clues in clinical evaluation[11]. The diagnostic and prognostic value of ANA patterns has increasingly been investigated because they show characteristic associations with some autoimmune diseases[12-14]. A limited number of studies in the literature have evaluated the discrimination of specific ANA patterns in JIA and JIA-associated uveitis[15,16]. However, the clinical reflection of specific patterns underlying ANA positivity and their relationship with the development of uveitis in JIA patients has not yet been fully elucidated. Accordingly, this study investigates ANA staining patterns and titers in patients with JIA-associated uveitis, idiopathic uveitis, and JIA without uveitis, aiming to delineate distinctive serologic markers that may inform the pathogenesis and guide clinical decision-making.

MATERIALS and METHODS

Patients and Data Collection

Patients admitted to our tertiary care center between June 2022 and June 2025 with a diagnosis of JIA and/or uveitis and a positive ANA test titer of 1/100 or higher were included in the study. Exclusion criteria included those with concomitant systemic autoimmune or autoinflammatory conditions or infectious uveitis, and those without sufficient clinical and laboratory data. Patients were divided into three groups according to their clinical diagnosis: JIA-associated uveitis, JIA without uveitis, and idiopathic uveitis. Patients with JIA were classified according to the International League of Associations for Rheumatology criteria[17]. Patients with uveitis were classified according to the Standardization of Uveitis Nomenclature criteria[18]. All uveitis diagnoses were established by ophthalmologists, and patients were followed jointly by pediatric rheumatology and ophthalmology clinics. In our cohort, all patients with JIA-associated uveitis were diagnosed with uveitis either at the time of JIA diagnosis or within the first year of follow-up. Demographic variables such as age at diagnosis and sex, laboratory parameters including acute phase reactants and ANA titers and patterns, clinical features such as JIA subtype and presence or type of uveitis, and as well as treatment data, were collected from patient records. In patients with JIA, disease activity at diagnosis was assessed using the juvenile arthritis disease activity score-71 (JADAS-71).

This study was conducted in compliance with the Helsinki Declaration as well as local laws and regulations. Informed consent was obtained from the patients and their legal caregivers. The ethics committee of Istanbul Medeniyet University Göztepe Training and Research Hospital tertiary center approved our study (approval number: 2023/0919, dated: 12023).

Evaluation of ANA Staining Patterns and Titers

ANA testing was performed using IIF assay on *HEp-2* cell substrates with the EUROPLUS ANA mosaic (EUROIMMUN, Lübeck, Germany). *HEp-2* IIF staining patterns and titer levels were evaluated according to the International Consensus on ANA Patterns guidelines[19]. ANA staining patterns and titer levels were compared between the three patient groups. In addition, patients with JIA were divided into subgroups according to JIA subtypes and ANA staining patterns, and titer levels were compared among these subgroups.

Statistical Analysis

We performed the statistical analysis using Statistical Package for the Social Sciences (SPSS) for Windows, version 26.0 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used to assess the distribution of continuous variables. While variables with a normal distribution were presented as mean ± standard deviation, distributed abnormally were presented as median (minimum-maximum). The chi-square test or Fisher's exact test was used to compare the categorical variables, which were expressed as numbers (percentages). For continuous variables, One-Way ANOVA or the Kruskal-Wallis test was used for comparisons across more than two groups, and the Mann-Whitney U test was applied for comparisons between two groups. Bonferroni

adjustment was applied in post-hoc analyses for multiple comparisons. In addition, analysis of JADAS-71 scores across ANA staining pattern groups in JIA patients was performed using One-Way ANOVA (or Kruskal-Wallis test where appropriate). A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of All Patients

A total of 91 patients were included in the study, of whom 21 (23%) had idiopathic uveitis, 12 (13.1%) had JIA-associated uveitis and 58 (63.7%) had JIA without uveitis. Gender distribution was 34% (n=31) male and 66% (n=60) female. The median age at diagnosis was 12.30 (3.50-16.50) years in the idiopathic uveitis group; 5.72 (1.50-13.50) years in the JIA-associated uveitis group; and 9.87 (1.00-16.42) years in the JIA without uveitis group. All patients with JIA-associated uveitis had chronic anterior uveitis and were classified in the oligoarticular JIA subtype. Among patients with JIA without uveitis, oligoarticular JIA was also the most common subtype (67.2%, n=39), followed by enthesitis-related arthritis (20.6%, n=12), while other subtypes were observed less frequently (Figure 1). In the idiopathic uveitis group, anterior uveitis was present in the majority of cases (85.7%, n=18), and panuveitis was seen in the remainder (14.2%, n=3). The AC-1 staining pattern was detected in all patients with JIA-associated uveitis. Among those without uveitis, AC-1 remained the most common pattern (58.6%), followed by AC-4/5 (22.4%) and AC-2 (12%). Patients with idiopathic uveitis most frequently exhibited AC-1 (52.3%), followed by AC-4/5 (28.5%) and AC-2 (19%) staining patterns (Figure 1). When the patients with idiopathic uveitis, JIA-associated uveitis, and JIA without uveitis were compared based on the presence/absence of the AC-1 pattern, the difference was statistically significant (Fisher's exact test, p=0.007).

Comparison of Clinical and Laboratory Characteristics Among All Patient Groups

The median age at diagnosis was significantly younger in the JIA-associated uveitis group compared to the other groups (p=0.006). In addition, C-reactive protein and erythrocyte sedimentation rate were significantly lower in the idiopathic uveitis group than in the other groups (p=0.012, p=0.010). There was no statistically significant difference in ANA staining patterns and titer levels between the groups (Table 1). Additionally, JADAS-71 scores demonstrated no significant variation across both ANA staining pattern groups (p=0.522) and ANA titer groups (p=0.247).

Comparative Analysis of ANA Profiles in JIA Subtypes

The three most prevalent patterns identified were AC-1 (44, 62.8%), AC-4/5 (13, 18.5%), and AC-2 (7, 10%). The AC-1 pattern was notably more common in oligoarticular JIA, while the AC-4/5 pattern was significantly more prevalent in enthesitis-related arthritis (ERA) compared to the other groups (p<0.001) (Table 2). An ANA titer of 1/320-1/1000 was significantly more frequent in oligoarticular JIA than in other groups, whereas a titer of 1/100 was more commonly observed in patients with ERA than in other groups (p<0.001) (Table 3).

DISCUSSION

This study compared ANA profiles among patients with JIA-associated uveitis, idiopathic uveitis, and JIA without uveitis, revealing serological differences between the groups. The AC-1 staining pattern was significantly more common in oligoarticular JIA patients, while the AC-4/5 pattern predominated in patients with ERA. In contrast, all patients with JIA-associated uveitis exhibited the AC-1 pattern. When the patients with idiopathic uveitis, JIA-associated uveitis, and JIA without uveitis, were compared according to the presence or absence of the pattern, a statistically significant difference was observed, indicating a marked enrichment of AC-1 in JIA-associated uveitis. ANA patterns in the other patient groups were more heterogeneous. This finding suggests that the AC-1 pattern may serve as a potential serological marker for uveitis risk in JIA; however, given the limited sample size, this observation should be interpreted with caution and requires validation in larger, prospective studies.

Uveitis is the most common extraarticular involvement in patients with JIA and may lead to permanent vision loss or blindness if left untreated[4]. It is critical to understand the risk of uveitis in the follow-up of JIA patients, due to its significant contribution to prognosis. ANA positivity in oligoarticular JIA is known to be a risk factor for the development of uveitis[5]. In our study, all JIA-associated uveitis patients were observed to exhibit AC-1 positivity, which is a nuclear homogenous staining pattern. This pattern is usually associated with autoantibodies against nuclear antigens such as double-stranded DNA, nucleosomes, histones, and chromatin[17]. Indeed, one study has shown that JIA-associated uveitis is associated with antihistone antibodies[20]. A proteomic analysis to identify specific novel autoantigens for JIA-associated uveitis revealed that 17 autoantigens were associated with uveitis, and five of them were against nuclear components[21]. Moreover, studies in adult rheumatoid arthritis (RA) have reported more frequent pulmonary

involvement in patients with nuclear homogeneous ANA staining, suggesting that this pattern may also be relevant to systemic organ involvement in other contexts.

