ACTA MEDICA NICOMEDIA

C. ***Olgu Sunumu***

D. ***Derleme***

E. ***Geri Çekilme Makalesi***

doi: 10.53446/actamednicomedia.1598274

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Letter to the Editor / Editöre Mektup

COMMENT ON: "FRAGMENTED QRS PATTERN PREDICTS POOR PROGNOSIS IN SEPSIS AND SEPTIC SHOCK"

YORUM: "FRAGMENTE QRS PATERNİ SEPSİS VE SEPTİK ŞOKTA KÖTÜ PROGNOZU ÖNGÖRÜR"

Ayşe Ayyıldız[1], Özge Turgay Yıldırım[2*]

[1]Department of Intensive Care, Eskişehir City Hospital, Eskişehir, Türkiye. [2]Department of Cardiology, Eskisehir City Hospital, Eskisehir, Türkiye.

Dear Editor,

We are writing to express our views on the recently published article titled "Fragmented QRS Pattern Predicts Poor Prognosis in Sepsis and Septic Shock" by Karabacak et al.[1] We commend the authors for addressing an important and clinically relevant topic that explores the prognostic value of fragmented QRS (fQRS) patterns in patients with sepsis and septic shock. The study provides valuable insights into the association between fQRS patterns and short-term overall survival in critically ill patients. The authors' findings that fQRS patterns independently predict worse outcomes, alongside the need for mechanical ventilation and its duration, are particularly noteworthy. This observation could significantly influence the clinical management and risk stratification of septic patients. However, we would like to share some constructive comments and raise questions that may further refine the understanding of this important subject.[1]

While the study provides valuable insights into the prognostic significance of fQRS patterns, the absence of echocardiographic data is a notable limitation. Echocardiography could have offered crucial insights into the structural and functional cardiac abnormalities associated with fQRS, such as myocardial scarring, ischemia, or reduced left ventricular ejection fraction. Including echocardiographic parameters would have strengthened the association between fQRS and myocardial dysfunction, providing a more comprehensive understanding of the underlying mechanisms. Furthermore, this data could have helped differentiate whether the fQRS patterns observed were primarily reflective of sepsis-induced cardiomyopathy or pre-existing cardiac conditions, thereby refining the study's conclusions. Future research incorporating echocardiographic assessment alongside ECG findings would significantly enhance the robustness and clinical applicability of these findings.

The lack of a control group of patients without sepsis limits the study's ability to isolate the impact of sepsis on fragmented QRS (fQRS) patterns and their prognostic implications. Including a comparison group without sepsis but with similar cardiovascular risk factors, such as patients with stable chronic conditions, could have clarified whether fQRS patterns are specific markers of septic myocardial dysfunction or merely reflective of pre-existing cardiac abnormalities.[2] For example, fQRS patterns have been linked to myocardial scarring and ischemia in non-septic conditions like coronary artery disease and dilated cardiomyopathy.[3,4] A comparative analysis might have revealed whether the observed fQRS patterns are amplified in septic states due to inflammatory and hemodynamic derangements unique to sepsis. This would also help differentiate sepsis-induced changes from baseline cardiac abnormalities present in critically ill patients. Future studies could benefit from stratifying participants into septic, non-septic critically ill, and healthy cohorts to establish the incremental predictive value of fQRS in sepsis. Such an approach would enhance the clinical utility of fQRS patterns as a specific marker for septic cardiomyopathy.

Corresponding author/İletişim kurulacak yazar: Özge Turgay Yıldırım, Department of Cardiology, Eskisehir City Hospital, Eskisehir, Türkiye.
Phone /Telefon: +90 (532) 687 66 26, **e-mail /e-posta** ozgeturgay@gmail.com
Submitted/Başvuru: 8.12.2024 • **Accepted/Kabul:** 25.03.2025 • **Published Online/Online Yayın:** 30.06.2025

doi: 10.53446/actamednicomedia.1388483

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

THE RELATIONSHIP BETWEEN ANXIETY LEVELS OF MOTHERS OF LATE PRETERM AND TERM INFANTS AND BREASTFEEDING SELF-EFFICACY AND BREASTFEEDING SUCCESS

GEÇ PRETERM VE TERM BEBEK ANNELERİNİN KAYGI DÜZEYLERİ İLE EMZİRME ÖZYETERLİLİK VE EMZİRME BAŞARISI İLİŞKİSİ

Gonca Karatas Baran[1*], Sevil Sahin[2], Dilek Sarici[3], Fatma Torun[3], Tulin Gundogdu[1]

[1]Ankara Etlik Zubeyde Hanim Women's Health Training and Research Hospital, Ankara. [2]Ankara Yıldırım Beyazıt University, Faculty of Health Sciences, Ankara, Turkiye. [3]Ankara Atatürk Sanatorium Training and Research Hospital, Neonatal Clinic, Ankara, Turkiye.

ABSTRACT

Objective: The aim of this study was to determine anxiety levels of mothers who gave birth to late preterm and term infants hospitalized in the Neonatal Intensive Care Units (NICU) and evaluate the associations with breastfeeding outcomes.

Methods: The descriptive cross-sectional and comparative study was carried out in two hospitals between June 2019 and December 2021. The research sample consisted of 50 late preterm and 50 term infant mothers. The state and trait anxiety scale, the LATCH Breastfeeding Diagnosis and Evaluation and the Breastfeeding Self-Efficacy Scale were administered to the mothers.

Results: While no statistically significant difference was found between mothers of late preterm and term infants in terms of trait anxiety scale scores in the study (p>0.05). The state anxiety scale scores of mothers of late preterm infants were found to be lower (p<0.05). When trait and state anxiety scale scores were compared, it was determined that the mean state anxiety scale score was 3.80±9.26 points less than the trait anxiety scale mean (p<0.001). No statistically significant difference was found between the research groups in the scores of the LATCH Breastfeeding Diagnosis and Evaluation and the Breastfeeding Self-Efficacy Scale (p>0.05).

Conclusion: In this study, the babies need for NICU did not increase the mothers' anxiety levels. Although state-trait anxiety negatively affect breastfeeding self-efficacy, breastfeeding support of NICU staff could positively affect breastfeeding success in mothers with babies in need of intensive care.

Keywords: Neonatal intensive care units, preterm infants, term infants, anxiety, breastfeeding

ÖZ

Amaç: Amaç: Bu çalışmanın amacı; bebeği Yenidoğan Yoğun Bakım Ünitelerinde (YYBÜ) yatan geç preterm ve term annelerin kaygı düzeylerini belirlemek ve bebeklerinin emzirmelerini değerlendirmektir.

Yöntem: Tanımlayıcı kesitsel ve karşılaştırmalı araştırma, iki hastanede Haziran 2019-Aralık 2021 tarihleri arasında gerçekleştirilmiştir. Araştırma örneklemi 50 geç preterm ve 50 term bebek annesinden oluşturulmuştur. Annelere durumluk ve sürekli kaygı ölçeği, LATCH Emzirmeyi Tanılama ve Değerlendirme Ölçeği ve Emzirme Öz yeterlilik Ölçeği uygulanmıştır.

Bulgular: Araştırma grubundan geç preterm annelerinin durumluk kaygı ölçeği puanları daha düşükken, sürekli kaygı ölçeği puanları yönünden gruplar arasında istatistiksel olarak anlamlı farklılık saptanmamıştır (p>0,05). Sürekli ve durumluk kaygı ölçek puanları karşılaştırıldığında durumluk kaygı ölçeği puanı ortalamasının süreklil kaygı ölçeği ortalamasından 3,80±9,26 puan az olduğu ve bu durumun istatistiksel olarak anlamlı olduğu belirlenmiştir (p<0,001). Araştırma grupları arasında LATCH Emzirmeyi Tanılama ve Değerlendirme Ölçeği ve Emzirme Öz yeterlilik Ölçeği puanları arasında istatistiksel olarak anlamlı farklılık saptanmamıştır (p>0,05).

Sonuç: Bu çalışmada bebeklerin YYBÜ'ye ihtiyaç duyması annelerin kaygı düzeylerini artırmamıştır. Durumluk ve sürekli kaygısı emzirme özyeterliliğini olumsuz etkilemekle birlikte, yenidoğan yoğun bakım personelinin emzirme desteği, yoğun bakım ihtiyacı olan bebekleri olan annelerde emzirme başarısını olumlu etkileyebilmektedir.

Anahtar Kelimeler: Yenidoğan yoğun bakım üniteleri, preterm bebekler, term bebekler, kaygı, emzirme

***Corresponding author/İletişim kurulacak yazar:** Gonca Karatas Baran; Ankara Etlik Zubeyde Hanim Women's Health Training and Research Hospital, Ankara, Turkiye.
Phone/Telefon: +90 (542) 605 81 61, **e-mail/e-posta:** goncabaran@gmail.com
Submitted/Başvuru: 09.11.2023 • **Accepted/Kabul:** 13.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Premature infants make up the majority of the Neonatal Intensive Care Units (NICU) patient population. The vast majority of premature infants are also late preterm infants. The term late preterm was used to describe infants at 34 (0/7 days) - 36 (6/7 days) gestational weeks.[1] Due to the fact that the birth occurred before the expected date and and babies had health problems, the relatives of the patients may be anxious during the intensive care unit hospitalization of their babies.[2-4]

Breastfeeding success in the early postpartum period is influenced by breastfeeding knowledge and physical and mental well-being.[5,6] While positive attitudes such as excitement or satisfaction increase breastfeeding self-efficacy; negative attitudes such as pain, fatigue, anxiety or stress reduce the perception of breastfeeding self-efficacy.[7] Pregnancy and postpartum stages involve complex processes that can increase daily stress and make breastfeeding difficult.[8] Researchers have shown that maternal psychosocial factors such as stress and social support are also the main determinants of successful breastfeeding.[9,10]

It is important for NICU nurses to evaluate parents' anxiety, perception and care competencies with a holistic approach to care. Studies on the effect of anxiety level of mothers with premature babies on breastfeeding success and self-confidence are limited. This study was planned to determine anxiety levels of mothers who gave birth to late preterm and term infants hospitalized in the NICU and evaluate the associations with breastfeeding outcomes. Determining the anxiety levels of mothers in the postpartum period and knowing the consequences of anxiety in terms of newborn nutrition will increase the quality of care given by nurses to mother and baby, expand their holistic perspective and provide nursing care that will produce positive health outcomes in the newborn.

Methods

The descriptive cross-sectional and comparative study was carried out in a training-research hospital and gynecology branch hospital between June 2019 and December 2021. In both hospitals, NICU nurses provide breastfeeding support to mothers. Nurses provide information to mothers about the benefits of breastfeeding, breastfeeding techniques, positions, and skin-to-skin contact with the baby. Every mother's situation is different, so each mother is provided with individual support according to her needs. Nurses closely monitor the baby's sucking strength and weight gain, helping the breastfeeding process progress. In addition, both hospitals have maternal adjustment rooms, in which mothers are individually trained by nurses on breastfeeding and care. All babies are accepted directly from the delivery room.

Two groups were formed as mothers of late preterm and term infants. For sample calculation, a similar research reference[11] was taken and 41 individuals from each group (mothers of late preterm and term infants) were required (confidence interval: 0.95, margin of error: 0.05, size of effect: 0.815). Data loss was calculated and a total sample of 100 mother was formed (50+50).

The data were obtained from the self-report of mothers who met the inclusion criteria (the baby was hospitalized in the NICU, the gestational week of the baby was 34.0-36.6 in the late preterms, 37.0-41.6 in the term, has not been breastfed the baby yet, there was no health problem in the baby (congenital malformation, necrotizing enterocolitis) that may prevent feeding, no psychiatric disease, who agreed to participate in the study, and who did not have communication problems). The data were collected by the researcher by face-to-face interview method, which lasted 20 minutes. The data collection form consisted of five parts.

In the first part; according to the literatüre [2,11-15] (sociodemographic characteristics, obstetric characteristics, breastfeeding experience, birth characteristics, infant characteristics) were questioned.

In the second part, The State-Trait Anxiety Inventory (STAI) was used. State anxiety is the subjective fear that an individual feels due to the stressful situation. Trait anxiety is the tendency of the individual to experience anxiety. STAI was developed by Spielberger et al. It was adapted into Turkish by Öner and Le Compte in 1970. It is a 4-point Likert-type scale consisting of 20 questions measuring state and trait anxiety levels.[16]

While direct statements in the scales indicate negative emotions, reversed statements indicate positive emotions. There are ten reversed statements (items 1, 2, 5, 8, 10, 11, 15, 16, 19, and 20) in the state anxiety scale and seven reversed statements in the trait anxiety scale (21, 26, 27, 30, 33, 36 and 39) exists. In the calculation, the total weight score of the reverse expressions is subtracted from the total weight score expressed directly and the predetermined constant value is added. This constant value is 50 for the state anxiety scale and 35 for the trait anxiety scale.[16] The most recent value is the individual's anxiety score. A high score indicates a high level of anxiety. In our study, the Cronbach's alpha value was found to be 0.86 for the state anxiety scale and 0.75 for the trait anxiety scale.

In the third part; The LATCH Breastfeeding Diagnostic and Evaluation Scale was used. The LATCH scale was developed in 1993 and is one of the widely used scales. The scale includes assessing holding the breast, seeing/hearing the baby's swallowing, nipple type, mother's comfort with regard to the breast and nipple, and the position of holding the baby. A high score from the scale indicates high breastfeeding success. The Turkish reliability of the scale was made by Yenal and Okumuş and it was found to be a suitable and reliable diagnostic tool for use. The Cronbach alpha value of the original tool was 0.93, while the Turkish version of the tool was found to be 0.95.[17] In our study, the Cronbach's alpha value was 0.62.

In the fourth chapter; The Breastfeeding Self-Efficacy Scale was used. This scale is a 33-item scale developed by

Dennis in 1999 that measures breastfeeding self-efficacy. It was later reduced to 14 items in 2003. The scale is in a 5-point Likert type and the minimum score is 14 and the maximum score is 70. The scale has no cut-off point and an increase in the score means that breastfeeding self-efficacy is high. Dennis stated that it is appropriate to apply this scale in the postnatal period.[18] The Cronbach alpha value of the Turkish version of the scale by Alush-Tokat and Okumuş (2010) was 0.86.[19] In our study, the Cronbach's alpha value was found to be 0.91.

In the fifth section, data on nutritional characteristics at discharge werw included. Based on the literature to determine the nutritional characteristics of the baby whose discharge was planned before discharge [4,11,14,20-22] questions are included. Gestational age, length of hospital stay, number of breast feeding/day, weight, height, diet at discharge, and food type at discharge were evaluated.

Small for gestational age (SGA) infants were evaluated at birth and at discharge using the Fenton's growth curve for premature infants.[23] A birth weight below the 10th percentile was defined as SGA. Reaching birth weight at discharge was also evaluated with Fenton's growth curve for premature infants.

Ethics committee approval was obtained from the Ankara Yildirim Beyazit University ethical committee with date 29.05.2019, number 55. Consent was obtained from mothers of late preterm and term infants by giving information about the study and having the informed consent form signed.

The analysis of the data was made in SPSS (Statistical Package for the Social Sciences) 20.0 ready-made statistical program. In the evaluation of the data; Number, percentage, mean and standard deviation were used as descriptive statistics, parametric (t test) and nonparametric (Mann-Whitney U test) methods were used in dependent and independent groups according to data characteristics. The appropriate one from the Pearson and Spearman Correlation Analysis was used in the relationship between the scales. The results were evaluated at the level of significance $p<0.05$ at the 95% confidence interval.

Results

No statistically significant difference was found between mothers of late preterm and term infants in terms of sociodemographic characteristics (age, employment status, income, family type, social security, place of residence) except education ($p>0.05$). There was a statistically significant difference between the groups in terms of education level ($p<0.05$) and mothers of term infants were more likely to graduate from primary school than mothers of late preterm infants (Table 1).

There was no statistically significant difference between the study groups in terms of general health and obstetric characteristics (first gestational age, time between previous birth and current birth, breastfeeding status of the previous baby, type of delivery, number of births, number of living children, current smoking status) ($p>0.05$) (Table 2).

There was no statistically significant difference between the research groups in terms of number of milk expression (day), intention to breastfeed, duration (year) of intention to breastfeed, milk expression status, feeding route and nutritional properties after hospitalization, feeding route and nutritional properties at the time of interview ($p>0.05$). When the reasons for hospitalization were examined, the most common causes of hospitalization in term infants were jaundice, respiratory distress, hypoglycemia and infection, while jaundice, prematurity and intrauterine growth restriction (IUGG) in the late preterm group (Table 3).

The median week of gestation at birth for mothers of term infants was 38.3 (37.0-41.0)/75.5, and the median week of gestation at birth for mothers of late preterm babies was 35.0 (34.0-36.6)/25.5. When the newborn characteristics of the research group were examined, the mean birth weight of term infants and late preterm infants was 3195.0±581 and 2324.7±576 grams, respectively.

Late preterm infants have a longer hospital stay than term babies and the difference between them was statistically significant ($p<0.001$). There was no statistically significant difference between the research groups in terms of the number of breastfeeding/day, food route, and food type ($p>0.05$) (Table 4).

The gestational age at discharge was 39.2±3.2 for term babies and 36.3±1.2 for late preterm babies. The mean birth weight of term babies and late preterm babies at discharge was 3173.2±592 and 2390.92±567 grams, respectively While no difference was found between term infants (n=7, 43.8%) and late preterm infants (n=8, 56.3%) in terms of SGA at birth ($p= 0.59$), there was a statistically significant difference between term infants (n=8, 32.0%) and late preterm infants (n=17, 68.0%) in case of failure to reach birth weight at discharge ($p= 0.04$).

While there was a significant difference between the state anxiety scale scores of the research group ($p<0.05$), no statistically significant difference was found between the trait anxiety scale scores ($p>0.05$). When trait and state anxiety scale scores were compared, it was determined that the mean of the state anxiety scale was 3.8±9.3 points less than the mean of the trait anxiety scale, which was statistically significant ($p<0.001$). There was no statistically significant difference between the research groups in the scores of the LATCH Scale ($p>0.05$) (Table 5).

In the correlation analysis, there was a moderate, significant and positive relationship between the state and trait anxiety scales ($r=0.50$, $p<0.001$). There was a moderate, significant and negative relationship between the state anxiety scale and the breastfeeding self-efficacy scale ($\rho= -0.35$, $p<0.001$) and moderate, significant and negative relationship ($\rho=-0.48$, $p <0.001$) between the the trait anxiety scale and breastfeeding self-efficacy scale. Significant, negative but weak relationship was found between income and trait anxiety scale. ($r= -0.23$,

Table 1. Sociodemographic characteristics

	Term Infant Mother		Late Preterm Infant Mother		Total		Analysis
	n	**Mean±SD**	**n**	**Mean±SD**	**n**	**Mean±SD**	
Age	50	29.48±6.02	50	29.36±5.39	100	29.42±5.68	t= 0.11 p= 0.92
	n	**Med((min-max) /Mean Rank**	**n**	**Med((min-max) /Mean Rank**	**n**	**Med((min-max)**	
BMI (before pregnancy)	50	24.91(17.26-42.10) /51.32	50	24.87(17.26-35.42) /49.68	100	24.91 (17.26-42.10)	z= -0.28 p=0.78
	n	**%**	**n**	**%**	**n**	**%**	
Education level							
Primary education	24	48.0	12	24.0	36	36.0	χ^2= 6.42 p= 0.04
High school	13	26.0	21	42.0	34	34.0	
University and above	13	26.0	17	34.0	30	30.0	
Working status							
Working	7	14.0	8	16.0	15	15.0	χ^2= 0.08 p= 0.78
Not working	43	86.0	42	74.0	85	85.0	
Health insurance							
There is	43	86.0	44	88.0	87	87.0	χ^2= 0.09 p=0.78
None	7	14.0	6	12.0	13	13.0	
Income status							
Income less than expenses	17	34.0	14	28.0	31	31.0	Fish. Ex. T p= 0.73
Income equals expense	29	58.0	33	66.0	62	62.0	
Income more than expenses	4	8.0	3	6.0	7	7.0	
Family type							
Nuclear family	38	76.0	38	76.0	76.0	76.0	χ^2= 0.00 p= 1.00
Wide family	12	24.0	12	24.0	24	24.0	
Living place							
Provincial center	32	64.0	29	58.0	61	61.0	χ^2= 0.38 p= 0.54
County, village, town	18	36.0	21	42.0	39	39.0	
Total	50	100.00	50	100.00	100	100.00	

Table 2. General health and obstetrics characteristics

	Term Infant Mother		Late Preterm Infant Mother		Total		Analysis
	n	**Med((min-max)/Mean Rank**	**n**	**Med((min-max)/Mean Rank**	**n**	**Med((min-max)**	
Mother's age at first pregnancy	50	21.5(16-36)/45.0	50	24.0(18-40)/56.0	100	23.0(16-40)	z= -1.89 p= 0.06
	n	**Mean±SD**	**n**	**Mean±SD**	**n**	**Mean±SD**	
Time between previous and current birth (months)	50	56.9±36.2	50	45.7±27.0	100	46.9±32.4	t= 1.40 p=0.17
	n	**%**	**n**	**%**	**n**	**%**	
Number of births							
1	17	34.0	19	38.0	36	36.0	χ^2= 0.17 p= 0.92
2	18	36.0	17	34.0	35	35.0	
3 and above	15	30.0	14	28.0	29	29.0	
Number of living children							
1	17	34.0	19	38.0	36	36.0	χ^2= 0.25 p= 0.88
2	18	36.0	18	36.0	36	36.0	
3 and above	15	30.0	13	26.0	28	28.0	
Current smoking status							
No	43	86.0	41	82.0	84	84.0	Fish. Ex. T p=0.23
Yes	1	2.0	5	10.0	6	6.0	
Left	6	12.0	4	8.0	10	10.0	
Type of birth							
Vaginal birth	23	46.0	18	36.0	41	41.0	χ^2= 1.03 p=0.31
Cesarean section	27	54.0	32	64.0	59	59.0	
Total	50	100.00	50	100.00	100	100.00	
Breastfeeding status in a previous birth							
No	1	3.0	5	15.6	6	9.2	Fish. Ex. T p=0.11
Yes	32	97.0	27	84.4	59	98.8	
Total	33	100.00	32	100.00	65	100.00	

Table 3. Newborn and nutritional characteristics

	Term Infant Mother		Late Preterm Infant Mother		Total		Analysis
	n	**Med((min-max) /Mean Rank**	**n**	**Med((min-max) /Mean Rank**	**n**	**Med((min-max)**	
Number of milking (days)	50	4(1-12)/36.8	50	5(1-12)/46.0	100	5(1-12)	z= -1.76 p=0.08
	n	**Mean±SD**	**n**	**Mean±SD**	**n**	**Mean±SD**	
Intended time to breastfeed (years)	49	2.7±1.4	50	2.6±1.3	99	2.7±1.3	t= 0.38 p=0.71
	n	**%**	**n**	**%**	**n**	**%**	
Nutritional route during hospitalization							
Vascular access	7	12.1	17	23.0	24	18.2	More than one option has been ticked.
Gastric tube	2	3.4	9	12.1	11	8.3	
Mouth	49	84.5	48	64.9	97	73.5	
Total	58	100.0	74	100.00	132	100.0	
Nutritional route during the interview							
Vascular access	1	2.0	1	2.00	2	2.0	More than one option has been ticked**.**
Gastric tube	0	0.0	0	0.0	0	0.0	
Mouth	50	98.0	50	98.0	100	98.0	
Total	51	100.00	51	50.0	102	100.0	
Food type during hospitalization							
breast milk	19	38.0	22	44.0	41	41.0	Fish.Ex. T. p= 0.58
Breast milk + formula	25	50.0	25	50.0	50	50.0	
Formula	6	12.0	3	6.0	9	9.0	
Type of food during the interview							
breast milk	29	58.0	37	74.0	66	66.0	Fish. Ex. T p= 0.01
Breast milk + formula	21	42.0	10	20.0	31	31.0	
Formula	0	0.0	3	6.0	3	3.0	
Milking status							
No	10	20.0	9	18.0	19	19.0	χ^2= 0.07 p= 0.80
Yes	40	80.0	41	82.0	81	81.0	
Intention to breastfeed							
No	1	2.0	0	0.0	1	1.0	Fish. Ex. T p=1.00
Yes	49	98.0	50	100.0	99	99.0	
Total	50	100.00	50	100.00	100	100.00	
Reason for hospitalization							
Jaundice	21	42.0	21	41.2	42	41.6	More than one option has been ticked
Respiratory distress	12	24.0	7	13.7	19	18.8	
Hypoglycemia	5	10.0	1	2.0	6	5.9	
Infection	4	8.0	1	2.0	5	5.0	
IUGG	1	2.0	7	13.7	8	7.9	
Prematurity	0	0.0	10	19.6	10	9.9	
Other*	7	14.0	4	7.8	11	10.8	
Total	50	100.00	51	100.00	101	100.0	

*Other: Heart rhythm problems, birth with meconium, cleft palate, dehydration, groaning, fetal anomaly, weight loss

Table 4. Newborn and Nutritional Characteristics at Discharge

	Term Infant Mother		Late Preterm Infant Mother		Total		Analysis
	n	**Mean±SD Med((min-max) /Mean Rank**	**n**	**Med((min-max)/Mean Rank**	**n**	**Med((min-max)**	
Length of stay in NICU (day)	50	5(1-23)/41.1	50	7(1-35)/59.0	100	5(1-35)	z= -3.25 p=0.001
Number of breastfeeds/day	50	8(3-24)/48.4	50	8(6-15)/51.7	100	8(3-24)	z= -0.61 p=0.54
	n	**%**	**n**	**%**	**n**	**%**	
Nutritional route at discharge							
Breast-feeding	50	80.6	49	81.6	99	81.1	More than one option has been ticked.
Bottle	12	19.4	11	18.4	23	18.9	
Total	62	100.00	60	100.00	122	100	
Type of food at discharge							
breast milk	32	64.0	36	72.0	68	68.0	Fish. Ex. T p= 0.39
Breast milk + formula	18	36.0	13	26.0	31	31.0	
Formula	0	0.0	1	2.0	1	1.0	
Total	50	100.00	50	100.00	100	100.00	

Table 5. State Anxiety Scale, Trait Anxiety Scale, LATCH and Breastfeeding Self-Efficacy Scale Scores of the Research Group

Scales	**Term Infant Mother**		**Late Preterm Infant Mother**		
	n	**Mean±SD Med((min-max)/Mean Rank**	**n**	**Mean±SD Med((min-max)/Mean Rank**	**Analysis**
State Anxiety Scale	50	38.6±10.7	50	34.6±8.7	t= 2.06 p=0.04
Trait Anxiety Scale	50	41.0±9.0	50	39.7±8.0	t= 0.76 p=0.45
LATCH Breastfeeding Diagnosis And Assessment Scale	50	10 (5-10)/53.2	49	9 (4-10)/46.8	z= -1.17 p=0.24
Breastfeeding Self-Efficacy Scale	50	63 (34-70)/49.2	49	63 (27-70)/50.8	z= -0.28 p=0.78
	State anxiety scale **Mean±SD**		Trait anxiety scale **Mean±SD**		
	36.6±9.9		40.4±8.5		t= -4.10** p<0.001
	State anxiety scale - Trait anxiety scale **Mean±SD =** -3.8±9.3				

p<0.05). There was a weak, significant and positive relationship between the LATCH scale and the breastfeeding self-efficacy scale (ρ=0.28, p<0.05).

Discussion

In this study, we determined the anxiety levels of mothers who were hospitalized in NICU and delivered late preterm and term infants. Studies have found that mothers with premature infants hospitalized in the NICU experience more psychological discomfort than mothers with term infants.[4,24] In Zanardo's study, trait and state anxiety were found to be higher in late preterm mothers compared to term mothers.[11] In our study, no statistically significant difference was found between the trait anxiety scale scores of the groups (p>0.05) and the state anxiety scale was found to be higher in the mothers of term infants (p<0.05). Similarly, in the study of Çelen and Taş, it was determined that mothers of preterm infants had a low mean score on the state anxiety scale and did not experience anxiety, and their trait anxiety levels were moderate.[25] In the study of Akgün Çalışkanyürek et al., it

was determined that the level of state anxiety increased as the gestational week progressed.[12] In our study, it was determined that the state anxiety level of term infant mothers was high and this difference due to term infants mother's lower education level. In the study of Miles et al., mothers' anxiety about their babies' health status was found to be higher in those with low education levels.

When trait and state anxiety scale scores were compared in our study, it was determined that the mean of the state anxiety scale was 3.80±9.26 points less than the trait anxiety scale ($p<0.001$). In this study, the babies need for NICU did not increase the mothers' anxiety levels, on the contrary, anxiety decreased in the process. It can be reasoned they were have resulted from the support of NICU nurses. The cut-off score of the Spielberger state and trait anxiety scale is 40 points.[26] In our study, the groups' trait anxiety scale was at the cut-off point, but the state anxiety scale score was below the cut-off point.[27] However, as trait anxiety increased, state anxiety also increased.

In this study, we examined the effects of anxiety on early breastfeeding in mothers whose babies were in the intensive care unit. The great benefits of breastfeeding preterm infants and the resulting increased survival rates have led to research on how to optimize the breastfeeding performance of mothers with premature infants.[28-30] In a systematic review, evidence correlated newborn nutritional outcomes with maternal mental health indices.[14] The study of Ziomkiewicz et al., showed that maternal stress has a significant and negative effect on the composition of breast milk in the postpartum period.[31] Trait anxiety inhibits the release of oxytocin and prolactin, which are hormones that support the milk let-down reflex.[32,33] Also, acute emotional stress (state anxiety) is associated with high cortisol and glucose levels. These hormones are effective in delaying the fullness of the breast and decreasing the first milk volume at birth. Second, it provides evidence that lactation results in endocrinological changes that buffer anxiety symptoms.[33] In our study, as state and trait anxiety increases, breastfeeding self-efficacy decreases, but there was no difference between the groups in breastfeeding success and self-efficacy.

Studies and clinical experience show that late preterm babies and their mothers have factors that that put them at risk for unsuccessful lactation that may result in breastfeeding failure.[21,34] In the study of Zanardo et al., only 21% of the late preterm sample was found to be exclusively breastfed.[11] In our study, while the feeding route and feeding type of newborns were similar after hospitalization, the rate of breastfeeding was higher in late preterm babies at the time of interview. In the evaluation at the time of discharge, the rate of exclusive breastfeeding of term mothers decreased and the rate of taking breastmilk + formula increased compared to the first intervention time, while the rate of taking only breast milk in preterm mothers was higher at discharge. This may be due to the high level of state anxiety and low education level of term infant mothers. In the study of Akgün Çalışkanyürek et al., it was determined that one unit increase in the state anxiety level decreased the breastfeeding attitude by 0.54 units.[12] It may be logical that the higher education level of preterm mothers in our study may have provided knowledge and interest in breastfeeding. In the study of He et al., it is stated that when mothers with late preterm infants believe in their ability to breastfeed, they will overcome the difficulties of doing so, have confidence in their abilities, and can correctly interpret and respond to the needs of their infant.[35]

In the study of Gupta et al., the rate of breastfeeding at discharge was 43.8% in late preterm infants.[15] In the study of Crippa et al., it was 16.0%.[36] Anticipating the help a mother may need to manage the first feeding at the breast, Casey et al. indicate in their study that NICU staff can improve the level of breastfeeding to these high-risk premature infants who need their therapeutic effects most.[20] In our study, breast feeding rates of late preterm and term infants (64.0% and 72.0%) were higher compared to other studies.[15,36] Breastfeeding among term-late preterm groups the similarity of success and breastfeeding self-efficacy was the reason for the presence of "mother adaptation" services in both hospitals and the support of NICU nurses about breastfeeding, and the interventions made increase the success of breastfeeding. In addition, in our study, it was determined that while the LATCH score observed in the first breastfeeding increased, breastfeeding self-efficacy also increased. Successful breastfeeding can be achieved by providing mothers with information on the normal physiology of breastfeeding and the correct methods of knowing whether the baby is receiving enough milk, increasing their confidence in breastfeeding, and receiving support from healthcare professionals when mothers encounter any breastfeeding problems. Thus, the use of formulas can be minimized and even avoided.[37]

In this study, the intention to breastfeed in mothers of late preterm infants (98.0%) and mothers of term infants (100.0%) were positive, the intended duration of breastfeeding in mothers of late preterm infants was 2.71±1.35 year and term mothers were 2.61±1.0 year. In this study, it was deduced that the intention to breastfeed positively affects the breastfeeding success of mothers in both groups.

In this study, it was determined that the rate of reaching birth weight at discharge was lower in late preterm infants than in term infants. Preterm infants may experience greater weight loss by suckling, and this is related to maturity. Considering that extrauterine weight gain is slower in preterm infants,[23] feeding this group with breast milk is important.

Breastfeeding self-efficacy may improve breastfeeding among mothers with late preterm infants.[38] It has been shown that breastfeeding self-efficacy is related to the duration of breastfeeding.[22] A Hong Kong study among 199 postpartum Chinese women with term babies found that breastfeeding self-efficacy significantly predicted the duration of breastfeeding.[39] In this study, breastfeeding self-efficacy of mothers in both groups was

similar (p>0.05) and it was concluded that this similarity led to similar results in breastfeeding success. (p>0.05).
In the study of Gupta et al., the hospitalization period was 5.0 weeks in very preterm infants and 1.2 weeks in late preterm infants.[15] In our study, the median hospital stay was 7(1-35)/day for late preterm infants and 5(1-23)/day for term infants, and the difference between them was statistically significant (p<0.001). When the reasons for hospitalization were examined, the most common reasons for hospitalization in term infants were jaundice, respiratory distress, hypoglycemia and infection, while jaundice, prematurity and IUGG in the late preterm group. In the study of Arayıcı et al., the reason for hospitalization to the NICU was respiratory distress in both groups, while IUGG and nutritional deficiency in late preterm infants; term infants have jaundice and polycythemia.[13]
In conclusion, in this study, the babies need for NICU did not increase the mothers' anxiety levels. Although state-trait anxiety negatively affect breastfeeding self-efficacy, breastfeeding support of NICU staff could positively affect breastfeeding success in mothers with babies in need of intensive care.
Developing appropriate strategies for ensuring the breastmilk intake of NICU infants and encouraging the interest of health care providers in certain demographics and psychological characteristics can assist mothers improve breastfeeding.
Breastfeeding self-efficacy and postnatal support positively affect breastfeeding success in mothers with babies in need of NICU. Nurses-midwives and health professionals should evaluate breastfeeding self-efficacy and create self-efficacy-enhancing breastfeeding support interventions to successfully exclusively breastfeed infants in need of NICU.
Limitations: The data were limited to the self-reports and observations of the mothers whose babies were hospitalized in the NICU in two hospitals.

Conflict of interest statement
The author declared no conflict of interest in the manuscript.

Ethics Committee Approval: Ankara Yildirim Beyazit University (AYBU) Ethics Committee. Date/No: 29.05.2019/55

Patient Consent: Written consent was obtained with the informed voluntary consent form.

Author Contributions: Concept – GKB; Design – GKB, SŞ, DS; Supervision – GKB, SŞ, DS, FT, TG; Resources – GKB, SŞ, DS, FT, TG; Data Collection and/or Processing – GKB, FT, TG; Analysis and/or Interpretation – GKB, SŞ, DS; Literature Review – GKB, SŞ, DS; Writing the Manuscript – GKB; Critical Review – GKB, SŞ, DS.

Financial Declaration: None.

References

1. Çakır SÇ, Dorum BA, Köksal N, Özkan H, Coşkun M, Özcan N. The Problems of Late Preterm Infants in Neonatal Period. *J Curr Pediatr.* 2018;16(1):2-18
2. Çalışır H, Şeker S, Güler F, Anaç GT, Türkmen M. (2018). The needs and anxiety levels of parents whose babies are hospitalized in the neonatal intensive care unit. *Journal of Cumhuriyet University School of Nursing.* 12(1), 31-44.
3. Çekin B. Stress level and coping methods of parents with a premature baby hospitalized in the neonatal intensive care unit of a university hospital. (Master's Thesis). Pamukkale University, Institute of Health Sciences, Child Health and Diseases Nursing Program. 2014, Denizli.
4. Wigert H, Johansson R, Berg M, Hellström AL. Mothers' experiences of having their newborn child in a neonatal intensive care unit. Scand J Caring Sci, 2006; 20(1):35-41.
5. Gaynes BN, Gavin N, Meltzer-Brody S. Lohr KN, Swinson T, Gartlehner G, Brody S, Miller WC. Perinatal Depression: Prevalence, Screening Accuracy, and Screening Outcomes. *Evid Rep Technol Assess*. 2005;119:1-8.
6. Koç GI, Tezcan S. (2005). Attitudes of Pregnant Women Towards Breastfeeding and Some Factors Affecting Breastfeeding Attitudes. *Journal of Hacettepe University School of Nursing*. 2005;*12*(2):1-13.
7. Stockdale J, Sinclair M, Kernohan G, Keller J. (2011). Understanding Motivational Theory and the Psychology of Breastfeeding. *In Theory for Midwifery Practice*.2011;2:92-106.
8. Isiguzo C, Mendez DD, Demirci JR, Youk A, Mendez G, Davis EM, Documet P. Stress, social support, and racial differences: Dominant drivers of exclusive breastfeeding. *Matern Child Nutr*. 2023 Apr;19(2):e13459. doi: 10.1111/mcn.13459.
9. de Jager E, Broadbent J, Fuller-Tyszkiewicz M, Skouteris H. The role of psychosocial factors in exclusive breastfeeding to six months postpartum. *Midwifery.* 2014; 30(6):657–666.
10. Islam MJ, Baird K, Mazerolle P, Broidy L. Exploring the influence of psychosocial factors on exclusive breastfeeding in Bangladesh. *Archives of Women's Mental Health.* 2017; 20(1):173-188.
11. Zanardo V, Gambina I, Begley C, Litta P, Cosmi E, Giustardi A, Trevisanuto D. Psychological distress and early lactation performance of mothers of preterm infants. *Early Hum Dev.* 2011;87(4):321-323.
12. Akgün Çalışkanyürek SS., Yıldırım Dİ, İnanli İ. The Effect of Mental Status of Mothers Aged 18-49 Years on Attitude to Breastfeeding. *Selcuk Med J.* 2022; *38*(1):30-39.
13. Arayıcı S, Kadıoğlu Şimşek G, Say B, Alyamaç Dizdar E, Uraş N, Canpolat FE, Oğuz ŞS. (2016). Late Preterm Neonates and Causes of Admission to the Neonatal Intensive Care Unit. *Turkish J Pediatr Dis.* 2016;1: 22-26. doi: 10.12956/tjpd.2015.177.
14. Fallon V, Bennett KM, Harrold JA. Prenatal anxiety and infant feding outcomes: a systematic review. *J Hum Lact*., 2016; 32(1):53-66. doi: 10.1177/0890334416662241.
15. Gupta S, Yuhas D, Wasylyshen-Velasco J, Stolfi A. Predictive Factors for Early Breastmilk Discontinuation in Premature Infants: A Retrospective Study. Medical Student Research Symposium Abstracts and Posters. *2021;*3. https://corescholar.libraries.wright.edu/msrs/2021/poster_presentations_6/3.
16. Öner N, Le Compte A. Handbook of State-Trait Anxiety Inventory. Istanbul: 1983. Boğaziçi University Printing House.