The heterogeneous ANA staining patterns, we found in the idiopathic uveitis group suggest that this disease group differs immunologically from JIA-associated uveitis and that it may have more diverse autoantibody profiles, perhaps playing a secondary or limited role in the pathogenesis of the disease. Indeed, a previous study has shown that ANA positivity in pediatric patients with noninfectious uveitis is not an independent risk factor for the need for biologic therapy or the development of uveitis-related complications[22]. This finding suggests that the clinical predictive power of ANA in idiopathic uveitis may not be as strong as in JIA-associated uveitis. Additionally, the significantly lower acute phase reactant levels observed in the idiopathic uveitis group compared to the JIA groups suggest a more limited inflammatory process and further support the distinct immunological nature of these disease entities.

Previous studies have reported that the most frequently observed staining patterns in JIA are nuclear homogeneous and fine granular patterns[10,15,16].

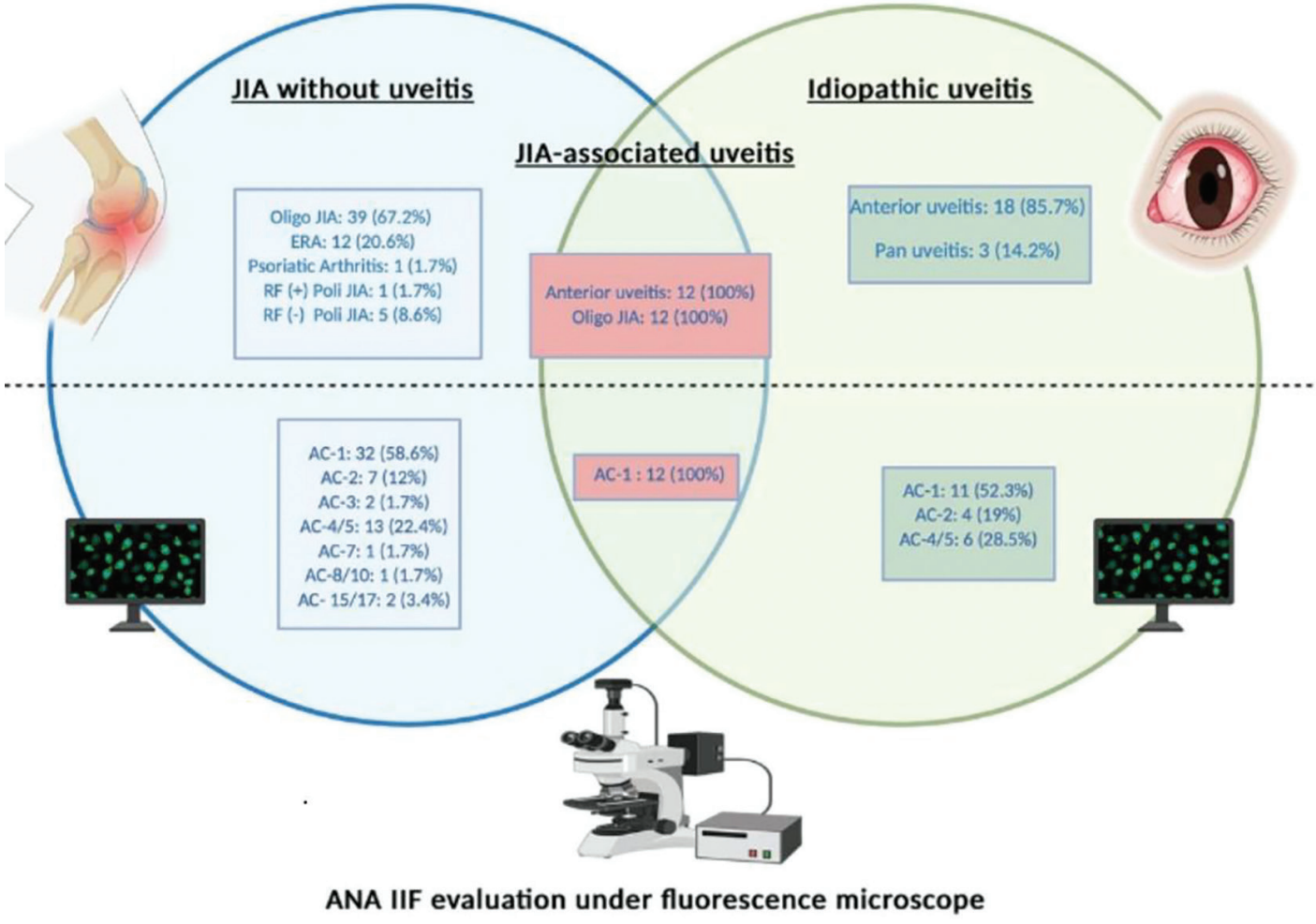

Figure 1. Distribution of antinuclear antibody (ANA) staining patterns and clinical subtypes among patients with juvenile idiopathic arthritis (JIA) with and without uveitis, and idiopathic uveitis. The Venn diagram illustrates the overlap and distinct features of JIA-associated uveitis. All patients in the JIA-associated uveitis group had anterior uveitis and were classified as oligoarticular JIA. ANA patterns were evaluated by indirect immunofluorescence using *HEp-2* cells and classified according to the International Consensus on ANA Patterns nomenclature. AC-1 pattern was detected in 100% of JIA-associated uveitis cases. In contrast, AC-4/5 pattern was more frequent in idiopathic uveitis and JIA without uveitis, particularly in patients with enthesitis-related arthritis (ERA).

Table 1. Comparison of clinical and laboratory characteristics among all patient groups.				
	Idiopathic uveitis group (n=21)	**JIA-associated uveitis group (n=12)**	**JIA without uveitis group (n=58)**	**p**
Male gender (n,%)	9 (42.9%)	1 (8.3%)	21 (36.2%)	0.112
Age at diagnosis (year) (median/min-max)	12.30 (3.50-16.50)	5.72 (1.50-13.50)	9.87 (1.00-16.42)	**0.006**
ANA titers				0.168
1/100	7 (33.3%)	0 (0%)	16 (27.6%)	
1/100-1/320	8 (38.1%)	6 (50%)	21 (36.2%)	
1/320-1/1000	4 (19.0%)	4 (33.3%)	14 (24.1%)	
1/1000-1/3200	2 (9.5%)	2 (16.7%)	7 (12.1%)	
>1/3200	0 (0%)	0 (0%)	0 (0%)	
ANA patterns				0.298
AC-1	11 (52.4%)	12 (100%)	34 (58.6%)	
AC-2	4 (19.0%)	0 (0%)	7 (12.1%)	
AC-3	0 (0%)	0 (0%)	1 (1.7%)	
AC-4/5	7 (33.3%)	2 (16.7%)	13 (22.4%)	
AC-7	0 (0%)	0 (0%)	1 (1.7%)	
AC-8/10	0 (0%)	0 (0%)	1 (1.7%)	
AC-15/17	0 (0%)	0 (0%)	2 (3.4%)	
Acute phase reactants at diagnosis				
CRP (mg/L) [median (min-max)]	2 (0.2-27)	4 (0.1-21)	8.06 (0.13-146)	**0.012**
ESR (mm/h) [median (min-max)]	10 (2-53)	22 (3-42)	29.5 (2-120)	**0.010**

AC: Anti-cell, AC-1: Nuclear homogeneous pattern, AC-2: Nuclear dense fine speckled pattern, AC-3: nuclear centromere pattern, AC-4/5: Nuclear fine speckled/coarse speckled patterns, AC-7: Nuclear few dots pattern, AC-8/10: Homogeneous nucleolar/punctate nucleolar; AC-15/17: Cytoplasmic fibrillar linear/cytoplasmic fibrillar segmental, ANA: Antinuclear antibody, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate,JIA: Juvenile idiopathic arthritis

Table 2. Comparison of anti-nuclear staining patterns among Juvenile idiopathic arthritis subgroups.						
	JIA subgroups (n,%)					
	Oligo JIA (51, 56%)	**ERA (12, 13.2%)**	**Psoriatic arthritis (1, 1.1%)**	**RF (+) poliJIA (1, 1.1%)**	**RF (-) poliJIA (5, 5.5%)**	***p**
ANA staining pattern						**<0.001**
AC-1	38 (74.5%)	2 (16.7%)	1 (100%)	0 (0%)	3 (60%)	
AC-2	4 (7.8%)	2 (16.7%)	0 (0%)	1 (100%)	0 (0%)	
AC-3	2 (3.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
AC-4/5	6 (11.7%)	6 (50%)	0 (0%)	0 (0%)	1 (20%)	
AC-7	1 (1.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
AC-8/10	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)	
AC-15/17	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	1 (20%)	

*The AC-1 pattern was significantly more frequent in oligoarticular JIA than in other subtypes. The AC-4/5 pattern was significantly more frequent in ERA than in other subtypes.