17. Yenal K., Okumuş, H. LATCH Emzirme tanılama ölçeğinin güvenirliğini inceleyen bir çalışma. HEMAR-G Dergisi. 2003; *5*(1):38-44.
18. Dennis CL, Faux S. Development and psychometric testing of the Breastfeeding Self-Efficacy Scale. *Research in Nursing and Health*. 1999; *22*:399-409.
19. Aluş-Tokat M, Okumuş H. Translation and psychometric assessment of the Breast-feeding Self-Efficacy Scale-Short Form among pregnant and postnatal women in Turkey. *Midwifery*. 2010; *26*(1):101-108.
20. Casey L, Fucile S, Dow KE. Determinants of Successful Direct Breastfeeding at Hospital Discharge in High-Risk Premature Infants. *Breastfeedıng Medıcıne.* 2018*;* 13(5):1-6.doi: 10.1089/bfm.2017.0209.
21. Sisk P, Lovelady C, Dillard R, Gruber K.J. Lactation counseling for mothers of very low birth weight infants: effect on maternal anxiety and infant intake of human milk. *Pediatrics.* 2006;117:67-75.
22. Wang Y, Briere CE, Xu W, Cong X. Factors affecting breastfeeding outcomes at six months in preterm infants. *Journal of Human Lactation.* 2019;*35*(1):80–89.
23. Fenton TR, Kim JH. A systematic review and metaanalysis to revise the Fenton growth chart for prematüre infants. *BMC Pediatrics.* 2013;13:59. http://www.biomedcentral.com/1471-2431/13/59
24. Miles MS, Holditch-Davis D, Schwartz M. Depressive symptoms in mothers of prematurely born infants. *J Dev Behav Pediatr.* 2007; 28:36-44.
25. Çelen R, Taş Arslan F. The anxiety levels of the parents of premature infants and related factors. *J Pediatr Res*. 2017; *4*(2):68-74.
26. Julian LJ. Measures of anxiety: state-trait anxiety inventory (STAI), beck anxiety inventory (BAI), and hospital anxiety and depression scale-anxiety (HADS-A). *Arthritis Care Res (Hoboken).* 2011;63:467-472.
27. Miles MS, Burchinal P, Holditch-Davis D, Brunssen S, Wilson SM. Perceptions of stress, worry, and support in Black and White mothers of hospitalized, medically fragile infants. *J Pediatr Nurs.* 2002;17(2): 82-88.
28. Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, Eidelman AI. Breastfeeding and the use of human milk. *Pediatrics.* 2005;115*:*496-506. doi: 10.1542/peds.2004-2491
29. Kent JC. How breastfeeding works. *J Midwifery Womens Health*. 2007;*52*:564-570.
30. Lau C, Hurst NM, Smith EO, Schanler RJ. Ethnic/racialdiversity, maternal stress, lactation and very low birth weight infants. *J Perinatol.* 2007; 27:399-408.
31. Ziomkiewicz A, Babiszewska M, Apanasewicz A, Piosek M, Wychowaniec P, Cierniak A, Barbarska O, Szołtysik M, Danel D, Wichary S. Psychosocial stress and cortisol stress reactivity predict breast milk composition. Sci Rep. 2021 Jun 2;11(1):11576. doi: 10.1038/s41598-021-90980-3.
32. Stuebe AM, Grewen K, Pedersen CA, Propper C, Meltzer Brody S. Failed lactation and perinatal depression: common problems with shared neuro endocrinemechanisms? *J Womens Health (Larchmt).* 2012; *21*(3):264-272. doi:10.1089/ jwh.2011.3083.
33. Lonstein JS. Regulation of anxiety during the postpartum period. *Front Neuro endocrinol*. 2007; 28(2-3):115-141. doi:10.1016/j.yfrne.2007.05.002.
34. Zanardo V, Buzzacchero R, Giustardi A, Trevisanuto D, Micaglio M. Breastfeeding the 'healthy' near-term infants after laryngeal mask airway ortraditional resuscitation methods. *J Mat-Fetal Neonatal Med.* 2009;22*:*92-95.
35. He J, Yimyam S, Namprom N. Breastfeeding self-efficacy, social support, and breastfeeding among Chinese mothers with late preterm infants*. Journal of Neonatal Nursing.* 2021;28(1):21-25. doi:10.1016/j.jnn.2021.07.005.
36. Crippa BL, Colombo L, Morniroli D, Consonni D, Bettinelli ME, Spreafico I, et al., Do a few weeks matter? Late preterm ınfants and breastfeeding Issues. *Nutrients.* 2019;1;11(2):312. doi: 10.3390/nu11020312.
37. Zhang K, Tang L, Wang H, Qiu LQ, Binns CW, Lee AH. Why do mothers of young infants choose to formula feed in China? Perceptions of mothers and hospital staff. *International Journal of Environmental Research and Public Health*. 2015;12(5):4520-4532.
38. Brockway M, Benzies KM, Carr E, Aziz K. Does breastfeeding self-efficacy theory apply to mothers of moderate and late pretermi nfants? A qualitative exploration. *Journal of Clinical Nursing*. 2020;29(15-16): 2872-2885. doi: 10.1111/jocn.15304.
39. Loke AY, Chan LKS. Maternal breastfeeding self-efficacy and the breastfeeding behaviors of newborns in thepractice of exclusive breastfeeding. *Journal of Obstetric, Gynecologic&Neonatal Nursing.* 2013;42(6): 672-684.

doi: 10.53446/actamednicomedia.1503454

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Araştırma Makalesi | Research Article

MEME KANSERİ HÜCRESİNDE METİLEN MAVİSİ ARACİLİ FOTODİNAMİK TERAPİ VE DOKSORUBİSİNİN KOMBİNASYONEL ETKİSİ

COMBINATIONAL EFFECT OF METHYLENE BLUE-MEDIATED PHOTODYNAMIC THERAPY AND DOXORUBICIN ON BREAST CANCER CELLS

Kübra Açıkalın Coşkun[1*], Elif Cansu Abay[2], Lütfi Tutar[3], Yusuf Tutar[4]

[1]İstanbul Aydın Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji Anabilim Dalı, İstanbul, Türkiye. [2]Sağlık Bilimleri Üniversitesi Hamidiye Tıp Fakültesi Tıbbi Biyoloji anabilim Dalı, İstanbul, Türkiye. [3]Ahievran Üniversitesi, Fen Fakültesi, Moleküler Biyoloji ve Genetik Anabilim Dalı, Kırşehir, Türkiye. [4]Recep Tayyip Erdoğan Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabili Dalı, Rize, Türkiye.

ÖZ

Amaç: Meme kanseri kadınlarda görülen en sık kanser olup görülme sıklığı giderek artmaktadır. Günümüzde meme kanserinin tedavisinde cerrahi tedavi, radyasyon tedavisi ve kemoterapi gibi birçok yöntem vardır. Kemoterapi tedavisinde yaygın olarak kullanılan kemoterapötiklerden birisi olan doksorubisin (DOX) belirli bir dozun üzerinde tehlikeli yan etkilere ve ilaç direncine neden olur. Mevcut tedavilere kıyasla kombinasyon tedaviler son zamanlarda bu sorunların üstesinden gelmek için önem kazanmıştır. Bir fotoduyarlaştırıcı ile uygulanan fotodinamik terapi klinikte birçok kanser türünde uygulanmaktadır. Bu çalışma ile meme kanseri tedavisinde kullanılan DOX'u fotodinamik terapi (PDT) ile aktive edilen metilen mavisi (MB) ile kombinasyon uygulayarak DOX'un uygulanmasında düşük toksisite ile yüksek etkinlik alınması amaçlanmıştır.

Yöntem: MCF-7 meme kanseri hücre hattı üzerinde farklı konsantrasyonlarda DOX, MB, MB-PDT, DOX-PDT, MB-PDT+DOX ve DOX+MB-PDT olacak şekilde ayrı ayrı ve kombinasyon tedaviler denenmiştir. Hücre canlılığını belirlemek için MTT testi gerçekleştirilmiş ve hücrelerin %50 canlılık gösterdiği IC_{50} değerleri hesaplanmıştır. Ayrıca, MB ve DOX arasındaki etkileşimin derecesini belirlemek için kombinasyon indeksi (CI) değeri bulunmuştur. Ayrıca DOX ve fotodinamik terapi kombinasyonunun meme kanseri hücrelerine karşı apoptotik potansiyelini belirlemek için apoptotik ve antiapoptotik belirteçlerin (Bax, Bcl-2) mRNA ekspresyon seviyelerine RT-PCR ile bakılmıştır.

Bulgular: MB-PDT ve ardından düşük konsnatrasyonda DOX (428.4 nM) kombinasyonunun, tek başına DOX'a ve önce DOX ardından MB-PDT'ye kıyasla kanser hücresi ölümünü indüklemede daha iyi bir etkiye sahip olduğunu göstermiştir. Aynı zamanda bu kombine uygulama Bcl-2 gen ekspresyonunu düşürürken, Bax gen ekspresyonunu yükselterek apoptotik potansiyelini göstermiştir.

Sonuç: Doksorubisin ve metilen mavisi ile indüklenmiş fotodinamik terapi kombine tedavisi meme kanseri hücreleri üzerinde sinerjik, sitotoksik ve apoptotik etkiler göstererek, umut verici bir yaklaşım sunmuştur.

Anahtar kelimeler: Meme kanseri, doksorubisin, fotodinamik terapi, metilen mavisi, kombinasyon tedavi.

ABSTRACT

Objective: Breast cancer is the most common cancer in women and its incidence is increasing. Today, there are many methods in the treatment of breast cancer, such as surgery, radiation therapy and chemotherapy. Doxorubicin (DOX), one of the chemotherapeutics commonly used in chemotherapy treatment, causes dangerous side effects and drug resistance above a certain dose. Thus, it reduces the effect of the treatment. Compared to existing treatments, combination therapies have recently gained importance to overcome these problems. Therefore, this study aimed to reduce the dose of DOX used in breast cancer treatment and increase the effectiveness of the treatment by combining it with methylene blue (MB) activated by photodynamic therapy (PDT).

Methods: Different concentrations of DOX, MB, MB-PDT, MB-PDT, DOX-PDT, MB-PDT+DOX and DOX+MB-PDT were tested separately and in combination on MCF-7 breast cancer cell line. MTT assay was performed to determine the cell viability and IC50 values were calculated where cells showed 50% viability. In addition, the combination index (CI) value was found to determine the degree of interaction between MB and DOX. In addition, mRNA expression levels of apoptotic and antiapoptotic markers (Bax, Bcl-2) were analysed by RT-PCR to determine the apoptotic potential of DOX and photodynamic therapy combination against breast cancer cells.

Results: The combination of MB-PDT followed by low concentrations of DOX (428.4 nM) had a better effect in inducing cancer cell death compared to DOX alone and DOX followed by MB-PDT. At the same time, this combined treatment showed its apoptotic potential by decreasing Bcl-2 gene expression and increasing Bax gene expression.

Conclusion: The combined treatment of doxorubicin and methylene blue-induced photodynamic therapy has shown synergistic, cytotoxic and apoptotic effects on breast cancer cells, offering a promising approach.

Keywords: Breast cancer, doxorubicin, photodynamic therapy, methylene blue, combination therapy.

***Corresponding author/İletişim kurulacak yazar:** Kübra Açıkalın Coşkun; İstanbul Aydın Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji Anabilim Dalı, İstanbul, Türkiye.
Phone/Telefon: +90 (532) 436 14 50, **e-mail/e-posta:** kubraacikalincoskun@aydin.edu.tr
Submitted/Başvuru: 22.06.2024 • **Accepted/Kabul:** 30.05.2025 • **Published Online/Online Yayın:** 30.06.2025

Giriş

En yaygın ve öldürücü hastalıklardan biri olan kanser, hücrelerin kontrolsüz bir şekilde çoğalması ve bulundukları bölge dışına yayılarak metastaz yapması ile gelişir. Akciğer kanserinden sonra ikinci sıklıkta görülen kanser çeşidi olan meme kanseri, kadınlarda en sık görülen kanser türünün başında gelir.[1]

Çeşitli kriter ve sınıflandırmalar olmasına rağmen, en temel meme kanseri türleri üç gruba ayrılabilir; meme kanseri eksprese eden hormon reseptörü (östrojen reseptörü (ER+) veya progesteron reseptörü) (PR+)), insan epidermal reseptörü 2'yi eksprese eden meme kanseri (HER2+) ve üçlü negatif meme kanseri (TNBC) (ER−, PR−, HER2−).[2] TNBC, ER, PR veya HER2 eksprese etmez ve meme kanserlerinin %15-20'sinde görülmektedir. HER2 ise meme kanserlerinin %20-25'ini oluşturur.[3] Meme kanseri türleri içerisinde en agresif olanı olan TNBC çoğunlukla beyin, kemikler, akciğerler ve karaciğere metastaz yapar. Bununla birlikte, çoğu zaman tanı anında ileri evrelerde fark edildiğinden yüksek nüks oranına ve düşük hayatta kalma oranına yol açar.[4]

Cerrahi müdahale, radyasyon ve kemoterapi kanser tedavisinde kullanılan 3 temel tedavi yöntemidir. Kullanılan en yaygın yöntem olan kemoterapi sürecinde birden fazla anti-kanser ilaç uygulanarak başarıya ulaşma hedeflenir. Ancak kanserin mutasyona uğraması, hastalarda ilaç direncinin gelişmesi, çeşitli yan etkilerin görülmesi ya da metastatik meme kanserinin son evrede tanısının konması bu başarıyı gölgelemektedir.[5] Bu nedenle pek çok anti-kanser ilaç, hastalar üzerinde beklenen etkisini gösterememekte ve hastalığın ilerlemesine neden olmaktadır. Bu kapsamda kombine tedaviler önem kazanmaktadır.

Fotodinamik terapi (PDT) malign tümörlere karşı seçici sitotoksik aktivite gösterirken normal dokuya düşük toksisite gösteren bir tedavi olup son yıllarda kanser tedavisinde büyük ilgi görmüştür.[6] Üç temel bileşeni vardır; ışığa duyarlılaştırıcılar (photosensitizer), belirli dalga boylarında ışık ve oksijen. Tek başlarına toksik değillerdir. Ancak, belirli bir dalga boyundaki ışıkla uyarılan ışığa duyarlılaştırıcıların oksijenle moleküler reaksiyona girmesiyle hedef dokuda reaktif oksijen türlerini oluşturur. Böylece apoptoz veya nekroz yoluyla tümör hücrelerinin ölümüne yol açar.[7] Metilen mavisi (MB), PDT tedavisinde ışığa duyarlaştırıcı olarak kullanılan bir boyadır. MB, metiltiyoninyum klorür olarak da bilinir. Hidrofilik ve fenotiyazin türevidir. 660 nm'de maksimum optik absorpsiyon sunar.[8] MB ışık varlığında aktive olurken karanlıkta toksisite üretmez. MB ayrıca düşük maliyetli bir ışığa duyarlılaştırıcıdır. MB, tüm bu özellikleriyle PDT'de kullanılan iyi bir ışığa duyarlaştırıcı seçeneğidir.[9]

Doksorubisin (DOX), *Streptomyces peucetius* türlerinden izole edilen bir antrasiklin antibiyotiktir. Adriamisin olarak da bilinir ve birçok kanser çeşidinde antikanser ilaç olarak kullanılmaktadır. DOX enterkalasyon yoluyla DNA ile etkileşerek DNA polimerazı inhibe eder. Böylece nükleik asit sentezini durdurur. Ayrıca, DNA replikasyonunda önemli rolü olan topoizomeraz II ile etkileşime girerek enzimin ilerlemesini engeller.[10] Ne yazık ki DOX oldukça etkili olmasına rağmen kanser hücreleri için seçici değildir ve toksisitesi kullanımını ciddi şekilde sınırlamaktadır. Bu toksisite sonucunda kalp, beyin, karaciğer ve böbrekler etkilenir ve bu etkilerin görülmesi yıllar alabilir.[11]

Apoptozun moleküler mekanizmalarındaki kilit düzenleyicilerden biri Bcl-2 ailesidir. Bu proteinler hücre membranıyla ilişkili veya sitozolde serbest halde bulunur. İntrinsik apoptozu düzenleyen mitokondrinin hücre içi membranlarında işlev görürler. Bu aile Bax gibi proapoptotik ve Bcl-2 gibi antiapoptotik üyeleri içerir. Hem sitozolik hem de mitokondriyal Bcl-2 ailesi üyelerinin ekspresyon seviyelerindeki değişime bağlı olarak aralarındaki iletişim, hücrenin ölüm veya hayatta kalma kaderini belirler[12,13].

Kombinasyon tedavisinde iki veya daha fazla tedavi kullanılarak tedavinin etkinliğini arttırmak amaçlanır. Birçok araştırma kombine tedavinin tümör büyümesini ve ilaç direncini azaltabileceğini göstermiştir.[14,15] Bu yüzden bu çalışmada MCF-7 meme kanseri hücre hattında DOX ve MB ile aktive edilen PDT'nin kombine tedavisi uygulanarak DOX toksisitesini ve yan etkilerini azaltmak amaçlanmıştır.

Yöntem

Hücre Kültürü

Ticari olarak temin edilen MCF-7 hücre hattı (HTB-22, The American Type Culture Collection;ATCC, Manassas, VA, USA). 37°C'de, %5 CO_2'li ve %95 nem içeren ortamda %10 fetal bovin serumu (FBS, 12103C, Sigma-Aldrich, USA), yüksek glikozlu Dulbecco modifiye Eagle's besiyeri (DMEM, ECB7501L, Euroclone S.p.A, Italy) ve 1% Penicillin- streptomycin (10,000 U/mL, Capricorn Scientific, Germany) içeren besiyerinde kültüre edilmiştir. %80-90 yoğunluğa ulaşan MCF-7 Dulbecco's fosfat buffer saline (DPBS, ECB4004, Euroclone S.p.A, Italy) ile yıkanıp %0.25 Tripsin-EDTA (T4049, Sigma-Aldrich, USA) ile kaldırılmıştır. Tripsin-EDTA-hücre karışımı 1200 rpm'de 3 dk santrifüj edilmiştir. Santrifüj sonrasında süpernatant uzaklaştırılmış ve taze besiyeri eklenmiştir. Hücreler 96 kuyucuklu plakalara ($6x10^4$ hücre/kuyucuk, Euroclone S.p.A., Italy) ekilerek inkübe edilmiştir.