AC: Anti-cell, AC-1: Nuclear homogeneous pattern, AC-2: Nuclear dense fine speckled pattern, AC-3: Nuclear centromere pattern, AC-4/5: Nuclear fine speckled/coarse speckled patterns, AC-7: Nuclear few dots pattern, AC-8/10: Homogeneous nucleolar/punctate nucleolar, AC-15/17: Cytoplasmic fibrillar linear/cytoplasmic fibrillar segmental, ANA: Antinuclear antibody, ERA: Enthesitis-related arthritis, RF: Rheumatoid factor, JIA: Juvenile idiopathic arthritis

Table 3. Comparison of anti-nuclear titers among juvenile idiopathic arthritis subgroups.						
	JIA subgroups (n,%)					
	Oligo JIA (51, 56%)	ERA (12, 13.2%)	Psoriatic arthritis (1, 1.1%)	RF (+) poliJIA (1, 1.1%)	RF (-) poliJIA (5, 5.5%)	*p
ANA titers						**<0.001**
1/100	5 (9.8%)	10 (83.3%)	0 (0%)	1 (100%)	0 (0%)	
1/100-1/320	22 (43.1%)	2 (16.6%)	0 (0%)	0 (0%)	3 (60%)	
1/320-1/1000	17 (33.3%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	
1000-1/3200	7 (13.7%)	0 (0%)	1 (100%)	0 (0%)	1 (20%)	

*ANA titer of "1/320-1/1000" was significantly more frequent in oligoarticular JIA than in other groups. A titer of 1/100 was more frequently observed in patients with ERA than in other groups.

ANA: Antinuclear antibody, ERA: Enthesitis-related arthritis, RF: Rheumatoid factor, JIA: Juvenile idiopathic arthritis

Consistently, the most common staining patterns in our JIA patients were AC-1 and AC-4/5. While AC-1 was the most common staining pattern in our patients with oligoarticular JIA, AC-4/5 was the most common one in those with ERA. This finding aligns with the results reported by Sener et al.[15], who also identified similar AC-1 positivity rates in oligoarticular JIA, although they noted AC-4/5 as the most common pattern. However, the AC-4/5 pattern was also the most frequent in their ERA patients, consistent with our observations. As is well established, human leukocyte antigens-B27 rather than ANA positivity is typically expected in ERA, in line with its immunogenetic background. In addition, ANA titer distribution showed a significant difference between JIA subtypes in our study, with medium and high titers predominating in oligoarticular JIA and low titers in the ERA subtype. The nuclear homogeneous staining pattern has been reported to be a risk factor for RA[23]. In contrast, we observed a high variability of titers and staining patterns of ANA among our JIA patients. While adult RA exhibits a homogeneous disease profile with symmetric polyarthritis, JIA is a clinically and immunologically heterogeneous disease with several subtypes[24,25]. Thus, we considered that our results may reflect the immunologic heterogeneity of JIA; and different autoantibody profiles may be at the forefront according to subtypes.

Study Limitations

The main limitations of our study are its retrospective design, the limited number of patients, and the fact that ANA testing was only evaluated at the time of diagnosis and not repeated during periods of disease remission or exacerbation. This may have resulted in missing serologic changes related to disease activity. However, the main strength of the study is it included not only patients with JIA but also patients with idiopathic uveitis, allowing comparison of serologic patterns and thus contributing to a better understanding of the pathogenesis of JIA and idiopathic uveitis. In addition, this is the second study in a limited number of studies, in which ANA staining patterns were evaluated in detail with AC codes, which is an important contribution to the literature.

CONCLUSION

Our findings show that ANA pattern differences in JIA subtypes may provide significant clues regarding disease pathogenesis and clinical prediction. In particular, the prominence of the AC-1 pattern in JIA-associated uveitis may suggest a potential biomarker for early identification of uveitis risk; however, this possibility requires confirmation in larger prospective studies before integration into clinical practice.

Ethics

Ethics Committee Approval: The ethics committee of Istanbul Medeniyet University Göztepe Training and Research Hospital tertiary center approved our study (approval number: 2023/0919, date: 13.12.2023).

Informed Consent: Informed consents were obtained from the patients and their legal caregivers.

Footnotes

Author Contributions

Concept: L.K., F.E., Ö.T., F.K., E.K., Z.A., E.N.D., H.K.D., M.Ö.B., F.H., K.Ö., Design: L.K., F.E., K.Ç., Ö.T., F.K., E.K., E.N.D., M.Ö.B., F.H., K.Ö., Data Collection and/or Processing: L.K., K.Ç., Ö.T., E.K., Z.A., H.K.D., F.H., K.Ö., Analysis and/or Interpretation: L.K., K.Ç., Ö.T., F.K., Z.A., H.K.D., M.Ö.B., Literature Search: L.K., Ö.T., F.K., Z.A., H.K.D., H.K.D., F.H., K.Ö., Writing: L.K., F.H., K.Ö.

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Review

Exploring the Link between DNA Hypermethylation and HPV in Salivary Gland Tumors

Tükürük Bezi Tümörlerinde DNA Hipermetilasyonu ve HPV Arasındaki Bağlantının Araştırılması

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ABSTRACT

Salivary gland tumors (SGTs) pose considerable diagnostic and treatment challenges due to their heterogeneous nature, diverse histogenesis, and unpredictable clinical outcomes. Benign tumors exhibit a known recurrence rate, whereas malignant tumors are associated with a poor prognosis and a low recovery rate. Nonetheless, despite the growing body of research, there is insufficient evidence to establish a link between SGTs, human papilloma virus (HPV) infection, and the hypermethylation of tumor suppressor genes. The aim of this study is to elucidate the relationship between DNA hypermethylation and HPV in SGTs, elucidate the role of DNA hypermethylation in HPV-associated SGTs, thereby offering insights into novel diagnostic, and prognostic markers. As epigenetic alterations significantly contribute to the development of carcinogenesis, addressing these epigenetic alterations may help in early treatment plans and early detection of SGTs.

Keywords: DNA hypermethylation, epigenetics, salivary gland tumors, human papilloma virus, tumor suppressor genes

ÖZ

Tükürük bezi tümörleri (SGT'ler), heterojen yapıları, çeşitli histogenezleri ve öngörülemez klinik sonuçları nedeniyle tanı ve tedavi açısından önemli zorluklar oluşturmaktadır. Benign tümörlerin belirli bir oranda nüksettiği bilinmekteyken, malign tümörler kötü prognoz ve düşük iyileşme oranıyla ilişkilidir. Bununla birlikte, artan araştırmalara rağmen, SGT'ler, insan papilloma virüsü (HPV) enfeksiyonu ve tümör baskılayıcı genlerin (TSG'ler) hipermetilasyonu arasındaki bağlantıyı kanıtlayacak yeterli kanıt bulunmamaktadır. Bu çalışmanın amacı, SGT'lerde DNA hipermetilasyonu ve HPV arasındaki ilişkiyi aydınlatmak, HPV ile ilişkili SGT'lerde DNA hipermetilasyonunun rolünü açıklamak ve böylece yeni tanı ve prognostik belirteçler hakkında bilgi sağlamaktır. Epigenetik değişiklikler karsinogenez gelişimine önemli ölçüde katkıda bulunduğundan, bu epigenetik değişikliklerin ele alınması, SGT'lerin erken tedavi planları ve erken teşhisinde yardımcı olabilir.