MTT Testi

MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromür) kolorimetrik bir yöntemdir. Hücre canlılığının, çoğalmasının ve sitotoksisitenin bir göstergesi olarak hücrelerin metabolik aktivitesini ölçmek için kullanılır. MTT sarı renkli bir tetrazolyum tuzudur ve metabolik olarak aktif hücreler tarafından mor formazan kristallerine indirgenir. Belirli bir miktar formazanın DMSO içerisinde çözünmesiyle oluşan bu mor çözeltinin absorbansı 570 nm'de spektrofotometrik olarak ölçülebilmektedir. Oluşan mor rengin miktarı canlı hücre sayısıyla doğru orantılıdır.[16]

Kombine olarak kullanımda Doksorubisinin ve MB'nin etkin dozunu (IC_{50}) belirlemek için MCF-7 hücreleri 96

kuyucuklu plakalara $6x10^4$ hücre/kuyucuk şekilde ekilmiştir. Hücrelerin 24 saat kuyucuklara tutunmasının ardından DOX ve MB için 32-16-8-4-2-1-0,5-0,25 µM konsantrasyonlarda 5 tekrar olacak şekilde 24 saat boyunca inkübe edilmiştir. Etken maddenin uygulanmadığı hücre grubu kontrol grubu olarak kabul edilmiştir. Deney süresi sonunda tüm kuyucuklardan besiyeri uzaklaştırılıp kuyucuklara 100 µL besiyeri ile 20 µL (5 mg/mL) MTT solüsyonu (T0793(DB0362),Bio Basic Inc., Markham ON, Canada) eklenmiştir. 3 saat inkübasyonun ardından her kuyucuğa 100 µL DMSO (MERCK) ilave edilmiştir. Absorbans değerleri 570 nm'de ELISA okuyucuda (Multiskan GO-Thermo) ölçülmüştür. Sonuçlar GraphPad Prism 9 (California, USA) programı kullanılarak hücre canlılığının %50 azalmasını sağlayan doz (IC_{50}) değerleri hesaplanmıştır. Tüm istatistiksel analizler GraphPad Prism 9 (California, USA) kullanılarak gerçekleştirilmiştir. ± SD olarak ifade edilmiştir. Veriler One-Way ANOVA ile analiz edilmiştir. 0.05'ten küçük p-değerleri istatistiksel olarak anlamlı kabul edilmiştir.

Fotodinamik Terapi

Hücreler taze kültür ortamı uygulanarak 96 kuyucuklu plakaya ekilmiş ve ardından 37°C'de 24 saat boyunca %5 CO2 altında inkübe edilmiştir. Ardından, hücrelere farklı konsantrasyonlarda MB (32-16-8-4-2-1-0,5-0,25 µM) ve DOX (32-16-8-4-2-1-0,5-0,25 µM içeren besiyerinde 1 saat inkübe edilmiştir. İnkübasyon sonrası hücreler PBS tamponu ile yıkandı ve ardından 660 nm ile 60 sn ışınlama yapılmıştır. 24 saat inkübasyon sonrasında Hücrelerin canlılığını belirlemek için MTT testi uygulanmıştır. Her deney 5 kez tekrarlanmıştır. Lazer ve ilaç uygulanmayan hücreler kontrol olarak eklenmiştir.

MCF-7 Hücrelerinde MB-PDT ve DOX ile Kombinasyon Tedavi

Bu çalışmalar için iki farklı kombinasyon yapılmıştır.

Önce MB-PDT Ardından DOX ile Tedavi

96 kuyucuklu plakalara $6x10^4$ hücre/kuyucuk olacak şekilde ekilen MCF-7 hücreleri tutunması için 24 saat 37°C'de, %5 CO_2'de inkübe edilmiştir. 24 saat sonunda hücrelere MB için hesaplanan IC_{50} değerinin altındaki bir konsantrasyon (3,5 µM) 5 tekrar olacak şekilde taze besiyeri ile eklenmiş ve 1 saat inkübe edilmiştir. İnkübasyonun ardından tüm kuyucuklardan besiyeri uzaklaştırılıp PBS ile yıkanmıştır. Hücrelere 660 nm dalga boyu ile 60 saniye lazer ışığı uygulanmıştır. Daha sonra hücrelere farklı konsantrasyonlarda DOX (32-16-8-4-2-1-0,5-0,25 µM) ilave edilip ve 24 saat inkübasyona bırakılmıştır. İnkübasyonun ardından hücre canlılığını belirlemek için MTT testi gerçekleştirilmiştir.

Önce DOX Ardından MB-PDT ile Tedavi

MCF-7 hücreleri $6x10^4$ hücre/kuyucuk olacak şekilde 96 kuyucuklu plakalara ekilmiş ve 24 saat inkübe edilmiştir. Daha sonra hücrelere farklı konsantrasyonlarda DOX (32-16-8-4-2-1-0,5-0,25 µM) ilave edilip ve 24 saat inkübasyona bırakılmıştır. İnkübasyonun sonunda tüm kuyucuklardan besiyeri uzaklaştırılıp PBS ile yıkanmıştır. 3,5 µM MB içeren taze besiyeri ile 1 saat inkübe edilmiştir. Ardından hücrelere 660 nm dalga boyu ile 60 saniye lazer ışığı uygulanmış olup hücre canlılığını belirlemek için MTT testi gerçekleştirilmiştir.

Ayrıca tedavileri daha iyi karşılaştırmak amacıyla DOX+PDT (lazer), MB+PDT, MCF-7+PDT tedavileri uygulanmıştır. Uygulanan DOX ve MB dozları 32-16-8-4-2-1-0,5-0,25 µM'dır. 5 tekrar olacak şekilde 24 saat boyunca inkübe edilmiştir. İnkübasyon sonrası 660 nm dalga boyu ile 60 saniye lazer ışığı uygulanmış ve hücre canlılığını belirlemek için MTT testi yapılmıştır.

Kombinasyon indeksi (CI) Hesaplanması

MB ve DOX arasındaki sinerjik etki, CompuSyn yazılım programı (Chou and Martin, 2005, Compusyn Inc, USA) kullanılarak hesaplanmıştır. Kullanılan formül CI = D1/(Dx)1 + D2/(Dx)2. (Dx)1 ve (Dx)2, belirlenen bir konsantrasyonda hücre büyümesini inhibe etmek için gereken ve tek başına uygulanan MB ve DOX dozlarıdır. D1 ve D2 ise, kombinasyon halinde uygulanan DOX ve MB dozlarıdır. 1'in altındaki CI değerleri sinerjiyi (CI < 1), 1'e eşit olan CI değerleri (C=1) aditif etkiyi ve 1'in üzerindeki CI değerleri antagonizmi (CI > 1) gösterir. Etkilenen fraksiyon (Fa) dozdan etkilenen büyüme inhibisyonunu belirtir.[17] Fa'ya ilişkin ortalama CI değerleri, DOX ve MB'ye ait sekiz kombinasyonu hesaplamak için kullanıldı.

Gene Ekspresyon Analizi

MCF-7 hücresinde anti-apoptotoik, pro-apoptotik genlerin (Bax ve Bcl-2) mRNA ekspresyon seviyeleri sadece DOX, DOX+MB-PDT ve MB-PDT+ DOX kombinasyonu kullanılarak qPCR ile belirlenmiştir. Hücrelere 24 saatlik inkübasyonda belirlenen IC50 değerlerinde DOX, MB ve 660 nm lazer uygulanmıştır. Ardından, total RNA izolasyonu ve ardından cDNA sentezi gerçekleştirilmiştir. Referans gen olarak GADPH kullanılmıştır. Primerler, Bax ve Bcl-2 Gen Bankasında yayınlanan gen dizisine göre Primer3 yazılımı kullanılarak tasarlanmıştır. Primer setleri Tablo 1'te verilmiştir. İfade düzeyleri 2-ΔΔCt yöntemi ile hesaplanmıştır.

Tablo 1. Primer dizileri

Primer	Dizi
Bax-F	TCAGGATGCGTCCACCAAGAAG
Bax-R	TGTGTCCACGGCGGCAATCATC
Bcl-2-F	ATCGCCCTGTGGATGACTGAGT
Bcl-2-R	GCCAGGAGAAATCAAACAGAGGC
GADPH-F	GTCTCCTCTGACTTCAACAGCG
GADPH-R	ACCACCCTGTTGCTGTAGCCAA

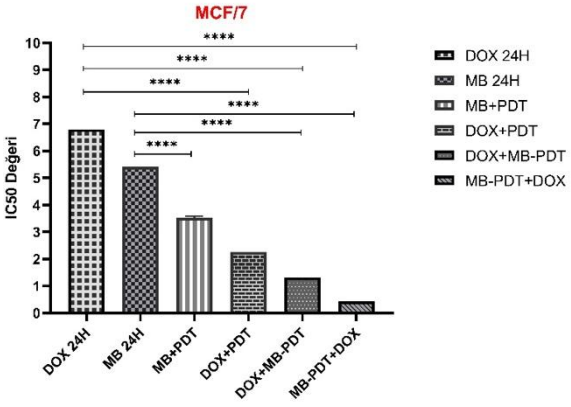
İstatiksel Analiz

Tüm istatistiksel analizler GraphPad Prism 9 (California, USA) kullanılarak gerçekleştirilmiştir. ± SD olarak ifade edilmiştir. Veriler One-Way ANOVA ile analiz edilmiştir. 0.05'ten küçük p-değerleri istatistiksel olarak anlamlı kabul edilmiştir.

Bulgular

DOX'un ve MB'nin MCF-7 Hücre Hattındaki Etkisi

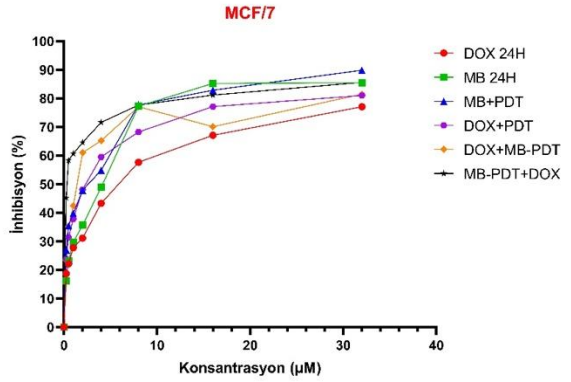
Farklı konsantrasyonlarda (32-16-8-4-2-1-0,5-0,25 µM) uygulanan DOX'un meme kanseri hücre hattı MCF-7'nin canlılığı üzerindeki etkisi araştırılmıştır. Sonuçlar, DOX ve MB'nin konsantrasyonunun artmasıyla hücre canlılığının azaldığını, inhibisyon değerinin arttığını göstermiştir. DOX'un 24 saatte MCF-7 hücre hattındaki IC_{50} değeri 6,8 µM ve MB'nin ise 5,42 µM olarak hesaplanmıştır (Şekil 1).



Şekil 1. Doksorubisin (DOX), metilen mavisi (MB) ve kombinasyon tedavilerin (MB+ fotodinamik terapi (PDT), DOX+PDT, DOX+MB-PDT, MB-PDT+DOX) IC_{50} değerleri (****p<0.0001).

DOX+PDT'nin ve MB+PDT'nin MCF-7 Hücre Hattındaki Etkisi

DOX'un ardından yapılan lazerin etkisi 32-16-8-4-2-1-0,5-0,25 µM konsantrasyonlarda uygulanarak incelenmiştir. Sadece DOX uygulaması ile karşılaştırıldığında 32-16-8-4-2-1-0,5-0,25 µM'da %77, %67, %57,6, %43,2, %31,1, %27,8, %22,1, %18,6 olan inhibisyon değerleri %80,9, %77,1, %68,2, %59,4, %48,1, %37,8, %31,4, %23,7'ye yükselmiştir (Şekil 2). Sadece DOX'un 24 saatteki IC_{50} değeri 6,8 µM iken bu değer DOX+PDT'nin birlikte uygulamasında 2,26 µM olarak bulunmuştur (****p<0.0001).



Şekil 2. Doksorubisin (DOX), metilen mavisi (MB), ve kombinasyon tedavilerin (MB+ fotodinamik terapi (PDT), DOX+PDT, DOX+MB-PDT, MB-PDT+DOX) MCF-7 hücre hattındaki inhibisyon grafiği.

Sonuçlar, 32-16-8-4-2-1-0,5-0,25 µM konsantrasyonlarda MB ardından uygulanan lazerin etkisi tek başına uygulanan MB'ye göre inhibisyon değerini daha da arttırdığını göstermiştir. %85,4, %85,2, %77,2, %48,8, %35,6, %29,6, %23,1, %16,1 olan inhibisyon değerleri %89,8, %82,8, %77,7, %54,8, %47,8, %39,7 %35,4 ve %26,9'a yükselmiştir. Ek olarak, 5,42 µM olan IC_{50} değeri lazerin etkisi ile 3,57 µM'a gerilemiştir.

DOX+MB-PDT'nin MCF-7 Hücre Hattındaki Etkisi

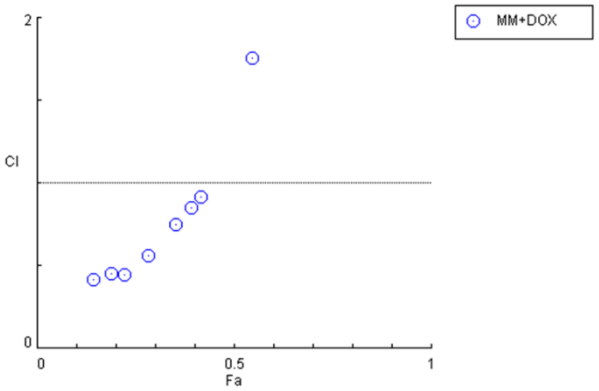
Şekil 2. ye göre 24 saatlik DOX tedavisinden sonra uygulanan MB-PDT'nin DOX+PDT'ye göre inhibisyon değerinde çok önemli bir artma görülmese de IC_{50} değeri 2,26 µM'dan 1,32 µM'a düşmüştür.

MB-PDT+DOX'un MCF-7 Hücre Hattındaki Etkisi

MB-PDT ardından uygulanan DOX tedavisi sonucunda inhibisyon değeri %85,5, %81,1, %77,6, %71,6, %64,5, %60,6, %58,3, %45,1'ye önemli ölçüde artmıştır. IC_{50} değeri 428.4 nM bulunmuştur. Böylece MB+PDT tedavisinde elde edilen IC_{50} değeri (3,57 µM) DOX etkisi ile 428.4 nM'a gerilemiştir. Sonuç olarak tüm tedaviler karşılaştırıldığında, en yüksek ölüm ve inhibisyon yüzdesi, hücreler MB-PDT ve ardından DOX tedavisi uygulandığında gözlenmiştir (****p<0.0001).

MB-PDT+DOX Tedavisinin MCF-7 Hücreleri Üzerindeki Kombinasyon İndeksi (CI) Analizi

MB-PDT ardından 32-16-8-4-2-1-0,5-0,25 µM konsantrasyonlarda uygulanan DOX tedavisinin MCF-7 hücrelerindeki kombinasyon indeksi (CI) analiz grafiği Şekil 3'te gösterilmiştir. CI <1, =1 ve >1 değerleri sırasıyla sinerjistik, aditif ve antagonistik etkileri temsil eder. İnhibitör oranını ise etkilenen fraksiyon (Fa) gösterir.



Şekil 3. MB-PDT'den (Metilen mavisi-Fotodinamik terapi) sonra uygulanan farklı konsantrasyonlardaki doksorubisin (DOX) tedavisinin MCF-7 hücrelerinde büyüme inhibisyonunun kombinasyon indeksi (CI) analizi.

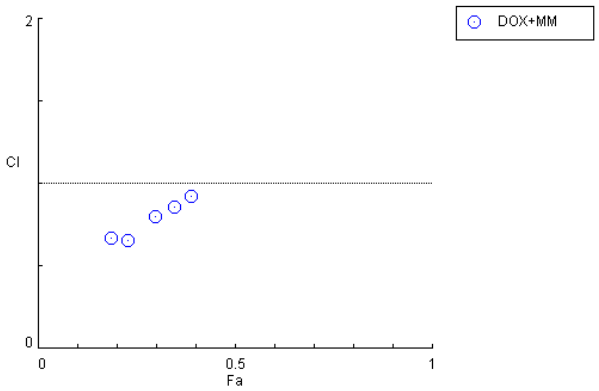
Analiz sonucu MB-PDT ardından DOX kombinasyonunun 0,25 µM hariç tüm konsantrasyonlarda CI değerinin 1'den düşük ve sinerjistik olduğunu ortaya çıkarmıştır (Tablo 2).

Tablo 2. MB-PDT'den (Metilen mavisi-Fotodinamik terapi) sonra uygulanan farklı konsantrasyonlardaki doksorubisin (DOX) tedavisinin kombinasyon indeksi (CI) analizi.

MB Dozu (µM)	DOX Dozu (µM)	CI Değerleri	Etkisi
3,5	32	0,41378	Sinerjizm
3,5	16	0,44999	Sinerjizm
3,5	8	0,44732	Sinerjizm
3,5	4	0,56188	Sinerjizm
3,5	2	0,75277	Sinerjizm
3,5	1	0,85384	Sinerjizm
3,5	0,5	0,91456	Sinerjizm
3,5	0,25	1,75517	Antagonizm

DOX+MB-PDT Tedavisinin MCF-7 Hücreleri Üzerindeki Kombinasyon İndeksi (CI) Analizi

DOX ardından uygulanan MB-PDT tedavisinin MCF-7 hücrelerindeki kombinasyon indeksi (CI) analiz grafiği Şekil 4'te gösterilmiştir.



Şekil 4. Farklı konsantrasyonlarda uygulanan doksorubisin (DOX) tedavisi ardından yapılan MB-PDT (Metilen mavisi-Fotodinamik terapi) tedavisinin MCF-7 hücrelerinde büyüme inhibisyonunun kombinasyon indeksi (CI) analizi.

Analiz sonuçları DOX ardından uygulanan MB-PDT tedavisinin 32-16-8-4-2 µM'da CI değeri 1'den düşük ve sinerjistik etki, 1-0,5-0,25 µM'da CI değeri 1'den büyük ve antagonist etki göstermiştir (Tablo 3).