Anahtar kelimeler: DNA hipermetilasyonu, epigenetik, tükürük bezi tümörleri, insan papilloma virüsü, tümör baskılayıcı genler

INTRODUCTION

Salivary gland tumors (SGTs) are rare and heterogeneous in nature, have diverse histogenesis, and show unpredictable clinical outcomes. World Health Organization (WHO) Global Cancer Observatory reported 53,083 new cases diagnosed worldwide in 2020, with an incidence rate of 0.56 and a death rate of 0.23 per 100,000 individual-years[1]. According to GLOBOCAN 2022, SGTs are rated 28th in incidence, comprising 0.56% of all cancer types. The mortality rate ranks 27th, with 23,942 fatalities, constituting 0.2% of all cancer locations[2]. Due to their morphological heterogeneity, encompassing over twenty recognized histologic subtypes, the diagnosis of such conditions is challenging and necessitates a combination of extensive molecular profiles and histological techniques. While a significant number may originate from minor salivary glands, the predominant region of SGTs is the major salivary glands (including parotid, submandibular, and sublingual glands). Most of these tumors are malignant and might be identified throughout the mucosal lining of the oral cavity[3].

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Importance of Epigenetic Modifications in SGTs

Epigenetics refers to heritable changes in gene expression that occur independently of alterations to the DNA sequence[4]. Genetic and epigenetic mechanisms are primarily responsible for the modifications in gene expression. Epigenetic modifications occur at the transcriptional level, whereas genetic alterations typically involve changes in the quantity or structure of specific genes. Methylation of CpG islands in the promoter region is a common epigenetic mechanism for regulating gene expression. CpG methylation has been shown in SGTs, oral squamous cell carcinoma, and esophageal squamous cell carcinoma as it influences the development of the tumor. This CpG methylation leads to inhibition of tumor suppressor genes (TSGs) such as p16, *MGMT*, *DAPK*, and *RASSF1A* which may cause DNA hypermethylation[5]. DNA methylation modifications influence the structure of DNA without modifying the genetic code, DNA hypermethylation is much resilient than other epigenetic alterations, rendering it a dependable biomarker for diagnostic applications, this variance is essential due to its significant implications[6]. Besides participating in multiple physiological processes, such as cell differentiation and embryogenesis, epigenetic modifications can also play a role in pathological conditions, including cancers (particularly SGTs) and disrupted cellular states[7]. Epigenetic alterations significantly influence the pathophysiology of SGTs and can potentially be utilized for targeted therapies. It could also serve in prognostic and diagnostic applications[8]. It is evident that, given the vast epigenetic reprogramming occurring during gametogenesis and embryogenesis sensitivity of these reactions, perturbations in this reprogramming could have substantial clinical consequences. Epigenetic modifications transpire throughout folliculogenesis and embryogenesis; thus, any disruption in the natural process throughout these critical phases may result in epigenetic alterations[9]. Several other mechanisms also contribute to the etiology of SGTs, including chromosomal translocations[10], deletions[11], point mutations[12], gene amplifying mutations[13], and epigenetic modifications[14].

Epidemiology and Categorization of SGT

SGTs comprise a varied collection of benign and malignant neoplasms distinguished by diverse, occasionally overlapping, unpredictable, and histological characteristics. Numerous molecular alterations are also observed[15]. In the year 2020, the male-to-female ratio was approximately 1.3:1 for these tumors. They exhibit a stable incidence rate according to epidemiological data, but are also more frequently identified in older individuals. A comprehensive method is typically employed in medical care, encompassing systemic therapies, radiation, and surgery tailored to the patient and the tumor's characteristics[16]. The classification of SGTs entails differentiating among major and minor salivary glands and determining whether tumors are benign or malignant. Clinicians often refer to "80/20 rule" for SGTs, which includes 80% of benign[17], with 70% originating from the parotid gland, around 10% in the submandibular gland, and less than 1% in the sublingual gland. Pleomorphic adenomas (PA) represent the predominant subtype of SGTs, accounting for 65% of cases and approximately 55% of large gland neoplasms and 50% of small gland neoplasms[18]. Warthin's tumors are the second most prevalent benign salivary tumors, mostly impacting the parotid gland, where they constitute 25-32% of its occurrences. They predominantly occur in Caucasian males with a smoking history, with ten to fifteen percent exhibiting bilateral presentation[19].

The histopathological variation among SGTs, which includes many subtypes categorized by WHO[17], poses obstacles in identifying and treating these tumors[20]. SGTs are typically classified as high-grade or low-grade depending on their conduct, which may vary from non-aggressive to aggressive. Approximately 70% of malignant SGTs arise in the parotid gland, 8% in the submandibular gland, and 22% in minor salivary glands[21]. Mucoepidermoid carcinoma (MEC) is among the most common malignant tumors, accounting up to 30% of parotid malignancies[22]. Adenoid cystic carcinoma (AdCC) is the next most prevalent malignant neoplasm of the salivary glands, generally manifesting during the fourth and fifth decades of life[23]. Acinic cell carcinoma, a low-grade neoplasm, constitutes 10-15% of parotid tumors and typically exhibits a protracted progression; nonetheless, it may demonstrate increased aggressiveness in the parotid gland compared to minor salivary glands. Salivary ductal carcinoma is an uncommon yet extremely aggressive neoplasm, primarily impacting older males, and linked to a dismal prognosis[24], evidenced by a 5-year mortality rate of 43%[25]. Carcinoma ex pleomorphic adenoma is an uncommon malignancy originating from persistent or repeated PA, with its prevalence increasing from 1.5% after five years to 10% after fifteen years[5].

The Implications of Hypermethylation in SGTs and Tumor Suppressor Gene Silencing

Hypermethylation leads to the silencing of a substantial number of TSGs which are essential in controlling the hallmarks of carcinogenesis in human cancers[26]. DNA methylation has been associated with several SGTs, both malignant and benign[27]. The epigenetic control of TSGs is being extensively investigated as a possible contributor

to the neoplastic development of salivary glands. The knockdown of TSGs is crucial in neoplasm progression because of their functions in controlling the cell cycle, apoptotic induction, DNA repair, and metastasis inhibition[28]. Despite extensive research on TSGs, their precise actions are still not fully elucidated[29]. However, aberrant methylation of promoter genes is recognized as one of the prevalent causes of TSGs silencing, acting as a catalyst during the initial phases of carcinogenesis[30]. This atypical methylation may facilitate the rendering of particular methylation patterns and is useful not just as a diagnosis and prognosis indicator but also as prospective treatment target[31]. Recent molecular investigations have enhanced comprehension of the significance of DNA methylation in the pathogenesis of SGTs as a mechanism for gene silencing[14]. The methylation status could indicate a new contributing element; however, additional research is required to elucidate its relationship with TSGs[32].

Research Connecting DNA Hypermethylation in SGTs Prognosis

DNA hypermethylation may function as a significant biomarker for monitoring tumor growth, forecasting malignancy, and offering prognostic information[33]. This molecular alteration results in the transcriptional suppression of promoter regions in TSGs[34]. Studies have repeatedly demonstrated that hypermethylation in TSGs such as *MGMT* and *DAPK* occurs in a substantial proportion of SGTs, suggesting its possible involvement in tumorigenesis and prognosis. It exhibits diverse expression patterns that occasionally are associated with tumor aggressiveness and clinical outcomes[35]. Hypermethylation of *MGMT* and *DAPK* frequencies range from twenty percent to forty percent in particular types of tumors such as AdCC and MEC. This methylation could be associated with higher-grade tumor behavior and a worse prognosis[36]. Healthy salivary samples often lack a methylation index; however, benign tumors such as PA and Warthin tumors exhibit methylation in genes like *RASSF1*, *MGMT*, and *DAPK*[37]. Table 1 summarizes malignant and benign SGTs and their prevalence highlighting associated genetic alterations and methylation patterns, reflecting emerging insights into their molecular etiologies.

Overview of Human Papilloma Virus (HPV) Infection in Tumorigenesis and Its Epigenetic Role in SGT: Contemporary Evidence

Human papilloma virus (HPV) is a small, circular, double-stranded DNA virus that primarily targets cutaneous and mucosal epithelial tissues. Although the complete mechanism of HPV infection remains incompletely understood, the widely accepted model suggests that the virus gains entry through micro-abrasions in the epithelial basement membrane. Following endocytosis, the viral genome is transported to the nucleus, where replication and transcription are

Table 1. Highly prevalent genetic alterations in benign and malignant salivary gland tumor.

No	Tumor type	Genetic alterations/ oncogenes	Methylation pattern	Prevalence	Key role in tumorigenesis	Ref
1	MEC	*MECT1-MAML2, EGFR, HER2*	Hypermethylation and genetic translocations	40-90%	Methylation status linked to tumor progression	(38)
2	AdCC	*EN1, FOXE1, TBX4, PITX1*	Hypermethylation of TSGs	~80%	Methylation status linked to tumorigenesis	(39)
3	SC	*ETV6-NTRK3* fusion	Showed unmethylated results	>90%	No molecular alteration	(40)
4	AciCC	*NR4A3* fusion, *RASSF1*	Hypermethylation	86%	Methylation status linked to tumorigenesis	(37)
5	MSA	*MEF2C-SS18* fusion	NA	>90%	NA	(40)
6	MAC	*AKT1 E17K* mutations	NA	100%	Mutant	(40)
7	Ca ex-Pa	*PLAG1* fusions	NA	73%	Amplification	(40)
8	PA	*PLAG1* fusions, *MGMT, DAPK*	Hypermethylation pattern	>50%	Methylation status partially linked to tumorigenesis	(37)
9	BCA	*CTNNB1*	NA	37-80%	Mutant	(40)
10	SP	*BRAF V600E*	NA	50%-100%	Mutant	(40)

MEC: Mucoepidermoid carcinoma, AdCC: Adeniod cyctic carcinoma, SC: Secretory carcinoma, AciCC: Acinic cell carcinoma, MSA: Microsecretory adenocarcinoma, MAC: Mucinous adenocarcinoma, Ca ex-Pa: Carcinoma ex pleomorphic adenoma, PA: Pleomorphic adenoma, BCA: Basal cell adenoma, SP: Sialadenoma papilliferum, NA: Not applicable

initiated using host cellular machinery. The HPV genome consists of eight open reading frames: six early genes (*E1, E2, E4, E5, E6*, and *E7*) and two late genes (*L1* and *L2*). The early genes are chiefly involved in viral replication, transcription, and modulation of host cellular pathways, while the late genes encode structural proteins essential for capsid formation. Among the early genes, *E6* and *E7* have been extensively studied for their oncogenic potential, particularly their ability to disrupt cell cycle regulation and inhibit tumor suppressor proteins such as p53 and Rb. The late genes, *L1* and *L2*, encapsulate the replicated viral genome into icosahedral virions, which are eventually released through the natural process of epithelial desquamation[41]. HPV infections are often asymptomatic and self-resolving; they can manifest as anogenital warts, respiratory papillomatosis, and precancerous or cancerous lesions in the cervical, penile, vulvar, vaginal, anal, and oropharyngeal regions[42]. In 2019, HPV accounted for around 620.000 new cases of cancer in females and 70.000 in males globally. Those with genital HPV infections exhibit a heightened risk of oral or anal HPV infections[43]. In females with cervical cancer, the virus has potential to be transmitted to their partner's oral cavity during sexual intercourse and eventually to one's own oral cavity[44].

The underlying causes of SGTs remain largely undefined, though various risk factors such as radiation, silica dust rubber chemicals, and viruses including Epstein-Barr virus and HPV are believed to contribute to their development and progression. Most tumors, however, do not have a discernible etiology[45]. Increasing evidence suggests that epigenetic alterations, categorized into five core mechanisms -DNA methylation, histone modification, RNA-based methylation, chromatin remodelling, and regulation by non-coding RNAs- play a significant role in the pathogenesis of these tumors. Notably, similar epigenetic alterations have been consistently observed in HPV-associated cancers, supporting the notion that epigenetic reprogramming is a key feature of HPV-driven tumorigenesis[46]. Recent studies have begun to explore the potential association between HPV infection and the development of SGTs. One case-control study conducted within a Taiwanese cohort investigated this relationship by comparing 416 patients with SGTs to 2,080 matched controls. The findings revealed a higher prevalence of prior HPV infection among cancer cases (10.8%) than in controls (6.2%). After adjusting for sociodemographic and health-related variables, individuals with a history of HPV infection demonstrated an 88% increased likelihood of developing SGTs (odds ratio: 1.885)[47]. These results suggest a possible correlation between HPV infection and increased risk of SGTs. If substantiated, this relationship may carry significant diagnostic and therapeutic implications, with potential relevance to patient prognosis and survival outcomes. However, the causal role of HPV, particularly in driving hypermethylation patterns observed in SGTs, remains unclear. This uncertainty largely stems from the rarity and heterogeneity of these tumors, which continue to challenge large-scale molecular investigations[48].

Possibility of Integrated SGTs Biomarker Strategies

Various diagnostic methodologies are being investigated to enhance the diagnosis and treatment planning for SGTs. The examination of morphology parameters, which include inflammatory biomarkers, such as the systemic immune-inflammation index, the systemic inflammation response index, the platelet-to-lymphocyte ratio, and the neutrophil-to-lymphocyte ratio, and radiomic features obtained from imaging techniques such as nuclear magnetic resonance image sequences and histopathology slides, is conducted as part of prospective diagnostic targets[49]. Furthermore, Artificial intelligence (AI)-enhanced salivary biomarker models are being studied for the detection of oral cancer[50]. Nonetheless, the use of AI in the analysis of SGTs remains in development, primarily due to the limited number of cases available at certain diagnostic centers[51].

CONCLUSION

We conclude that studies establishing correlations, conducting comprehensive clinical trials, and examining the predictive and therapeutic relevance of hypermethylation and SGTs are unclear and vary across study populations. This could be due to multiple reasons, such as geographical locations, environmental factors, and delayed diagnosis, where two-thirds of cases are identified at stage 3 or 4 and the lack of molecular indicators to forecast tumor behavior and facilitate patient stratification for customized treatment[52]. Timely identification and ongoing monitoring substantially influence survival rates and clinical outcomes. Whereas the correlation between methylation and HPV status is promising, it requires more research via more extensive, longitudinal investigations and advanced studies. Advancements in diagnostic imaging, particularly MRI and targeted radiomic signatures, are facilitating the differentiation between benign and malignant SGTs[16]. Epigenetic modifications may contribute to the progression of SGTs. The detection of prognostic markers such as *RASSF1*, *MGMT*, and *DAPK* may aid in early cancer treatment strategies, potentially resulting in better treatment outcomes for patients. Further studies

are warranted to better understand their role in tumor development and progression in SGTs.

Footnotes

Author Contributions

Surgical and Medical Practices: A.A.M.Z., R.M., N.M.L., Concept: S.S., A.A.M.Z., N.M.L., Design: A.M., N.M.L., Data Collection and/or Processing: A.M., Analysis or Interpretation: A.M., Literature Search: A.M., Writing: A.M.

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Case Report

Pi*M Palermo Mutation in Bronchiectasis due to Alpha-1 Antitrypsin Deficiency: A Rare Genetic Cause

*Alfa-1 Antitripsin Eksikliğine Bağlı Bronşektazide Pi*M Palermo Mutasyonu: Nadir Bir Genetik Sebep*

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ABSTRACT

Bronchiectasis, defined as the permanent dilation of the bronchial wall, is a chronic inflammatory disease with nearly thirty known causes. The most common cause is recurrent and inadequately treated lower respiratory tract infections. Among the rarer causes is alpha-1 antitrypsin (AAT) deficiency, an anti-protease and anti-inflammatory protein deficiency. To date, approximately 500 variants of AAT deficiency have been identified, with the PI*S and PI*Z mutations being the most commonly associated with bronchiectasis. Here, we present a case diagnosed with bronchiectasis secondary to AAT deficiency during an advanced clinical workup, in which the rare Pi*M Palermo mutation was identified. This case is discussed in the context of the existing literature.