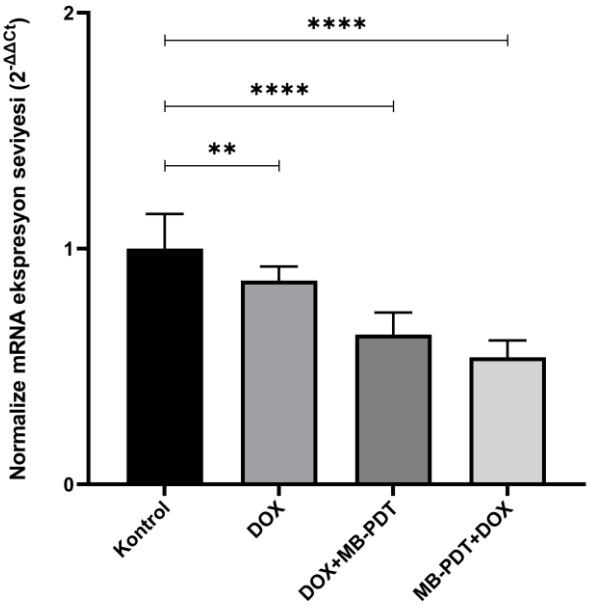
Tablo 3. Farklı konsantrasyonlarda uygulanan doksorubisin (DOX) tedavisi ardından yapılan MB-PDT (Metilen mavisi-Fotodinamik terapi) tedavisinin kombinasyon indeksi (CI) analizi.

MB Dozu (µM)	DOX Dozu (µM)	CI Değerleri	Etkisi
3,5	32	0,67315	Sinerjizm
3,5	16	0,65838	Sinerjizm
3,5	8	0,80353	Sinerjizm
3,5	4	0,85953	Sinerjizm
3,5	2	0,92748	Sinerjizm
3,5	1	2,30276	Antagonizm
3,5	0,5	3,19629	Antagonizm
3,5	0,25	6,88738	Antagonizm

Gen Ekspresyon Sonuçları

MCF-7 hücrelerinde tek başına DOX uygulnmasına kıyasla kombine DOX ve MB aracılı fotodinamik terapi tedavisi MCF-7 hücrelerinde Bcl-2 ve Bax genlerinin mRNA ekspresyon seviyelerini değiştirmiştir. Bu değişim Önce MB-PDT sonra DOX uygulanmasında daha fazla olmuştur. Sonuçlar kontrole göre normalize edilmiş ve istatiksel açıdan anlamlı olması değerlendirilmiştir.

Bcl-2'nin mRNA gen ekspresyon seviyeleri yalnızca Dox için MCF-7'de istatistiksel olarak anlamlı bir şekilde kontrole göre 0.86 kat, DOX+MB-PDT kombinasyonu için 0.63 kat ve MB-PDT +DOX kombinasyonu için 0.53 kat azalmıştır (Şekil 5).



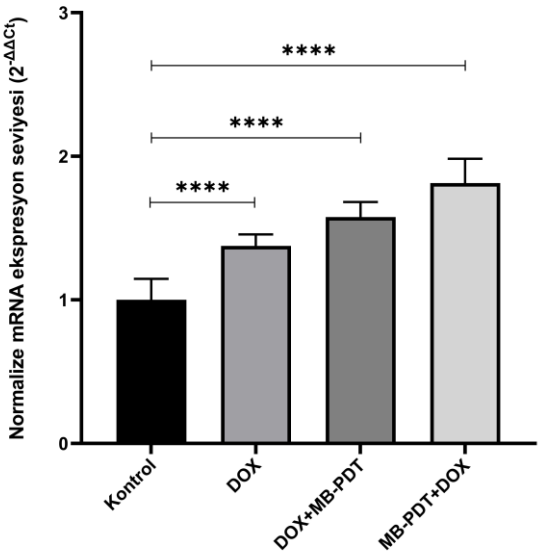
Şekil 5. DOX tek başına ve kombinasyon halinde 24 saatlik inkübasyonda Bcl-2 mRNA ekspresyon seviyeleri üzerindeki etkisi. Veriler kontrole normalize edilmiştir. (n=3, **p < 0.01, ****p < 0.0001).

Bax için mRNA ekspresyon seviyeleri sadece hücerelere DOX uygulandığında kontrole göre gen ifadesi 1.37 kat, önce DOX ardından MB-PBT uygulandığında 1.57 kat ve önce MB-PDT ardından DOX uygulandığında 1.81 kat artmıştır (Şekil 6). Bu artışlar üç grup içinde kontrole göre istatiksel açıdan anlamlı bulunmuştur.

Tartışma

Antrasiklin antibiyotik doksorubisin (DOX) yıllardır meme kanseri, yumurtalık kanseri, akciğer karsinomu, akut lösemi gibi pek çok kanser çeşidinde kullanılan bir anti-kanser ajandır[10]. Klinikte kullanımı yaygın olmasına rağmen sitostatik etkisi genellikle terapötik dozlarda yetersizdir. Daha yüksek dozlarda kullanımı ise kalp, beyin, karaciğer ve böbrekler dahil olmak üzere çeşitli organlarda çeşitli olumsuz etkilere neden olabilir. Ayrıca kanser hücrelerinde DOX direncinin gelişmesi tedavinin etkinliğini azaltmaktadır.[18] Bu sorunların üstesinden gelmek ve DOX'un etkinliğini artırmak için çeşitli

çalışmalar yapılmıştır. Bu çalışmalardan bir tanesi PDT ile kombinasyon tedavidir.



Şekil 6. DOX tek başına ve kombinasyon halinde 24 saatlik inkübasyonda Bax mRNA ekspresyon seviyeleri üzerindeki etkisi. Veriler kontrole normalize edilmiştir. (n=3, ****p<0.0001).

Metilen mavisi (MB), fotodinamik terapide (PDT) ışığa duyarlılaştırıcı olarak kullanılan bir fenotiyazinil boyadır ve antikanser tedavisi için umut verici olmuştur. Akridin boyalarına benzer düzlemsel yapısı nedeniyle MB, DOX ile benzer şekilde DNA ile kolayca etkileşime girebilir.[19] Bu yüzden birçok çalışma PDT ile DOX'u kombinasyon uygulayarak tedavi etkisinin arttırabileceğini göstermiştir.

Aniogo ve ark.[20] DOX ile sülfonatlı çinko ftalosiyanin (ZnPcS) PDT ile anti-kanser etkisini MCF-7 hücre hattında ayrı ayrı ve kombinasyon halinde incelediler. DOX ve ZnPcS konsantrasyonları giderek arttırıldı. Bu çalışmanın sonuçları, DOX-PDT kombinasyon tedavisinin MCF-7 hücrelerinin çoğalmasını ve büyümesini daha etkili bir şekilde engellediğini gösterdi. Ayrıca PDT'nin daha düşük dozlarda DOX ile kombinasyonunun meme kanseri tedavisinde umut verici bir kombinasyon terapi stratejisi olduğunu göstermektedir. Zakaria ve ark.[19] MCF-7 hücrelerinde DOX ile 5-Aminolevulinic acid (ALA)'nın PDT ile etkisini araştırdılar. DOX + ALA/PDT ile tedavi edilen hücrelerde, bireysel etkileriyle karşılaştırıldığında daha fazla hücre ölümü görüldü.

Dos Santos ve ark.[22] üç meme epitel hücre hattında MB kullanarak PTD'nin hücre öldürme potansiyelini değerlendirdi. MDA-MB-231 (TNBC), MCF-7 hücre hatlarında ve neoplastik olmayan MCF-10A hücre hattında MB varlığında ve yokluğunda 660 nm'de PDT uygulandı. En yüksek ölüm malign hücrelerde sırasıyla MDA-MB-231, MCF-7 ve MCF 10A hatlarında gözlendi. Yousefi Sadeghloo ve ark.[23] farklı konsantrasyonlarda DOX, MB ve MB-DOX'un kombinasyon terapisinin MDA-MB-231 hücre hattı üzerindeki etkisini araştırdı. Önce DOX ardından MB-PDT tedavisi ve önce MB-PDT sonra DOX tedavisi olacak şekilde iki farklı uygulama yapılmıştır. Sonuçlar, MB-PDT ve ardından DOX'un (düşük konsantrasyonlarda bile) kullanıldığı kombinasyonun, kanser hücresi ölümünü tetiklemede tek başına DOX'tan daha etkili olduğunu göstermiştir.

Bu çalışmada DOX'un MCF-7 meme kanseri hücreleri üzerinde ayrı ayrı ve MB-PDT varlığında DOX'un etkilerini değerlendirdik. Böylece MB ile fotodinamik tedavinin etkinliğini arttırarak hücre ölüm oranının arttırılması ve ilaç dozunu azaltarak yan etkilerinin azaltılması hedeflenmiştir. DOX, MB, MB-PDT, DOX-PDT, MB-PDT+DOX ve DOX+MB-PDT olacak şekilde ayrı ayrı ve kombinasyon tedaviler denenmiştir. Sonuçlar MB-PDT ardından uygulanan DOX tedavisinin DOX ardından MB-PDT tedavisine göre ve tek başına uygulanan her tedaviye göre MCF-7 hücrelerinde en yüksek ölüm ve inhibisyon gösterdiğini ortaya koymuştur. Ayrıca, MCF-7 hücrelerinin önce DOX ardından MB-PDT tedavisi sonucunda 32-16-8-4-2 µM'da sinerjizm, ve 1-0,5-0,25 µM'da antagonist etki gösterse de MCF-7 hücrelerinin önce MB-PDT ve daha sonra DOX ile tedavi edilmesi durumunda terapide sinerji oluşmuş ve düşük konsantrasyonlarda da (1 ve 0,5 µM) DOX'un artan etkisiyle sonuçlanmıştır. Böylece bu çalışmanın sonuçları önceki yapılan sonuçlar ile uyumlu olmuştur ve yapılan çalışmalara benzer şekilde MB, DOX ile kombinasyon halinde etkinliği arttırmış ve ilaç konsantrasyonunu azaltmıştır.

Doksorubisin gibi sitotoksik ajanlar, duyarlı hedef hücrelerde ölüm sinyal yollarını başlatarak apoptozu indükleyebilirler. Apoptoz belirteçleri olarak bilinen çeşitli genlerin ekspreyon seviyeleri ile ajanların apoptoz sürecindeki etkisi belirlenmektedir. Bunlardan Bcl-2 geni apoptozun başlmasını aktive ederken, Bax geninin ekspresyondaki artış apoptozu indüklemektedir. Çok çeşitli deneysel ve klinik raporlar doksorubisin için biyolojik etkiler göstermiştir. Benzer sonuçlar Bcl-2 ekspresyonunda azalmaya ve Bax ekspresyonunda artışa neden olan doksorubisin ile de gösterilmiştir[13].

Bu çalışmada, MCF-7 hücrelerinde doksorubisin ile apoptozisin uyarılmasını takiben ayrıca fotodimamik terapi uygulanarakl Bcl-2 (anti-apoptotik), Bax (pro-apoptotik) genlerinin ekspresyonu, seviyesindeki değişim rapor edilmiştir. Sonuçlara bakıldığında DOX ile tedavi edilen MCF-7 hücrelerinde Bcl-2 geninin ekspresyonundaki azalma (0.86 kat) fotodinamik terapinin DOX ile muamelesinden önce yada sonra verilmesine bağlı olarak değişmiştir. Önce MB+PDT sonra DOX uygulandığı zman Bcl-2 gen ekspresyonununda azalma (0.53 kat) olmuştur. Ancak hücrelere önce DOX sonra MB+PDT uygulanmasıyla bile (0.63 kat) sadece DOX muamelesine göre Bcl-2 geni daha fazla aşağı regüle olmuştur.

Aynı şekilde Bax geninin ekpresyon seviyelerina baktığımız zaman gruplae arasındaki değişimler Bcl-2 gen değişimleriyle aynı paterni göstermiştir. Bax geni hücreleri apoptoza yönlendiren bir aktivatör olarak rol oynadığı için ekspresyonda yükselme gözlenmesi yaptığımız çalışmayı desteklemektedir. Yalnız DOX uygulandığı zaman Bax ekspresyonu 1.37 kat, önce DOX

sonra MB+PDT uygulandığında 1.57 kat ve önce MB+PDT sonra DOX uygulandığı zaman ise 1.81 kat artmıştır.

Gen ekspresyonundaki bu değişimler literatürde daha önce yapılan çalışmaları [24] desteklediği gibi yalnızca doksorubisin kullanımı değil beraberinde fotodinamaik terapinin de uygulnması hem doksorubisinin etkinlğini, artırmış ve hücrelerin daha fazla apoptoza sürüklenmesini sağladığını desteklemiştir.

Sonuç olarak, MB-PDT ardından uygulanan DOX'un IC_{50} değeri (428.4 nM) tek başına uygulanan DOX'un IC_{50} değerine (6,8 µM) göre oldukça düşük bir değer bulunmuş ve DOx ile fotodinamik terapi sinerjik bir etki göstermiştir. Bu etkiye parelel olarak antiapoptotik ve proapoptotik gen değişimleri de bu sonuçlarla körele olarak artış ve azalış göstererek sitotoksisite sonuçlarını desteklemiştir. Böylece bu çalışmanın sonuçları PDT tedavisinde MB ile kombine olarak uygulanan DOX dozunun azaldığını göstermiştir. Bu, DOX'un yan etkilerinin en aza indirilmesiyle sonuçlanabilir. Dolayısıyla bu yaklaşım gelecekte meme kanserinin tedavisi için umut verici bir strateji olabilir. Daha fazla gen ve protein ekspresyon seviyelerindeki değişimler yolak analizleri floresan görüntülemelerde bu sonuçların doğruluğu daha artırılabilir. Ayrıca meme kanserinin farklı alt tiplerindeki hücre hatlarında daha çok çalışmaya ihtiyaç vardır.

Etik Standartlara Uygunluk

Bu çalışma etik kurul onayı gerektirmemektedir.

Çıkar Çatışması

Yazarların konuyla ve/veya herhangi başka bir yazar ile ilgili maddi veya manevi bir çıkar çatışması yoktur.

Yazar Katkıları

Yazarlar eşit katkıda bulunmuşlardır.

Finansal Açıklama

Yazarlar tarafından finansal destek almadıkları bildirilmiştir.

Kaynaklar

1. Sun YS, Zhao Z, Yang ZN, et al. Risk Factors and Preventions of Breast Cancer. *Int J Biol Sci*. 2017;13(11):1387-1397. doi:10.7150/ijbs.21635
2. Jin X, Mu P. Targeting Breast Cancer Metastasis. *Breast Cancer (Auckl)*. 2015;9(Suppl 1):23-34. doi:10.4137/BCBCR.S25460
3. Ferrando-Díez A, Felip E, Pous A, Bergamino Sirven M, Margelí M. Targeted Therapeutic Options and Future Perspectives for HER2-Positive Breast Cancer. *Cancers (Basel).* 2022;14(14):3305. doi:10.3390/cancers14143305
4. Elsawaf Z, Sinn HP. Triple-Negative Breast Cancer: Clinical and Histological Correlations. *Breast Care (Basel).* 2011;6(4):273-278. doi:10.1159/000331643
5. Mangla B, Kohli K. Combination of Natural Agent with Synthetic Drug for the Breast Cancer Therapy. *Int J Drug Dev & Res*. 2018; 10: 22–26.
6. Correia JH, Rodrigues JA, Pimenta S, Dong T, Yang Z. Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions. *Pharmaceutics*. 2021;13(9):1332. doi:10.3390/pharmaceutics13091332
7. Agostinis P, Berg K, Cengel KA, et al. Photodynamic therapy of cancer: an update. *CA Cancer J Clin.* 2011;61(4):250-281. doi:10.3322/caac.20114
8. Chen CW, Chan YC, Hsiao M, Liu RS. Plasmon-Enhanced Photodynamic Cancer Therapy by Upconversion Nanoparticles Conjugated with Au Nanorods*. ACS Appl Mater Interfaces*. 2016;8(47):32108-32119. doi:10.1021/acsami.6b07770
9. Tardivo JP, Del Giglio A, de Oliveira CS, et al. Methylene blue in photodynamic therapy: From basic mechanisms to clinical applications. *Photodiagnosis Photodyn Ther*. 2005;2(3):175-191. doi:10.1016/S1572-1000(05)00097-9
10. Carvalho C, Santos RX, Cardoso S, et al. Doxorubicin: the good, the bad and the ugly effect. *Curr Med Chem.* 2009;16(25):3267-3285. doi:10.2174/092986709788803312
11. Meredith AM, Dass CR. Increasing role of the cancer chemotherapeutic doxorubicin in cellular metabolism*. J Pharm Pharmacol*. 2016;68(6):729-741. doi:10.1111/jphp.12539
12. Khodapasand E, Jafarzadeh N, et al. Is Bax/Bcl-2 Ratio Considered as a Prognostic Marker with Age and Tumor Location in Colorectal Cancer? Iran Biomed J. 2015; 19(2): 69–75. 10.6091/ibj.1366.2015
13. Sharifi S, Barar J, et al. Doxorubicin Changes Bax /Bcl-xL Ratio, Caspase-8 and 9 in Breast Cancer Cells. Adv Pharm Bull. 2015 Sep; 5(3): 351–359. https://doi.org/10.15171%2Fapb.2015.049
14. Fisusi FA, Akala EO. Drug Combinations in Breast Cancer Therapy. *Pharm Nanotechnol.* 2019;7(1):3-23. doi:10.2174/2211738507666190122111224
15. Palmer AC, Sorger PK. Combination Cancer Therapy Can Confer Benefit via Patient-to-Patient Variability without Drug Additivity or Synergy. *Cell.* 2017;171(7):1678-1691.e13. doi:10.1016/j.cell.2017.11.009
16. van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol*. 2011;731:237-245. doi:10.1007/978-1-61779-080-5_20
17. Palmer AC, Sorger PK. Combination Cancer Therapy Can Confer Benefit via Patient-to-Patient Variability without Drug Additivity or Synergy. *Cell.* 2017;171(7):1678-1691.e13. doi:10.1016/j.cell.2017.11.009
18. Hanušová V, Boušová I, Skálová L. Possibilities to increase the effectiveness of doxorubicin in cancer cells killing. *Drug Metab Rev.* 2011;43(4):540-557. doi:10.3109/03602532.2011.609174
19. Usacheva MN, Teichert MC, Biel MA. The role of the methylene blue and toluidine blue monomers and dimers in the photoinactivation of bacteria. *J Photochem Photobiol B*. 2003;71(1-3):87-98. doi:10.1016/j.jphotobiol.2003.06.002
20. Aniogo EC, George BPA, Abrahamse H. In vitro combined effect of Doxorubicin and sulfonated zinc Phthalocyanine-mediated photodynamic therapy on MCF-7 breast cancer cells. *Tumour Biol*. 2017;39(10):1010428317727278. doi:10.1177/1010428317727278
21. Zakaria S, Gamal-Eldeen AM, El-Daly SM, Saleh S. Synergistic apoptotic effect of Doxil ® and aminolevulinic acid-based photodynamic therapy on human breast adenocarcinoma cells. *Photodiagnosis Photodyn Ther.* 2014;11(2):227-238. doi:10.1016/j.pdpdt.2014.03.001
22. Dos Santos AF, Terra LF, Wailemann RA, et al. Methylene blue photodynamic therapy induces selective and massive

cell death in human breast cancer cells. *BMC Cancer*. 2017;17(1):194. doi:10.1186/s12885-017-3179-7

23. Yousefi Sadeghloo A, Khorsandi K, Kianmehr Z. Synergistic effect of photodynamic treatment and doxorubicin on triple negative breast cancer cells. *Photochem Photobiol Sci*. 2020;19(11):1580-1589. doi:10.1039/d0pp00132e
24. Ferreto NP, Calaf GM. Influence of doxorubicin on apoptosis and oxidative stress in breast cancer cell lines. *International Journal of Oncology*. 2016; 3(6):753-762. doi.org/10.3892/ijo.2016.3558. 10.15171/apb.2015.049

doi: 10.53446/actamednicomedia.1586536

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

RISK OF HEPATITIS B VIRUS REACTIVATION IN PATIENTS WITH NEUROLOGICAL DISEASES RECEIVING ANTI-CD20 THERAPIES

NÖROLOJİK HASTALIĞI BULUNAN VE ANTİ-CD20 TEDAVİSİ ALAN HASTALARDA HEPATİT B VİRÜSÜ REAKTİVASYON RİSKİ

Ipek Gungor Dogan[1*] Feyzullah Yadi[1] Damla Cetinkaya Tezer[1] Serkan Demir[1]

[1] University of Health Sciences, Sancaktepe Sehit Prof Dr Ilhan Varank Training and Research Hospital, Department of Neurology, Istanbul, Turkiye.