Keywords: Alpha-1 antitrypsin, bronchiectasis, M Palermo mutation

ÖZ

Bronş duvarının kalıcı dilatasyonu olarak tanımlanan ve yaklaşık otuza yakın sebebi tanımlanmış kronik enflamatuvar bir hastalık olan bronşektazinin en sık nedeni tekrarlayan, iyi tedavi edilemeyen alt solunum yolu enfeksiyonlarıdır. Bunun dışında görülen nadir sebeplerinden birisi de bir anti-proteaz ve anti-enflamatuvar protein olan alfa-1 antitripsin (AAT) eksikliğidir. AAT eksikliğinin bugün için tanımlanmış yaklaşık 500 varyantı vardır. Bunlar içerisinde PI*S mutasyonu ve PI*Z mutasyonları en sık bronşektazi ile ilişkilendirilmiş varyantlardır. Kliniğimizde bronşektazi ileri tetkiki sırasında bronşektazinin nadir bir nedeni olan AAT eksikliği tanısı alan yine nadir bir mutasyonun (Pi*M Palermo mutasyonu) tespit edilen olgumuz literatür eşliğinde sunulmuştur.

Anahtar kelimeler: Alfa-1 antitripsin, bronşektazi, M Palermo mutasyonu

INTRODUCTION

Alpha-1 antitrypsin (AAT) deficiency is a genetic disorder characterized by low serum levels of AAT due to mutations in the *SERPINA1* gene, which result in impaired production of AAT. AAT is a crucial anti-protease and anti-inflammatory protein known to inhibit the destructive effects of major proteases such as neutrophil elastase. It is a potent serine protease inhibitor (PI) and acts as an acute-phase reactant that protects the lungs from serine proteases, particularly neutrophil elastase. Under normal conditions, PIs neutralize the effects of proteases and help preserve the alveolar architecture. A disruption in the protease/anti-protease balance in the lungs may lead to damaging effects on lung parenchyma. AAT deficiency has been associated with diseases such as bronchiectasis, panacinar emphysema, chronic hepatitis, cirrhosis, and panniculitis[1,2].

The clinical presentation of AAT deficiency most commonly includes emphysema. It remains a subject of debate whether bronchiectasis in these patients is directly associated with AAT deficiency or is secondary to emphysematous changes. Some studies support the theory that bronchiectasis develops secondary to emphysema[3]. It has also been suggested that in AAT deficiency, impaired immune function in the damaged lung through both direct and complex indirect mechanisms may be a critical component in the development of bronchiectasis. Another hypothesis proposes that AAT deficiency may be considered a subcategory of non-cystic fibrosis bronchiectasis, as

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bronchiectasis is observed only in certain subgroups of AAT deficiency[4].

When analyzing the etiology of bronchiectasis, approximately 29 distinct causes can be classified under six major categories (Table 1). Among these, AAT deficiency is considered one of the rarest causes. We present the case of a patient diagnosed with AAT deficiency during the evaluation of widespread bronchiectasis at an advanced age. Genetic analysis revealed the Pi*M Palermo mutation, a rare genetic variant associated with bronchiectasis, which further contributes to the uniqueness of this case.

Table 1. Causes associated with bronchiectasis.

Category	Subgroups
Infections	• **Bacterial:** *B. pertussis, P. aeruginosa, H. influenzae, Mycoplasma, Mycobacterium avium* complex • **Viral:** Measles, HIV, EBV, Influenza, HTLV-1, Herpes simplex virus, Adenovirus types 7 and 21 • **Fungal:** Aspergillosis
Immunodeficiencies	• Primary and secondary immunodeficiency • Complement deficiency • Waldenström macroglobulinemia • Hypogammaglobulinemia • Chronic granulomatous disease
Genetic disorders	• Cystic fibrosis • Alpha-1 antitrypsin deficiency • Williams-Campbell syndrome • Swyer-James syndrome • Mounier-Kuhn syndrome
Clearance defects	• Primary ciliary dyskinesia • Kartagener syndrome • Young syndrome
Systemic diseases	• Rheumatoid arthritis • Sjögren's syndrome • Ulcerative colitis • Crohn's disease • Yellow nail syndrome • Celiac disease
Other causes	• Toxic chemical exposure • Aspiration of gastric contents • Heroin use • Foreign body aspiration • Pulmonary fibrosis • Bronchial tree malformations • Tumor compression

HIV: Human immunodeficiency virus, EBV: Epstein-Barr virus, HTLV-1: Human T-cell lymphotropic virus

CASE REPORT

A 78-year-old female patient presented to the emergency department with complaints of fever, cough, and green-colored sputum. Her medical history included diagnoses of bronchiectasis and hypertension (HT). She had no history of smoking or tuberculosis exposure. The patient was diagnosed with bronchiectasis approximately ten years ago during the evaluation for recurrent lower respiratory tract infections at a healthcare facility. It was also noted that two of the patient's sisters had localized bronchiectasis, suggesting a familial predisposition. On physical examination, early inspiratory crackles were noted in the bilateral mid to lower lung fields. Vital signs were as follows: temperature 39 °C, heart rate 85 bpm, blood pressure 130/70 mmHg, and oxygen saturation 91% on room air, as measured by pulse oximetry. Other systemic examinations revealed no significant findings. Laboratory tests showed elevated acute-phase reactants, with no other remarkable pathological abnormalities (Table 2).

Table 2. Laboratory values of the case.

Parameter	Result	Reference range
WBC (10^3 uL)	**18.2**	4.00-10.00
Neutrophil count (10^3 uL)	**14.55**	2.00-7.00
Neutrophil (%)	**80.2**	40.00-80.00
Lymphocyte count (10^3 uL)	**2.5**	0.80-4.00
Lymphocyte (%)	**13.5**	10.00-50.00
Hemoglobin (g/dL)	10.6	12-16
Hematocrit (%)	33	36.00-47.00
Urea (mg/dL)	37	16.6-48.5
Creatinine (mg/dL)	0.72	0.5-0.9
ALT (U/L)	8	0-33
AST (U/L)	17	0-32
LDH (U/L)	**165**	135-214
CRP (mg/L)	**98.49**	0-5
Procalcitonin (µg/L)	**0.348**	<0.5
Sedimentation (mm/hour)	**47**	0-20
Glucose (mg/dL)	97	74-106
Total bilirubin (mg/dL)	0.4	0-1.2
Uric acid (mg/dL)	6	2.4-5.7
Sodium (mmol/L)	133	135-145
Potassium (mmol/L)	4.8	3.1-5.1
Alkaline phosphatase (U/L)	141	35-104

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, LDH: Lactate dehydrogenase, WBC: White blood cell (Count)

A posteroanterior chest radiograph revealed bilateral diffuse density increases, with cystic lucencies scattered throughout both lungs (Figure 1). Thoracic computed tomography (CT) showed "bilateral tubular cystic bronchiectasis and sequelae of emphysematous changes" (Figure 2).

Further history revealed that two of the patient's sisters had also been diagnosed with bronchiectasis. The AAT levels of both sisters were at the lower limit of the normal range. There was no known diagnosis of cystic fibrosis in the family that could account for the etiology of bronchiectasis, and our patient had no history of infertility. The patient did not recall any severe pulmonary infections during childhood, and no other identifiable causes of bronchiectasis were found. Therefore, serum AAT levels were measured, and the result was 0.29 g/L (reference range: 0.9-2.0 g/L). A medical genetics consultation was requested. Next-generation sequencing of the *SERPINA1* gene revealed a pathogenic variant as shown in Figure 3.

Sputum culture grew *Pseudomonas aeruginosa*, and treatment with piperacillin-tazobactam 4×4.5 g/day was initiated. Acid-fast bacilli testing of the sputum was negative. After clinical improvement, pulmonary function tests were performed; forced vital capacity (FVC) was 1.33 L (92% predicted), forced expiratory volume in 1 second (FEV1) was 2.35 L (68% predicted), and FEV1/FVC ratio was 57%. Diffusing capacity for carbon monoxide could not be measured due to poor patient cooperation.

The patient's overall condition improved, and her symptoms regressed. She was discharged with a diagnosis of AAT deficiency and was referred to the outpatient pulmonology clinic for follow-up. Written informed consent was obtained from the patient.

DISCUSSION

In this report, we present a case of AAT deficiency, which is a rare cause of bronchiectasis that was diagnosed at an advanced age, and which may be overlooked in routine pulmonary clinical practice if not specifically considered.

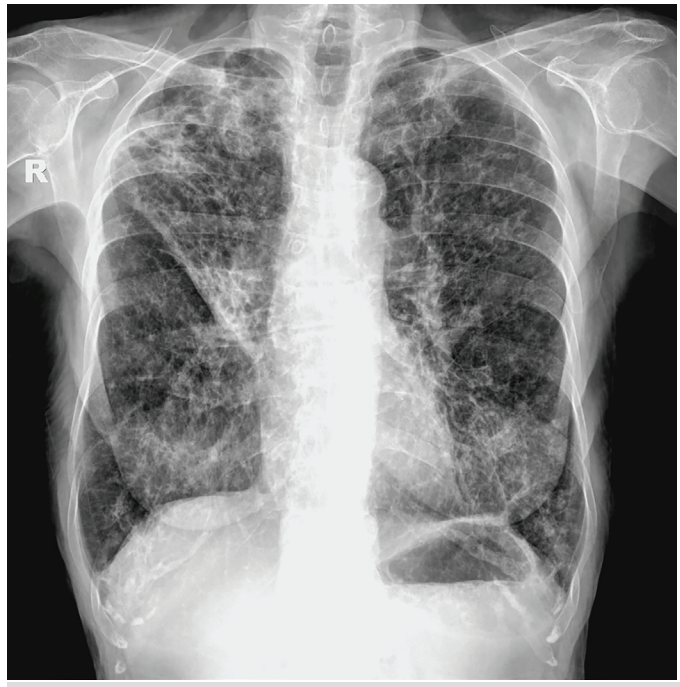

Figure 1. Chest X-ray taken at the time of admission showing increased densities with scattered cystic lucencies throughout all zones of both lungs.

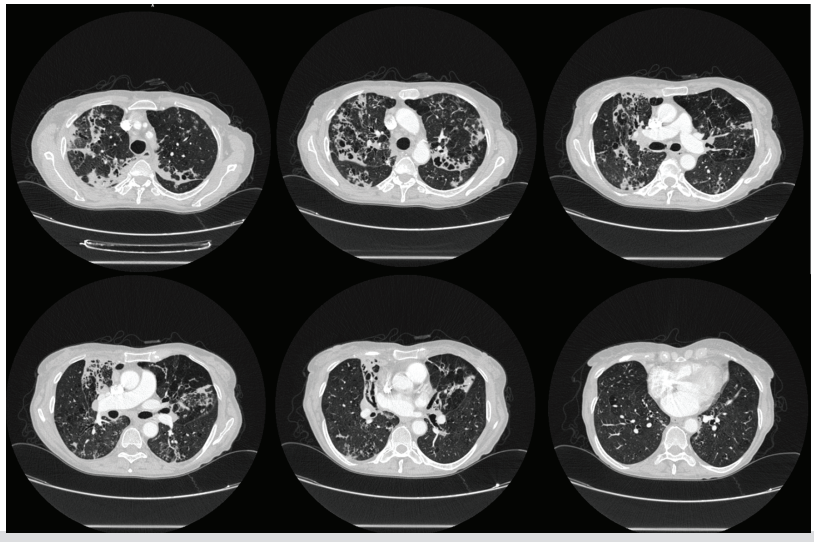

Figure 2. Thoracic computed tomography of the patient revealing bilateral tubular cystic bronchiectasis

Genomic Position	Gene Name / Ref Seq	Effet	Zygosity (Ref/Alt)	Classification
chr14:94849345 GAGA>G rs775982338	SERPINA1 NM_001127701.2 c.227_229del Exon 4/7	inframe_deletion NP_001121173.1 p.Phe76del	HOM 268/1524 VF %85	**Muhtemel Patojenik** PS3,PM4,PP5
Genomic Position	**Gene Name / Ref Seq**	**Effet**	**Zygosity (Ref/Alt)**	**Classification**
chr14:94849348 A>G rs1555369172	SERPINA1 NM_001127701.2 c.227T>C Exon 4/7	missense_variant NP_001121173.1 p.Phe76Ser	HET 159/68 VF %30	VUS PM2,PP3,PP5

Figure 3. Pathogenic variants detected in the *SERPINA1* gene through next-generation sequencing analysis.

AAT deficiency affects both sexes equally and is typically diagnosed in adults during the fifth decade of life (around ages 40-45)[5]. A comprehensive study evaluating the disease burden of AAT deficiency highlighted its significant pulmonary and hepatic morbidity. Beyond chronic obstructive pulmonary disease (COPD), emphysema, and bronchiectasis, patients with AAT deficiency may also present with panniculitis, HT, diabetes mellitus, cardiac disease, and pulmonary HT, underlining the substantial impact of the condition on both patients and healthcare systems[6,7]. Our patient was 78 years old and apart from HT, had no evidence of liver involvement or other comorbidities.

A recent study based on data from the European Alpha-1 Research Collaboration, a deep phenotyping registry supported by the European Respiratory Society, analyzed patients diagnosed with AAT deficiency and bronchiectasis[8]. Among 418 patients stratified by chest CT findings, 38 had only bronchiectasis, 190 had only emphysema, and 113 had both conditions. The average age was 55, the majority were female (53.3%), 41% had never smoked, and the mean FEV1 was 59%. Our patient shares similar characteristics with this cohort.

The two most common AAT deficiency mutations worldwide are PIS and PIZ (accounting for ~95% of cases); however, over 500 variants have been described. In our case, the rare PIM Palermo variant was identified. This mutation was first reported by Faber et al.[9] in 1994 as one of fifteen newly characterized AAT variants. They highlighted three rare variants PIQ0 Saarbruecken, PIQ0 Lisbon, and PIM Palermo as clinically significant due to their extremely low or undetectable serum AAT levels.

In 1997, Jardí et al.[10] detected the PIM Palermo mutation in six members across three generations of the same family; interestingly, none of these individuals had clinical evidence of lung disease. Karadoğan et al.[11], in their study conducted in Türkiye on the clinical implications of *SERPINA1* variants, reported serum AAT levels ranging between 0.2 and 0.9 g/L in individuals with the PIM Palermo mutation. They emphasized that MMalton, MNichinan, and MPalermo mutations were particularly associated with hepatic dysfunction and emphysema, especially in homozygous individuals. However, as their study focused on AAT deficiency and COPD, no specific data regarding the prevalence of bronchiectasis were provided.

Lepiorz et al.[12] reported bronchiectasis in a 51-year-old woman with the PIM Palermo mutation in the absence of COPD. Similarly, Feitosa et al.[13] categorized the PIM Palermo mutation as one of the variants that could lead to severe liver disease and extensive emphysema.

In conclusion, AAT deficiency is a known contributor to the development of bronchiectasis. While PIS and PIZ are the most commonly implicated mutations, studies specifically linking the PI*M Palermo mutation to bronchiectasis remain limited. This case contributes to the literature by highlighting the potential role of this rare mutation in the pathogenesis of bronchiectasis.

Ethics

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

Footnotes

Author Contributions

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

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

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Letter to the Editor

Comment on "the Role of Combined C-reactive Protein and Albumin Indices in Predicting Prolonged Hospital Stay in Acute Pancreatitis: A Prospective Observational Study"

"Akut Pankreatitte Uzun Süreli Hastane Yatışını Tahmin Etmede Kombine C-Reaktif Protein ve Albümin İndekslerinin Rolü: Prospektif Gözlemsel Çalışma"

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Keywords: Acute pancreatitis, hospital stay, CRP to albumin ratio, Glasgow Prognostic score, inflammation biomarkers
Anahtar kelimeler: Akut pankreatit, hastane yatışı, CRP/albümin oranı, Glasgow Prognostik skoru, enflamasyon biyobelirteçleri

Dear Editor,

We read with great interest the study by Algin et al.[1], evaluating the prognostic utility of combined C-reactive protein (CRP) and albumin indices in acute pancreatitis. The authors provide prospective data suggesting that CRP to albumin ratio (CAR), Glasgow Prognostic score (GPS), and modified GPS (mGPS) are independent predictors of prolonged hospitalization. This is a timely and clinically relevant inquiry, especially in emergency settings where early risk stratification tools are needed. However, some methodological considerations warrant further discussion.