ABSTRACT

Objective: Anti-CD20 therapies may increase the risk of hepatitis B virus (HBV) reactivation, particularly in patients with prior HBV exposure. Despite the recognized preventive measures for managing HBV reactivation, specific data regarding the safety of anti-CD20 therapies in this context remain limited. This retrospective study aims to evaluate the risk of HBV reactivation with prior HBV exposure among patients with neurological disorders treated with anti-CD20 therapies in a single-center cohort from Türkiye.

Methods: We reviewed the records of 580 patients who received at least one dose of anti-CD20 therapies between July 2018 and March 2024. Patients were stratified according to their HBV serostatus, with particular emphasis on anti-HBc positive individuals, who are considered at risk for HBV reactivation. Quantitative anti-HBs titers and rates of antiviral prophylaxis were also documented.

Results: Among the 71 patients who were anti-HBc positive (12.24% of the total cohort), anti-HBs positivity was detected in 50 patients (70.42%). The majority of patients received antiviral prophylaxis (78%), while 22% did not, reflecting some physicians' preference to withhold prophylaxis based on high anti-HBs titers. In contrast, all anti-HBs negative patients (n=21) were administered prophylaxis (100%). Importantly, no cases of HBV seroconversion or clinically meaningful HBV DNA elevation were observed in any subgroup, including anti-HBs positive patients who did not receive prophylaxis.

Conclusion: Our findings suggest that anti-CD20 therapy does not confer a detectable risk of HBV reactivation in anti-HBc positive patients, including those who are anti-HBs positive and did not receive prophylaxis.

Keywords: Anti-CD20 Therapies, HBV Reactivation, Multiple Sclerosis, Ocrelizumab, Antiviral Prophylaxis

ÖZ

Amaç: Anti-CD20 tedaviler, immün aracılı nörolojik hastalıkların tedavisinde yaygın olarak kullanılmaktadır. Ancak bu tedaviler, özellikle daha önce hepatit B virüsü (HBV) ile karşılaşmış hastalarda HBV reaktivasyon riskini artırabilir. HBV reaktivasyonunun önlenmesine yönelik çeşitli stratejiler mevcut olsa da, anti-CD20 tedavilerinin bu bağlamdaki güvenliğiyle ilgili özgül veriler sınırlıdır. Bu retrospektif çalışmada, Türkiye'de tek merkezde izlenen bir hasta kohortunda, nörolojik hastalıklar nedeniyle anti-CD20 tedavisi alan ve daha önce hepatit B virüsü ile karşılaşmış bireylerde HBV reaktivasyon riskini değerlendirmeyi amaçladık.

Yöntem: Temmuz 2018 ile Mart 2024 tarihleri arasında en az bir doz anti-CD20 tedavisi almış 580 hastanın verileri retrospektif olarak incelendi. Hastalar HBV serolojik durumlarına göre sınıflandırıldı; özellikle HBV reaktivasyon riski taşıyan anti-HBc pozitif bireyler değerlendirmeye alındı. Kantitatif anti-HBs titreleri ve antiviral profilaksi oranları belgelendi.

Bulgular: Anti-HBc pozitif olan 71 hastanın (toplam kohortun %12,24'ü) 50'sinde (%70,42) aynı zamanda anti-HBs pozitifliği mevcuttu. Hastaların çoğuna antiviral profilaksi uygulanmıştı (%78), ancak %22'lik bir gruba uygulanmamıştı; bu durum, bazı hekimlerin yüksek anti-HBs titresi temelinde profilaksiyi vermeme yönündeki tercihlerini yansıttı. Öte yandan, anti-HBs negatif olan tüm hastalara (n=21) profilaksi verilmişti (%100). Takip süresince, profilaksi almayan anti-HBs pozitif hastalar da dahil olmak üzere hiçbir alt grupta HBV serokonversiyonu veya klinik olarak anlamlı HBV DNA artışı gözlemlenmedi.

Sonuç: Bulgularımız, anti-CD20 tedavisinin anti-HBc pozitif hastalarda, anti-HBs pozitifliğinde profilaksi almayan bireylerde dahi belirgin bir HBV reaktivasyon riski oluşturmadığını göstermektedir.

Anahtar Kelimeler: Anti-CD20 tedavileri, HBV reaktivasyonu, Multiple Skleroz, Okrelizumab, Antiviral Profilaksi

***Corresponding author/İletişim kurulacak yazar:** Ipek Gungor Dogan; Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital, Neurology Department, Sancaktepe, Istanbul, Türkiye
Phone/Telefon: +90 (535) 510 65 77, **e-mail/e-posta:** dripekgngr@gmail.com
Submitted/Başvuru: 16.11.2024 • **Accepted/Kabul:** 27.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Hepatitis B virus (HBV) reactivation can occur in patients treated with immunosuppressive medications. Fundamentally, risk stratification for HBV reactivation depends on HBV serology indicating past or chronic HBV infection, the host immune response, and the type of immunosuppression.[1–3] Although it is well-recognized that this is a preventable consequence of hepatic decompensation or acute liver failure, there are still unclear aspects of preventive care.[2]

Recent advances in understanding the pathophysiology of immune-mediated neurological disorders have led to an increased use of B cell strategies, particularly through anti-CD20 therapies. These therapies, such as ocrelizumab, ofatumumab, and rituximab are commercially available in Türkiye and play a considerable role in our practice for immune-mediated neurological disorders. Current guidelines from major societies recommend screening for HBV in all patients planning to receive anti-CD20 therapies. Given the potentially serious outcomes of HBV reactivation, patients who are supposed to be treated with anti-CD20 therapies with either HBsAg positivity or anti-HBc positivity (regardless of HBsAg status) are considered at elevated risk.[2–4]

However, due to limited real-world data—particularly concerning ocrelizumab—existing guidelines primarily base their recommendations on rituximab and, to a lesser extent, ofatumumab.

Based on the 2010 epidemiological study, which revealed a high frequency of HBV infection in Türkiye[5], this article is specifically tailored to explore the impact of anti-CD20 therapy on HBV courses in neurology practice. We focus specifically on patients who are anti-HBc positive to explore the relationship between anti-HBs status, quantitative antibody titers, prophylaxis implementation, and the occurrence of seroconversion. Our findings will be considered within the context of existing literature to provide clinically relevant insights.

Methods

Sample collection

We conducted a retrospective study to analyze the data from patients (n=580) who received at least one dose of anti-CD20 therapies (ocrelizumab, n=469; ofatumumab, n=12; rituximab, n=99) at our neuroimmunology clinic of Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital between July 2018 and March 2024. The study includes baseline and six-month follow-up serological patterns for HBsAg, Anti-HBs, Anti-HBc IgM, Anti-HBc Ig G, HBV DNA (if available), liver enzymes, and antiviral prophylaxis. Basic demography for age, sex, and indications for anti-CD20 therapy are also recorded. Data was obtained from the hospital information management system and the personal health record system of the Turkish Ministry of Health.

Since the risk of HBV reactivation primarily affects HBsAg carriers and anti-HBc positive individuals undergoing immunosuppressive therapy, seroconversion analyses were specifically limited to these subgroups, which represent the population at virological risk.[2] Baseline HBV status was categorized into three groups: anti-HBc positive, anti-HBs positive, HbsAg negative; anti-HBc positive, anti-HBs negative, HBsAg negative, and anti-HBc positive, anti-HBs negative, and HbsAg positive for each treatment arm. This classification was created in accordance with the recommended guidelines for a risk-based approach. Use of prophylaxis and antiviral medication preference were also recorded for each category and treatment arm.

Serological follow-up data, repeated every 3-6 months, were reviewed for seroconversion*.

*Seroconversion analysis is based on the definition of the American Association for the Study of Liver Diseases (AASLD).[3]

The criteria for HBV reactivation are defined as the following:

- For HBsAg positive and anti-HBc positive patients: HBV DNA level that increases 100-fold (2-log) or greater compared to the baseline level; HBV DNA level of 1,000 IU/mL or greater in a person with a previously undetectable level (given that HBV-DNA levels fluctuate); or HBV DNA level of 10,000 IU/mL or greater if the baseline level is not available.
- For HBsAg negative and anti-HBc positive patients: detectable HBV DNA or reappearance of HBsAg.

Data Analysis

All statistical analyses were performed using GraphPad Software. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data or as median with interquartile range (IQR) for non-normally distributed data. Categorical variables were expressed as counts and percentages. Comparisons of anti-HBs titers between patients who received antiviral prophylaxis and those who did not were made using the Mann–Whitney U test due to the non-normal distribution of the data. Chi-square or Fisher's exact test was used to compare categorical variables. A two-tailed p-value of <0.05 was considered statistically significant.

Results

A total of 580 patients treated with anti-CD20 therapies (ocrelizumab n=469; rituximab, n=99; ofatumumab, n=12) are retrospectively analyzed. HBV screening results according to methodological category at baseline are given in Table 1. At baseline, anti-HBc positivity was identified in 71 out of 580 patients (12.24%). Specifically, 63 patients (15.57%) in the ocrelizumab group and 8 patients (8.08%) in the rituximab group tested positive for anti-HBc. However, this difference was not statistically significant (p=0.195), indicating a comparable

distribution of prior HBV exposure between the two treatment arms. As shown in Table 1, anti-HBc positive patients—particularly those with or without anti-HBs or with HBsAg positivity—represent the virologically at-risk population for HBV reactivation. Accordingly, analyses related to seroconversion and antiviral prophylaxis were primarily concentrated on these subgroups. Since none of the 12 patients receiving ofatumumab were anti-HBc positive, this arm was excluded from HBV risk analysis.

HBsAg positivity was 1.21% (n=7) across all treatment arms, with a rate of 9.86% in the anti-HBc positive population. The mean age of the 71 patients showing anti-HBc positivity was 50.05 +/- 9.13 years, with 40 (56.4%) of them being female. The median duration of diagnosis that necessitates anti-CD20 therapy was 12 years (0.75-42 years). The patients were receiving a median of 4 (1-13) cycles of anti-CD20 therapy. Table 2 provides a detailed summary of the basic demographic data of patients with anti-HBc positivity, along with the treatment arms of ocrelizumab and rituximab. Patients in the ocrelizumab group were older on average (50.72 ± 8.93 years) compared to the rituximab group (44.75 ± 9.56 years). Both groups had a female predominance, consistent with the gender distribution typically seen in immune-mediated neurological diseases such as Multiple Sclerosis (MS). Ocrelizumab was used exclusively in MS patients (100%). In comparison, the rituximab group included a heterogeneous mix: relapsing optic neuritis (ON) (12.5%), MS (25%), Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease (MOGAD) (25%), and Neuromyelitis Optica (NMO) (37.5%) reflecting a broader off-label use of rituximab in various neuroimmunological conditions.

When all the groups were evaluated, the prophylaxis rate was 84.51%. Among the anti-HBs positive group, the prophylaxis rate was 78%, while it was 100% in the anti-HBs negative side. Prophylaxis rates and preferred treatments according to serological status are shown in Table 3. All patients who were anti-HBs positive and did not receive prophylaxis (n=11; 22%) were those who received ocrelizumab (Anti-CD20 therapy cycles, median (IQR) 3 (2-6). The median antibody titer of patients who did not receive prophylaxis was 1000 IU/L (IQR: 340–1000), which was higher than that of patients who received prophylaxis (462 IU/L; IQR: 88–758.5), although the difference did not reach statistical significance (p= 0.064).

Two patients who were HBsAg and anti-HBc positive and were under prophylaxis showed detectable HBV DNA levels during follow-up (18 and 40 IU/mL, respectively). However, these levels did not meet the AASLD seroconversion criteria, and there was no deterioration in liver functions. No other patients were showing detectable HBV DNA levels, suggesting seroconversion.

Table 1. HBV screening results according to methodological category at baseline

HBV screening	**Anti-HBc positive**			**Anti-HBc negative**
	Anti-HBs positive HBsAg negative	**Anti-HBs negative HBsAg negative**	**Anti-HBs negative HBsAg positive**	
Rituximab **(n=99)**	5	2	1	91
Ocrelizumab **(n=469)**	45	12	6	406
Ofatumumab* **(n=12)**	0	0	0	12
Total (n)	50	14	7	509

*Ofatumumab-treated patients were included for cohort representation but not analyzed for HBV reactivation risk due to absence of anti-HBc positivity in this group.

Table 2. Detailed summary of the basic demographic data of patients with anti-Hbc positivity, along with the treatment arms of ocrelizumab and rituximab

Patient characteristics	**Anti-HBc positive patients in ocrelizumab arm (n=63)**	**Anti-HBc positive patients in rituximab arm (n=8)**
Age, mean SD	50.72 +/-8.93	44.75+/-9.56
Sex %	55.55% female 44.45% male	62.5% female 37.5% male
Duration of diseases (years), median (IQR)	12 (1-42)	2 (0.75-26)
Disease distribution	MS 100%	Relapsing ON: 12.5% MS: 25% MOGAD: 25% NMO: 37.5%
Anti-CD20 therapy cycles, median (IQR)	4 (1-13)	2 (1-11)

MS; Multiple Sclerosis, ON; Optik Neuritis, MOGAD; Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease, NMO; Neuromiyelitis Optica

Three patients under prophylaxis with initial serum anti-HBs positivity tested negative after receiving ocrelizumab infusions during follow-up. Their antibody titers were low at baseline (13, 14, and 17 IU/L, respectively). The total number of anti-CD20 therapy cycles leading to anti-HBs loss was 4, 1, and 1 cycles, respectively.

After the infusions, it's worth noting that one patient in the ocrelizumab group experienced a loss of anti-HBc after the second infusion, while three patients in the rituximab group experienced anti-HBc loss after their first infusions.

Considering all sera, seroconversion to HBsAg positivity was not observed in any patient, regardless of whether they were under prophylaxis or not.

Table 3. Prophylaxis rates and preferred treatments according to serological status

		Anti-HBc positive	
	Anti-HBs positive HBsAg negative (n=50)	**Anti-HBs negative HBsAg negative (n=14)**	**Anti-HBs negative HBsAg positive (n=7)**
Prophylaxis in rituximab (100%)	5 of 5 (1T* 4E**)	2 of 2 (2E*)	1 of 1 (1E*)
Prophylaxis in ocrelizumab (82.54%)	34 of 45 (9T** 25E*)	12 of 12 (2T** 10E*)	6 of 6 (6E*)
Total prophylaxis (84.51%)	39 (78%)	14 (100%)	7 (%100)
Seroconversion	0	0	0

*(E): Entecavir; **(T): Tenofovir

Discussion

This study provides real-world data on the management of patients with prior HBV exposure (anti-HBc positive) undergoing anti-CD20 therapies for neurological diseases, focusing on prophylaxis decisions, anti-HBs antibody levels, and seroconversion outcomes. The scarcity of data on HBV reactivation risk, particularly with ocrelizumab treatment, highlights the potential of our study to inform future research in this field.

In a study conducted by the Turkish Association for the Study of the Liver between 2009 and 2010, 4% of adults tested positive for HBsAg, and 30.6% tested positive for anti-HBc, indicating a high prevalence of hepatitis in Turkey.[5] Our anti-HBc positivity was 12.24%, while the HBsAg positivity was 1.21% across all treatment arms. The decrease in positivity rates may be attributed to the implementation of more comprehensive vaccination policies over the years.

Our results demonstrate that none of the patients experienced seroconversion to HBsAg positivity while previously negative or showing significant HBV DNA levels that met the reactivation criteria across all serological subgroups. Additionally, among patients treated with ocrelizumab, those who did not receive prophylaxis (22%) due to their anti-HBs positivity also did not show seroconversion. Generally, a person remains antibody-positive for life following HBV infection. However, under immunosuppressive conditions, both anti-HBc and anti-HBs antibodies may become negative.[6,7] In our sera, we observed a loss of anti-HBs in three out of 50 patients (6%), particularly those with low baseline antibody titers. This finding aligns with data indicating that low antibody levels are a risk factor for anti-HBs loss in individuals undergoing immunosuppression.[8] Additionally, four out of 71 patients (5.63%) experienced a loss of anti-HBc. However, it's important to note that neither of these losses appeared to be a risk factor for HBV reactivation.

To emphasize, the prophylaxis rate was 78% in the anti-HBs positive group and 100% in the anti-HBs negative group. The 22% loss of prophylaxis rate in the anti-HBs positive group can be attributed to the physician's discretion. In real-world clinical practice, the administration of prophylaxis to patients who are anti-HBs positive is inconsistent due to a lack of definitive, universally accepted guidelines. In our study, prophylaxis was not given according to a standardized protocol; instead, it was determined at the physician's discretion. Notably, some clinicians chose to start prophylaxis even for patients with high anti-HBs titers, while others decided against it in similar cases. This variability in clinical practice may have introduced a selection bias, potentially affecting the distribution of antibody titers between the prophylaxis and non-prophylaxis groups. Although the median anti-HBs titer was numerically higher in the non-prophylaxis group, the difference was not statistically significant. These findings highlight the need for more specific guidelines for this subgroup.[7,9] Although current evidence is insufficient to recommend anti-HBs titers as a standalone criterion for prophylaxis decisions, our findings suggest that the decision to administer or withhold prophylaxis did not affect clinical outcomes in our cohort.

Our findings are in line with those of a Spanish prospective study, which demonstrated that anti-CD20 monotherapy (rituximab, n = 22; ocrelizumab, n = 6) did not pose a detectable risk of HBV reactivation in HBsAg-negative/anti-HBc-positive patients with NMOSD and MS, even in the absence of antiviral prophylaxis.[10] Similarly, data from an Italian cohort reported no cases of HBV reactivation, despite the fact that 53% of patients with anti-HBs levels below 100 mIU/mL and 30% with levels above 100 mIU/mL did not receive either prophylaxis or active monitoring.[11] A recent study from our region presents findings that contrast with previous results, including our own. Among three patients undergoing ocrelizumab therapy who experienced HBV reactivation, two out of seven (28.6%) had not received

antiviral prophylaxis, while one patient failed to adhere to the prescribed prophylaxis regimen.[12]

We achieved significant results in our study, though it is important to recognize some limitations. First, as a retrospective observational study, it has certain constraints. While this research represents the largest cohort of patients treated with ocrelizumab in the available literature concerning hepatitis seroconversion, the sample size in the rituximab treatment group was comparatively small. Additionally, we lacked data on vaccine-induced HBV immunity. Addressing these limitations in future research could provide even more comprehensive insights.