First, while the definition of prolonged stay as more than 7 days is consistent with prior work, the median duration in the prolonged group was 19 days. This discrepancy suggests that the chosen cut-off may not have captured the more clinically severe population. A higher threshold might have provided a better correlation with significant complications such as necrosis or multiorgan dysfunction. Additionally, the decision to dichotomize hospital stay could have limited the discriminatory performance of the indices. Modeling length of stay as a continuous outcome may have better captured the incremental prognostic value.

Second, although the area under the curve (AUC) for CAR was the highest among the indices, its value of 0.677 suggests limited predictive power in isolation. The AUCs for GPS and mGPS were similarly modest, and the statistical superiority of CAR over mGPS was not established. These findings imply that, although statistically significant, the clinical performance of these indices may not suffice for standalone use in triage or disposition planning.

Moreover, the study did not report whether albumin or CRP levels were influenced by therapeutic interventions such as fluid resuscitation, nutrition support, or early antibiotics. These factors can alter biomarker levels independently of disease severity and

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potentially confound risk scores[2]. The absence of data on albumin infusion or parenteral nutrition during early hospitalization introduces uncertainty about the stability and timing of these markers.

Another concern is the lack of stratification by etiology of pancreatitis. Disease progression and inflammatory burden often vary between gallstone-induced, alcoholic, or idiopathic pancreatitis, which may influence CRP and albumin kinetics[3]. Without subgroup analysis, it is unclear whether these scores perform consistently across etiological profiles.

Finally, imaging-based severity markers such as Balthazar grade or computed tomography severity index were not included, limiting comparison with established tools. While CRP and albumin indices offer low-cost accessibility, their optimal utility may lie in complementing, not replacing, radiological assessment and clinical scores, like APACHE II[4].

In summary, while this study contributes useful preliminary evidence, the modest diagnostic accuracy, unmeasured confounders, and limited stratification suggest that CRP and albumin indices should be used cautiously and as part of a broader assessment framework. Further multicenter studies integrating biochemical, imaging, and etiological data are needed to define their clinical role.

Footnotes

Author Contributions

Concept: R.M., Design: R.M., Data Collection and/or Processing: R.S., Analysis or Interpretation: R.S., Literature Search: R.M., R.S., Writing: R.M., R.S.

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

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Response to the Letter to the Editor Regarding Our Research Article on "The Role of Combined C-reactive Protein and Albumin Indices in Predicting Prolonged Hospital Stay in Acute Pancreatitis: A Prospective Observational Study"

"Akut Pankreatitte Uzamış Hastanede Kalış Süresini Öngörmede Kombine C-reaktif Protein ve Albümin İndekslerinin Rolü: Prospektif Gözlemsel Bir Çalışma" Başlıklı Araştırma Makalemize Gelen Editöre Mektuba Yanıt

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Keywords: Acute pancreatitis, C-reactive protein/albumin ratio (CAR), prolonged hospital stay
Anahtar kelimeler: Akut pankreatit, C-reaktif protein/albümin oranı (CAR), uzamış hastanede kalış süresi

Dear Editor,

We sincerely thank the authors for their interest in our article, "The Role of Combined C-Reactive Protein and Albumin Indices in Predicting Prolonged Hospital Stay in Acute Pancreatitis: A Prospective Observational Study", and for their constructive comments[1].

Our choice of the >7- day threshold for the definition of prolonged hospital stay was based on previous literature identifying this cut-off as clinically meaningful in predicting complication risk and increased healthcare utilization in acute pancreatitis[2-4]. Importantly, our institution is one of the largest gastroenterology referral centers in the region, and a substantial proportion of complicated cases present directly to our emergency department from outpatient settings. This referral pattern naturally leads to a skewed length-of-stay (LOS) distribution, as illustrated in Figure 1, with a marked clustering of patients requiring prolonged hospitalization. Thus, the greater than seven-day threshold allowed comparability with existing studies, capturing a broader at-risk population-not solely the most severe cases with necrosis or multi-organ failure.

We agree that modeling LOS as a continuous variable could provide additional insights. However, our primary objective was to evaluate the discriminatory performance of inflammation-based indices for a binary outcome,

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which we believe to be more relevant for early triage and disposition decisions in the emergency department.

With respect to the reported area under the curve values, we acknowledge that the predictive performance of C-reactive protein to albumin ratio (CAR), Glasgow Prognostic score (GPS), and modified Glasgow Prognostic score (mGPS) is modest in isolation[5]. Nonetheless, all three indices remained statistically significant independent predictors in multivariate analysis. In line with prior evidence, we emphasize that these indices are best used as adjuncts rather than standalone tools, complementing comprehensive clinical evaluation. Their simplicity, rapid availability, and low cost make them especially valuable in high-volume emergency departments and resource-limited healthcare environments.

Concerning potential confounding from therapeutic interventions, we clarify that CRP and albumin levels were obtained at the time of presentation to the emergency department, before initiation of targeted treatments such as albumin infusion, nutritional support, or antibiotics. This approach minimized the likelihood of treatment-related bias in biomarker measurements.

Although etiology-specific subgroup analysis was not performed due to limited sample sizes within certain categories, we agree that inflammatory responses may differ between gallstone-induced, alcoholic, and idiopathic pancreatitis. This is an important consideration for future multicenter studies with larger and more balanced cohorts.

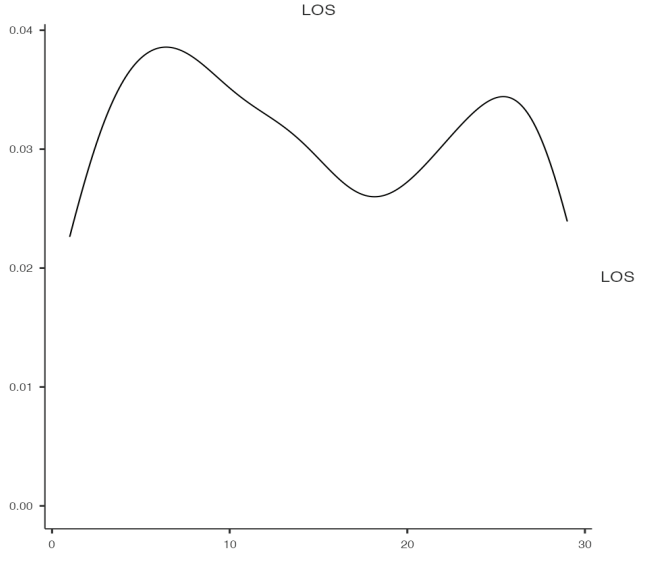

Figure 1. Distribution of length of stay (LOS) among pancreatitis patients

Similarly, advanced imaging-based severity scores such as the Balthazar grade or computed tomography severity index were not included in our study. This was a deliberate decision, as our primary aim was to evaluate the prognostic value of easily accessible biochemical indices-particularly relevant in emergency settings where advanced imaging may be delayed. We agree that integrating biochemical scores with imaging findings and clinical scoring systems such as Acute Physiology and Chronic Health Evaluation II may enhance prognostic accuracy, and this represents a valuable direction for future research.

In conclusion, our findings support CAR, GPS, and mGPS as independent predictors of prolonged hospital stay in patients with acute pancreatitis. These indices, especially in referral centers with a high proportion of complicated cases, may serve as practical tools for early risk stratification. We appreciate the opportunity to clarify these points and agree that further multicenter studies incorporating continuous LOS modeling, etiological stratification, and multimodal prognostic approaches are warranted.

Footnotes

Author Contributions

Surgical and Medical Practices: A.A., S.O., K.Y., M.A.A., Concept: A.A., A.O., Design: A.A., A.O., K.Y., M.A.A., Data Collection and/or Processing: A.A., S.O., M.A.A., Analysis or Interpretation: A.A., S.O., Literature Search: A.A., S.O., A.O., M.A.A., Writing: A.A., A.O., K.Y.

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

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

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