In conclusion, our research suggests that monotherapy with anti-CD20 is not associated with a detectable risk of HBV reactivation in our neuroimmunological practice. Moreover, the absence of antiviral prophylaxis in patients with anti-HBs positivity in the ocrelizumab group was also not linked to a detectable risk of HBV reactivation. However, prospective studies involving a larger number of patients and extended follow-up periods are needed to confirm these findings and clarify the existing literature.

Ethical Approval

The study has received ethical committee approval with the number E-46059653-050.99-225242407 (25.09.2023) from the Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital ethical committee.

Conflict of Interest

The author declares no conflicts interests.

Author Contributions

SD, IGD: Concept-Design; IGD, FD, DCT: Data Collection and/or Processing; SD, IGD: Analysis and/or Interpretation; IGD, FY, DCT: Literature Review; IGD: Writer; SD, DCT: Critical Review

Financial Support

None

Data Availability:

The datasets that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Aygen B, Demir AM, Gümüş M, et al. Immunosuppressive therapy and the risk of hepatitis B reactivation: Consensus report. Turkish J Gastroenterol Off J Turkish Soc Gastroenterol. 2018;29(3):259-269. doi:10.5152/tjg.2018.18263
2. Reddy KR, Beavers KL, Hammond SP, Lim JK, Falck-Ytter YT. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. Gastroenterology. 2015;148(1):215-217. doi:10.1053/j.gastro.2014.10.039
3. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67(4):1560-1599. doi:10.1002/hep.29800
4. Lau G, Yu M-L, Wong G, et al. APASL clinical practice guideline on hepatitis B reactivation related to the use of immunosuppressive therapy. Hepatol Int. 2021;15(5):1031-1048. doi:10.1007/s12072-021-10239-x
5. Tozun N, Ozdogan O, Cakaloglu Y, et al. Seroprevalence of hepatitis B and C virus infections and risk factors in Turkey: a fieldwork TURHEP study. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2015;21(11):1020-1026. doi:10.1016/j.cmi.2015.06.028
6. Holtkamp C, Fiedler M, Dittmer U, Anastasiou OE. The Course of Anti-HBc Antibodies over Time in Immunocompromised Hosts. Vaccines. 2022;10(2). doi:10.3390/vaccines10020137
7. Pei S-N, Ma M-C, Wang M-C, et al. Analysis of hepatitis B surface antibody titers in B cell lymphoma patients after rituximab therapy. Ann Hematol. 2012;91(7):1007-1012. doi:10.1007/s00277-012-1405-6
8. Hung M-H, Tien Y-C, Chiu Y-M. Risk factors for losing hepatitis B virus surface antibody in patients with HBV surface antigen negative/surface antibody positive serostatus receiving biologic disease-modifying anti-rheumatic drugs: a nested case-control study. Adv Rheumatol (London, England). 2021;61(1):22. doi:10.1186/s42358-021-00173-9
9. Cho Y, Yu SJ, Cho EJ, et al. High titers of anti-HBs prevent rituximab-related viral reactivation in resolved hepatitis B patient with non-Hodgkin's lymphoma. J Med Virol. 2016;88(6):1010-1017. doi:10.1002/jmv.24423
10. Marzo B, Vidal-Jordana A, Castilló J, et al. Hepatitis B reactivation is a rare event among patients with resolved infection undergoing anti-CD20 antibodies in monotherapy without antiviral prophylaxis: results from the HEBEM study. J Neurol. 2024;271(1):134-140. doi:10.1007/s00415-023-11973-y
11. Buonomo AR, Viceconte G, Calabrese M, et al. Management of hepatitis B virus prophylaxis in patients treated with disease-modifying therapies for multiple sclerosis: a multicentric Italian retrospective study. J Neurol. 2022;269(6):3301-3307. doi:10.1007/s00415-022-11009-x
12. Çelik M, Baba C, Irmak Ç, Özakbaş S, Avkan-Oğuz V. Risk of hepatitis B virus reactivation in people with multiple sclerosis treated with ocrelizumab: an observational study from Turkey. J Neurol. 2024;271(7):4131-4137. doi:10.1007/s00415-024-12333-0

doi: 10.53446/actamednicomedia.1589884

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Araştırma Makalesi | Research Article

ROSMARİNİK ASİTİN İNSAN KOLON KANSERİNDE OTOFAJİK ETKİSİ

AUTOPHAGIC EFFECT OF ROSMARINIC ACID ON HUMAN COLON CANCER

İlkay Çorumluoğlu[1*], Ebru Alimoğulları[1]

[1]Ankara Yıldırım Beyazıt Üniversitesi ,Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Ankara, Türkiye.

ÖZ

Amaç: Kolon kanseri en agresif kanser türüdür ve tedavilerin yetersiz olmasından kaynaklı ileri aşamalarda kötü prognoz ile karakterizedir. Rosmarinik asit (RA) kolon, pankreas, prostat, karaciğer ve multipl miyelom dahil olmak üzere çeşitli kanserlerdeki lezyonlara karşı etkili olduğu kanıtlanmıştır. Çalışmamızda lipopolisakkarit (LPS) ile oluşturulan hücresel hasar ve otofaji üzerine rosmarinik asitin kolon kanseri hücrelerine etkisini araştırmayı amaçladık.

Yöntem: Çalışmamızda insan kolon kanseri Caco-2 (HTB-37) hücre hattı kullanıldı. Kontrol, LPS, RA ve LPS+RA olmak üzere 4 grup dizayn edildi. Caco-2 için 200 µg/mL RA dozu ve LPS dozu 10 µg/ml kullanıldı. Caco-2 hücre hatları kültüre edildikten sonra Beclin-1, p62 ve LC3 proteinlerinin hücresel lokalizasyonlarını belirlemek için immünohistokimyasal analizler gerçekleştirildi. Otofajik yolaktaki bu proteinlerin ifadesini belirlemek için western blot analizleri yapıldı.

Bulgular: LPS hasarı oluşturulan grupta kontrol grubuna göre otofajik protein ifadesi yüksek bulunmuştur ($p<0,05$). LPS+RA verilen grupta diğer gruplar ile karşılaştırıldığında istatistiksel olarak anlamlı fark bulunmuştur ($p<0,05$).

Sonuç: Sonuç olarak, Caco-2 kolon kanser hücrelerinde LPS ile hasar oluşturulduğunda otofaji yolağındaki Beclin 1, p62 ve LC3 önemli proteinlerin ifadelerinde azalma ancak rosmarinik asit ile tedavi edilen grupta protein ifadelerinde artış olduğunu tespit ettik. Bu çalışma ile rosmarinik asitin kolon kanseri hücrelerinin otofaji yolağında önemli proteinlerin degredasyonuna karşı klinik etkilerinin tespit edilebilmesi için daha kapsamlı çalışmaların yapılması gerektiğini önermekteyiz.

Anahtar Kelimeler: Caco-2, rosmarinik asit, Beclin 1, p62, LC3, LPS

ABSTRACT

Objective: Colon cancer is the most aggressive type of cancer and is characterized by a inefficient prognosis in advanced stages due to inadequate treatment. Rosmarinic acid (RA) has been shown to be effective against lesions in various cancers, including colon, pancreatic, prostate, liver and multiple myeloma. In our study, we aimed to investigate the effect of rosmarinic acid on lipopolysaccharide (LPS)-induced cell damage and autophagy in colorectal cancer cells.

Method: The human colon cancer cell line Caco-2 (HTB-37) was used in our study. 4 groups were formed: Control, LPS, RA and LPS+RA. For Caco-2, an RA dose of 200 µg/ml and an LPS dose of 10 µg/ml were used. After the Caco-2 cell lines were cultured, immunohistochemical analyzes were performed to determine the cellular localizations of Beclin-1, p62 and LC3 proteins. Western blot analyzes were performed to determine the expression of these proteins in the autophagic pathway.

Results: Autophagic protein expression was found to be higher in the LPS-damaged group compared to the control group ($p<0,05$). It was found to be statistically significant in the LPS+RA group compared to the other groups ($p<0,05$).

Conclusion: As a result, we found that when LPS-induced damage was induced in Caco-2 colon cancer cells, there was a decrease in the expression of important Beclin 1, p62 and LC3 proteins in the autophagy pathway, but an increase in their protein expression in the rosmarinicacid-treated group. In conclusion, with this study we suggest that more comprehensive studies should be conducted to determine the clinical effect of rosmarinic acid against the degradation of key proteins in the autophagy pathway in colorectal cancer cells.

Keywords: Caco-2, rosmarinic acid, Beclin-1, p62, LC3, LPS

*** İletişim kurulacak yazar/Corresponding author:** İlkay Çorumluoğlu; Ankara Yıldırım Beyazıt Üniversitesi ,Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Ankara, Türkiye.
Telefon/Phone: +90 (542) 513 09 36, **e-mail/e-posta:** ilkaycorumluoglu@gmail.com
Başvuru/Submitted: 22.11.2024 • **Kabul/Accepted:** 30.04.2025 • **Online Yayın/Published Online:** 30.06.2025

Giriş

Kolorektal kanser (CRC), sindirim sisteminin sık görülen malign tümörlerinden biridir ve görülme sıklığı mide ve özofagus kanserlerinden sonra ikinci sıradadır[1]. Rektum kanserlerinin %90'ından fazlası, cerrahi eksizyonla çıkarılabilen adenokarsinomlardır. Ancak cerrahi eksizyonun sonuçları rektum kanserinin evresine bağlıdır. Tek başına cerrahi eksizyonun ve cerrahi eksizyonu takiben radyoterapi (RT) ile karşılaştırıldığında, rektum kanserinin lokal tekrarlama riskinin daha yüksek olduğu bulunmuştur[2]. Kolorektal kanser (CRC), erkeklerde üçüncü, kadınlarda ise ikinci en sık görülen tümördür ve dünya çapındaki tüm tümör çeşitlerinin %10'unu oluşturur. Erkeklerde görülme sıklığı %25 daha yüksektir ve ülkeler arasında büyük farklılıklar gösterir[3,4].

Kanser hücrelerinde ubikuitin-proteazom sistemininde (UPS) içinde olduğu protein hemostasızın (proteostaz) sekteye uğradığı bilinmektedir[5]. Genom sekanslama çalışmaları, kanser hücre genomunun protein kodlama sekanslarında yüzlerce nokta mutasyonlarının olduğunu ortaya çıkarmıştır[6]. Bu durumda, kanser hücrelerindeki bu proteinler, protein kalite kontrol sistemine aşırı yük oluşturacak ve UPS aracılığıyla yıkıma uğratılacaktır[7]. Yapılan çalışmalar, kanserin başlangıcında otofaji aktivitörlerinin kanser gelişimini engellediği[8], fakat ileri düzey kanserlerde hem aktive edici hem de inhibe edici ajanların tedaviye yönelik kullanımı önerilmiştir[9]. Yine kolorektal kanserli hastalarda p97/VCP (ATPaz valosin-containing protein)'nin fazlaca ekspre olduğu ve bu ekspresyonun hücre büyümesi ve hücre canlılığı ile ilişkili olduğu belirlenmiştir[10]. Otofaji ve apoptoz, hücre ölümü ve hücre canlılığının düzenlenmesinde yer aldıklarından, bağlantılı ve korunmuş süreçlerdir[11]. Otofaji proteinleri, membrana bağlı otofagozom oluşumu ve olgunlaşmasında rol oynar. Otofagozomların oluşumu Autophagy-related protein 8 (ATG8) / Microtubule-associated protein light chain 3 (LC3) proteinlerini gerektirir. LC3, otofagozomların en iyi bilinen belirtecidir. Otofajinin aktivasyonu, LC3I'yi katalize ederek otofajik membran ile ilişkili LC3-II'yi oluşturur[12,13]. Bu reaksiyon, moleküler ağırlığının 18 kDa'dan 16 kDa'ya değişmesine neden olur ve bu, genellikle otofaji tespiti için bir test olarak kullanılır. P62/SOSMT1 (Polyubiquitin-binding protein) proteini ayrıca otofajik akıyı incelemek için kullanılan başka bir belirteçtir ve otofajik bozunmayı kolaylaştırmak için LC3II'ye bağlanır. Daha sonra, otofagozomlar lizozomlarla birleşerek otolizozomlar haline gelirler ve burada içerikleri bozulur[14].

İki ana hücresel temizleme yolu olan UPS ve otofaji, proteinlerin bozulmasını sürdürmek için birbirleriyle işbirliği yapar[15,16]. UPS, substratların proteazom tarafından bozunması için ubikuitin ile tanındığı ve etiketlendiği bir proteolitik sistemdir. Otofaji aynı zamanda proteinleri ve diğer hücresel materyalleri parçalamak için lizozomal hidrolazları kullanan parçalayıcı bir sistemdir. Hem UPS hem de otofajinin farklı, birbirinden bağımsız işlevleri ve mekanizmaları vardır, ancak protein bileşenleri ve her hücrede bulunma gibi birçok açıdan benzerliği paylaşırlar. Ubikuitin (Ub) proteini, ubikuitin aktive edici (E1), ubikuitin konjuge edici (E2) ve ubikuitin ligaz (E3) enzimlerinin ortak aktivitesi yoluyla proteinlerin lizin kalıntılarına konjuge edilmiş 76 amino asitli bir proteindir[17]. BECN1 geni tarafından kodlanan protein olan beclin 1, otofaji için gereklidir. Beclin 1, endositotik yolakta ve LC3 (mikrotübül ilişkili protein 1 hafif zincir 3) ilişkili fagositoz dahil olmak üzere diğer yolaklarda da yer almaktadır[18]. Beclin 1, Bcl-2 ile etkileşen yeni bir protein olarak tanımlanan ilk memeli otofaji proteinidir[19]. Beclin 1 aynı zamanda dönüm noktası niteliğinde bir otofaji proteini olarak da tanımlanmaktadır[19,20]. BECLIN1, hem otofaji hem de membran trafiği işlevlerini, başlıca vakuolar protein sıralama ilişkili protein 15 (VPS15), VPS34, UV radyasyon direnci ilişkili gen ürünü (UVRAG) ve otofaji ilişkili protein 14 (ATG14) olmak üzere birkaç başka proteinle etkileşime girerek gerçekleştirir. Bunlar birlikte, sırasıyla ATG14 veya UVRAG'ın mevcut olup olmamasına bağlı olarak iki farklı Sınıf III PI3K kompleksi, Kompleks 1 (C1) ve Kompleks 2 (C2) halinde birleşir. Bu kompleksler içinde, katalitik lipid kinaz alt birimi VSP34, PtdIns'in fosforilasyonundan sorumludur ve bu da daha sonra efektör proteinlerin işe alınması yoluyla otofaji ve/veya membran trafiği işlevlerine aracılık eder[21]. Otofajik fonksiyona ek olarak, son çalışmalarda Beclin 1'in tümör proliferasyonunda düşük ekspresyonu ve baskılayıcı fonksiyonu olduğu gösterilmiştir[20].

Biberiye (Rosmarinus officinalis) labiat ailesine (Lamiaceae) aittir. Bu bitki ailesinin aktif bileşenleri fenolik diterpenler ve triterpenlerdir. Bu bileşiklerin yüksek antioksidan etkileri vardır. Biberiyenin ana aktif bileşikleri arasında kafeik asit, rosmarinik asit (RA), ursolik asit (UA), karnosik asit (CA) ve karnosol bulunur. Biberiyenin toplam antioksidan aktivitesinin yaklaşık %90'ı karnosol ve CA'dan türetilmiştir. Rosmarinik asit, fenolik halkalarından birini kafeik asit aracılığıyla fenilalaninden, diğer halkasını ise 3,4-dihidroksifenillaktik asit aracılığıyla tirozinden elde eden kafeik türevlerden biridir. Ursolik asit, antikanser ve anti-inflamatuar özelliklere sahip bir pentasiklik triterpenoiddir[22].

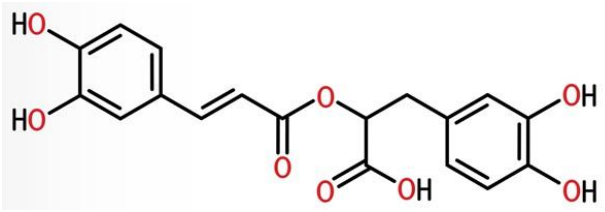
Bakteriyel lipopolisakkarit (LPS), birçok doku ve organda inflamasyona neden olur ve sistemik inflamasyonu uyararak sepsise neden olabilir. LPS, gram negatif bakterilerin hücre duvarının toksik bir bileşenidir. Gram negatif bakterilerin hücre duvarı; bir iç peptidoglikan tabakası ve bir dış LPS, protein ve fosfolipid tabakasından oluşur. LPS katmanındaki endotoksin molekülü hücre zarında kaldığı sürece etkisizdir. Hızlı hücre proliferasyonu veya hücre yıkımı sırasında salınan endotoksin, sepsis/endotoksemi olaylarını başlatan anahtar moleküldür. LPS, optimal bir bakteriyel enfeksiyon modeli oluşturmak için yaygın olarak kullanılmaktadır[23].

Caco-2 (HTB-37) hücre hattında rosmarinik asit ve LPS varlığında ilk olarak Beclin-1, p62 ve LC3 ekspresyonları immunohistokimya ve western blot yöntemleriyle belirlenmesi amaçlanmıştır. Literatürde ilk kez kolon karsinomlarında rosmarinik asit ve otofaji arasındaki ilişki ortaya konulup, kanser hastalıklarında otofajik proteinlerin önemine dair ön bilgiler elde edilmesi hedeflenmiştir.

Yöntem

Kimyasallar ve Antikorlar

Bu çalışmada kullanılan rosmarinik asit Sigma Aldrich (St. Louis, Mo., ABD) firmasından satın alınmıştır ve saflığı %95'tir (HPLC) (Şekil 1). Primer antikorlardan rabbit-LC3B (BS-55120R, Bioss, USA), rabbit- p62 (bs-55207R, Bioss, USA), rabbit Beclin-1 (PA5-20171, Invitrogen, USA), rabbit beta-aktin (1:1000, rabbit, bs-0061R, Bioss, USA), sekonder antikor olarak goat anti-rabbit IgG (H+L) konjuge peroksidaz (UK293475, Thermo scientific, USA), ve DAPI(2328994, Invitrogen, USA) kullanılmıştır.



Şekil 1. Rosmarinik asitin moleküler yapısı ($C_{18}H_{16}O_8$).

Hücre Kültürü Yöntemi

Çalışmamızda ATCC (American Type Culture Collection) tarafından sağlanan Caco-2 (ATCC® Cat. No. HTB-37™) hücre hattı kullanılmıştır. Hücreler %1 penisilin/streptomisin ve %10 fetal sığır serumu (Hyclone, ABD) ile desteklenen DMEM kültür ortamında (Gibco, ABD) 37°C'de %5'lik CO_2 içeren inkübatörde kültüre edilmiştir. Western blot ve immünohistokimyasal analizleri için hücreler, %70 çoğalma kapasitesine ulaştığında %0,25 trypsin kullanılarak kültür kaplarından ayrılmaları sağlanıp 6 kuyucuklu kültür kaplarına ekimleri yapılmıştır [24]. Ön çalışmalar sayesinde Caco-2 hücreleri için belirlenen 200 µg/mL RA dozu[25] ve LPS dozu (10 µg/ml)[26] kullanılmıştır. Çalışmamız kontrol, LPS, RA ve LPS+RA olmak üzere 4 gruba ayrılmıştır.

İmmünofloresan Çalışmaları

Caco-2 hücreleri coverslip (15x15mm, yuvarlak, Nest) eklenen 6 kuyucuklu kültür kaplarında inkübe edilmiştir. Caco-2 hücreleri 3 kez PBS (Phosphate-buffered saline) ile yıkandıktan sonra %4'lük formaldehit ile tespit işlemi gerçekleştirilmiştir. Fiksasyondan sonra 3 kez tekrardan PBS ile yıkandıktan sonra, 1 saat oda sıcaklığında bloklama solüsyonunda (%3'lük Bovine serum albümin-%10'luk rabbit serum) bekletilmiştir. Caco-2 hücrelerinin üzerine, bloklama sonrasında yıkama işlemi yapılmadan LC3B (1:300, BS55120R, Bioss, USA), p62 (1:200 bs-55207R, Bioss, USA), Beclin 1 (1:200, PA5-20171, Invitrogen, USA) primer antikorları damlatılıp 4°C'de gece boyunca buzdolabında inkübe edilmiştir. İnkübasyon sonrası 3 kez PBS ile yıkandıktan sonra primer antikorlara uygun sekonder antikorlar (Goat-anti-rabbit IgG (H+L) FITC konjuge peroksidaz, (UK293475, Thermo scientific, USA) ile 1 saat oda sıcaklığında inkübe edilmiştir. Süre bitiminde 3 kez PBS ile yıkanmıştır. Hücreler inkübasyon sonrasında immünofloresan kapatma medyumu DAPI (2328994, Invitrogen, USA) ile kapatılmıştır. Olympus CKX41 marka floresan mikroskobu ile görüntülenmesi gerçekleştirilmiştir.

Western Blot Analizi

Western blotlama için Caco-2 hücreleri 6 kuyucuklu kültür kaplarına kültüre edilmiştir. Protein konsantrasyonunun belirlenmesi için Bradford yöntemi kullanılmıştır. Hücreler 20 µg protein, gradyan jeline (Invitrogen) yüklenmiş ve bir nitroselüloz membran üzerine yarı kuru transfer gerçekleştirilmiştir. Membran blokajı için PBS içindeki %5 süt kullanılmıştır ve ardından LC3B (1:300, BS-55120R, rabbit, Bioss, USA), p62 (1:200, bs-55207R, rabbit, Bioss, USA), Beclin-1 (1:200, PA5-20171, Rabbit, Invitrogen, USA), beta-aktin (1:1000, rabbit, bs-0061R, Bioss, USA) primer antikorları ile gece boyunca 4 °C'de membranlar inkübe edilmiştir. Yıkamanın ardından 1 saat oda sıcaklığında HRP-konjuge-sekonder antikorlar (anti-rabbit 1:2000 oranında) ile inkübe edilmiştir. HRP Luminol/Enhancer ve Peroxide buffer (Abcam) blotlanan membranlara 5 dakika boyunca kemilüminesans (ab133406, Abcam) uygulanmıştır[27]. Western blot bantları jel belgeleme sistemi (UVP) ile görselleştirilmiş ve bant yoğunlukları IMAGE J Versiyon 1.46 (NIH) ile değerlendirilmiştir.

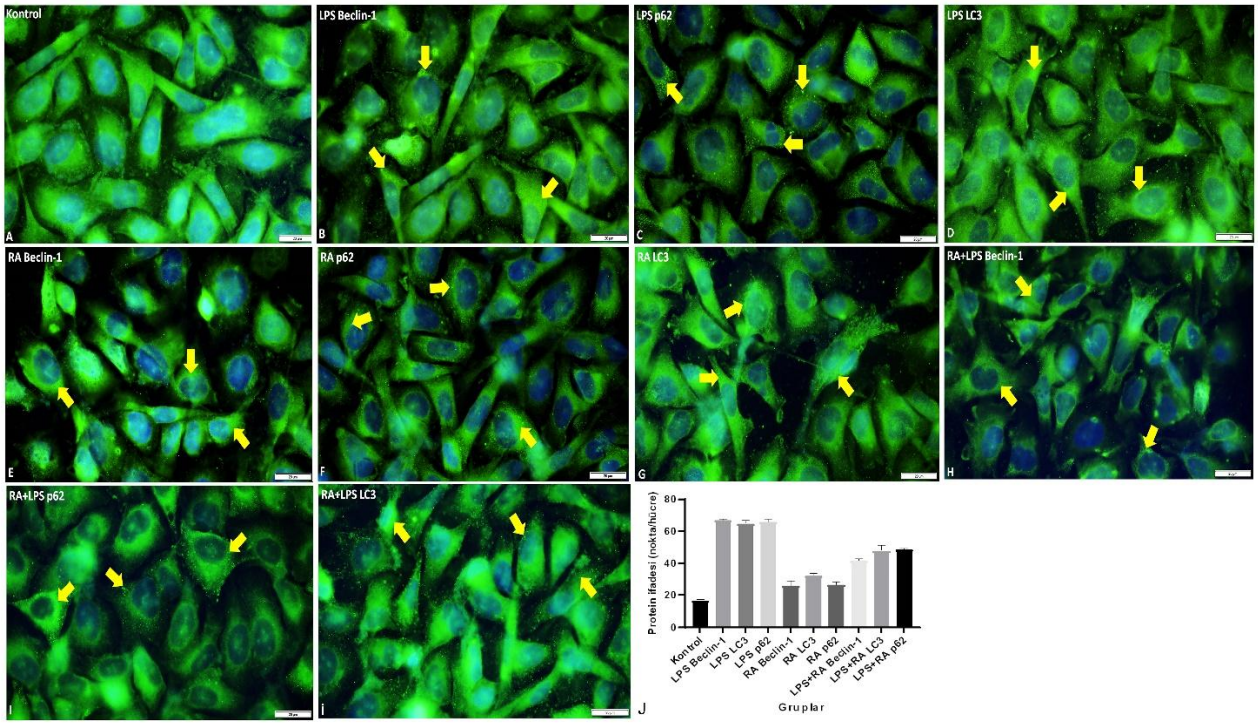
Biyoistatistiksel Analiz

Western blot analiz sonrası band yoğunlukları ve gruplar arası karşılaştırmalar Image J analiz programı kullanılarak analiz edilmiştir. İmmünofloresans sonuçlarının analizi GraphPad Prism version 8.4.2. programında One-way Brown-Forsythe testi uygulanarak belirlendi. Tüm grupların tanımlayıcı istatistiksel ANOVA değerleri yapılmıştır. Western blot analiz sonuçları GraphPad Prism version 8.4.2. programında One-way ANOVA Tukey'nin çoklu karşılaştırma testinde analizi yapılmıştır. İstatistiksel anlamlılık düzeyi $p<0,05$ olarak kabul edilmiştir.

Bulgular

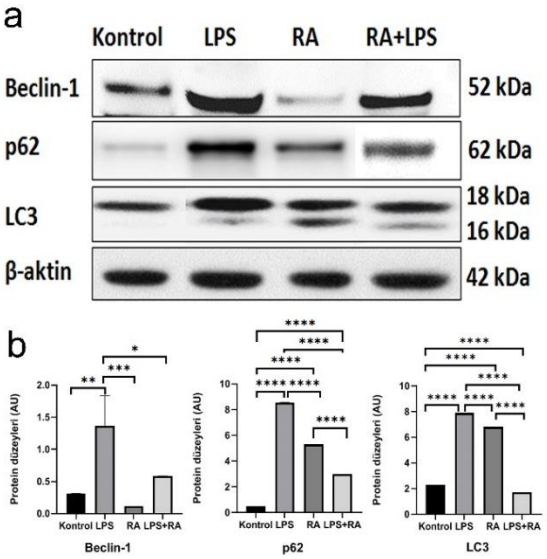
İmmünofloresans analizlerine göre LPS gruplarında otofajik yolaklardaki önemli belirteçlerden Beclin-1, p62 ve LCB proteinleri sitoplazmada ve perinükleer alanlarda yoğun olarak görülmüştür. Kontrol grubu ile LPS, RA ve LPS+RA grupları arasında Beclin-1, p62 ve LC3 protein ifadelerinde istatistiksel olarak anlamlı farklılık bulunmuştur ($p<0,0001$). LPS grubunda Beclin-1, p62, LC3 protein ifadelerinde artış tespit edilmiştir. RA grubundaki Beclin-1, p62, LC3 protein ifadelerinde azalma ve her iki grup arasında istatistiksel olarak anlamlı farklılık bulunmuştur ($p<0,0001$). Beclin-1, p62, LC3 proteinleri RA grubunda yüksek düzeyde ifade edilmiştir. RA grubu, LPS+RA grubu ile karşılaştırıldığında proteinlerin ifadesinde azalma görülmüştür ve istatistiksel olarak anlamlı fark bulunmuştur ($p<0,0001$) (Şekil 2). Hücresel hasar oluşturulan LPS grubuna RA verildiğinde otofajik yolak proteinlerinde yıkım normal olarak gerçekleştiği için protein ifade düzeylerinde azalma gözlenmiştir. RA gruplarındaki her üç proteinin kontrol grubuna göre protein ifadelerinde azalma görülmüştür.

LPS Beclin-1 ile LPS LC3 (p=0,9375), LPS Beclin-1 ile LPS p62 (p=0,9986), LPS LC3 ile LPS p62 (p=>0,9999), RA Beclin-1 ile RA p62 (p=>0,9999), RA LC3 ile RA p62 (p=0,0573) ve LPS+RA LC3 ile LPS+RA p62 (p>0,9999) grupları arasında protein ifadeleri açısından istatistiksel olarak anlamlı fark bulunmamıştır. Analiz sonuçlarımıza göre, hücresel hasar oluşturulan LPS grubuna verilen etken maddenin rejeneratif etkisi olduğu hem gözlemsel hem de istatistiksel olarak anlamlılık göstermiştir (Şekil 2).



Şekil 2. İmmünofloresans analiz fotomikrografları. A) Kontrol, B) LPS Beclin-1 grubu, C) LPS p62 grubu, D) LPS LC3 grubu, E) RA Beclin-1 grubu, F) RA p62 grubu, G) RA LC3 grubu, H) LPS+RA Beclin-1 grubu, I) LPS+RA p62 grubu, İ) LPS+RA LC3 grubu, J) Proteinlerin nokta/hücre oranları histogram grafiğinde verilmiştir. Hücrelerdeki protein ifadesi nokta/hücre şeklinde image j programında hesaplanmıştır. Gruplar arasındaki protein ifade düzeyleri istatistiksel olarak anlamlı bulunmuştur (p<0,05). Her grup 3 farklı alandan bağımsız şekilde değerlendirilmiştir. Sarı oklar protein ifadesinin yoğun olduğu alanları işaret etmektedir. (Olympus CKX41-büyütme 100x), Bar: 20 µm.

Western blot analizinde bant yoğunlukları incelendiğinde özellikle LPS gruplarında Beclin-1, p62 ve LC3 proteinlerinin ifadelerinde artış belirlenmiştir. Hücresel hasar oluşturulan LPS grubunda otofaji yolağındaki bozukluklar sebebi ile hücrede Beclin-1, p62 ve LC3 proteinlerinin birikime yol açmıştır. Hücrelerdeki hasarı tamir etmek için rosmarinik asit verilen LPS+RA grubunda Beclin-1, p62 ve LC3 proteinlerinin ifadelerinde RA ve kontrol gruplarına göre artış saptanmıştır (p<0,0001). LPS+RA grubunda otofaji yolağındaki protein yıkımının düzelmesi sebebi ile jel bantlarında LPS grubuna göre daha az protein ifadesi gözlemlenmiştir (Şekil 3). Beclin-1 protein ifadesine bakıldığında, kontrol ile LPS (p=0,0028), LPS ile RA (p=0,0009) ve LPS ile LPS+RA (p=0,0161) gruplarında istatistiksel olarak anlamlı farklılık bulunmuştur. Kontrol ile RA (p=0,7327), kontrol ile LPS+RA (p=0,5313) ve RA ile LPS+RA (p=0,1456) grupları karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık bulunmamıştır. LC3 ve p62 protein ifadeleri tüm gruplar içerisinde birbirleri ile karşılaştırıldığı istatistiksel olarak anlamlı farklılık olduğu belirlenmiştir (p<0,0001) (Şekil 3).



Şekil 3. Western blot analizi. A) Beclin-1 (52 kDa), p62 (62 kDa), LC3 (18 kDa ve 16 kDa) ve beta-aktin (42 kDa) protein ifadeleri western blot analizinde gösterilmiştir. Deneyler 3 kez tekrarlanmıştır, image J programında jel yoğunlukları ölçülmüştür ve istatistiksel olarak GraphPad prism 8.4.2 programında değerlendirilmiştir. B) Protein düzeylerine (AU) ait histogram grafiği verilmiştir. Beclin-1 proteini (*LPS vs. LPS+RA, p=0,0161; **Kontrol vs. LPS; p=0,0028; ***LPS vs. RA, p=0,0009), p62 ve LC3 protein ifade düzeyleri gruplar arasında ****p<0,0001 olarak gösterilmiştir.

Tartışma

Kolorektal kanserin ilerlemesi inflamasyon ile yakından bağlantılıdır[28]. Kolorektal kanser genellikle adenomatöz polipler adı verilen kanser öncesi büyümelerden kaynaklanır. Bu büyümeler, kolorektal sistemi kaplayan epitel hücrelerinde aşırı hücre çoğalmasına neden olur. Apoptozis gibi programlanmış hücre ölümünü bozan genetik mutasyonların birikmesi nedeniyle adenokarsinomaya kötü huylu dönüşüm geçirebilir[29]. Kolorektal kanserin erken teşhisi hastaların klinik sonuçlarını önemli ölçüde iyileştirebilse de, ileri evre III veya evre IV hastalık tanısı konulanlar için 5 yıllık sağkalım oranı endişe verici derecede düşük kalmaya devam etmektedir[30].

CRC insidansı giderek artmaktadır ve dünya çapında en yaygın kanserlerden biri haline gelmiştir. Bu nedenle, kolonla ilişkili tümörlerin yıkıcı etkisini bastırmada etkili olacak maliyeti ucuz, doğal olarak oluşan bileşikleri keşfetmek önemlidir. Bitkisel kökenli bileşiklerden biri olan RA, kanser önleme ve tedavisi için bir ajan olarak kullanılmak üzere çekici özelliklere sahiptir[31]. RA'nın özellikle fenolik bileşenlerinin, çeşitli mekanizmalar aracılığıyla kolon kanseri ve diğer kanser türleri üzerinde koruyucu etkiler gösterdiği de bulunmuştur[22].

Çalışmamızda, çeşitli bitkilerde bol miktarda bulunan polifenolik bir bileşik olan RA'nın, CRC hücrelerine karşı anti-kanser etkilerini güçlendirebileceğini gösterebilmek hedeflenmiştir. RA tedavisinin CRC hücre hattı Caco-2'de otofaji yolağındaki Beclin-1, p62 ve LC3 gibi önemli proteinlerin ifade düzeylerinde düzelme olduğunu tespit ettik. Bir çalışmada, RA'nın tümör oluşumu ve dolaşımdaki oksidan-antioksidan durumu üzerindeki etkisi değerlendirilerek, RA'nın azoksimetan (AOM) kaynaklı sıçan kolon karsinogenezisini önleme yeteneği araştırılmıştır ve çalışmamızla benzer sonuçlara ulaşmışlardır[31].

Programlanmış hücre ölümü olan apoptozis, doku homeostazının korunmasında önemli bir rol oynar ve düzensizliği kanser gelişimi ve ilerlemesine neden olur. RA'nın diğer kanser tiplerindeki pro-apoptotik etkilerine ilişkin önceki yayınlarla tutarlı olarak[28,32], RA'nın sisplatin ile birlikte kolorektal kanser hücrelerinde apoptozu tetiklediğini, bunun da kaspazların ve pro-apoptotik protein Bax'ın aktivasyonu ve anti-apoptotik protein Bcl-2'nin ifadesinin düzenlenmesiyle kanıtlandığını son çalışmalarda gözlemlediler. Bu bulgular, RA'nın içsel mitokondriyal yol aracılığıyla apoptozu indüklemedeki önerilen mekanizma ile uyumlu olduğu bulunmuştur[29]. RA, doğrudan sitotoksik etkilerinin yanı sıra tümör mikroçevresini düzenleyerek dolaylı antikanser etkileri de gösterebilir. Çok sayıda çalışma, RA'nın anti-inflamatuar, antioksidan ve antianjiyojenik özelliklerini vurgulamıştır[33-35], bu da tümör promoting faktör, oksidatif stres ve neovaskülarizasyonu baskılayarak anti-kanser aktivitesine potansiyel olarak katkıda bulunabilir.

LPS, bakteriyel orijinli bir endotoksindir ve vücutta septik şoku etkilediği bilinmektedir. Bağışıklık hücreleri tarafından salgılanan önemli inflamatuvar olan Tümör Nekroz Faktör- α (TNF-α), İnterlökinler (IL-1β, IL-6 ve IL-10) ve pro- ve anti-inflamatuvar sitokinlerin LPS hasarında immün cevabın oluşturulmasına neden olduğu gösterilmiştir[36]. Bir çalışmada, MTT testi kullanarak RA'nın MC38 hücreleri üzerindeki metastazı önleyici konsantrasyonu belirlenmiş ve RA 150 µM'nin MC38 hücrelerinin göçünü ve invazyonunu önemli ölçüde engellediği ve bunun *in vitro* tümör metastaz inhibitörü olarak potansiyelini göstermişlerdir[28].

Karnosol (%1 diyette) kolorektal kanseri, bağırsak tümör çeşitliliğini azalttığı belirlenmiştir[37]. Ursolik asit (%0,11 diyette) kolorektal adenom gelişiminin en erken öncülerinden biri olan anormal kript odaklarının ve özellikle tümör başlangıç evre insidansını azaltmıştır[38]. Araştırılan diğer kanser türleri arasında meme, karaciğer ve cilt kanseri yer almaktadır. Biberiye özütü, karnosol ve UA meme tümörünün görülme sıklığını ve/veya çokluğunu azaltmıştır[39-41]. Osakabe ve ark. tarafından bildirilen %68 RA içeren bir Perilla frutescens özütünün, iki aşamalı bir fare deri modelinde tümör oluşumunu belirgin şekilde azalttığını bildirmiştir[42].

Otofaji, uzun ömürlü proteinlerin, sitozolik bileşenlerin veya hasarlı organellerin yenilenmesinde rol oynayan oldukça düzenli bir süreçtir[43]. ROS'un ER stresini indüklemedeki rolüne ek olarak, Yang ve ark. tarafından gerçekleştirilen *in vivo* çalışmalar, bozulmuş otofajinin de ER stresine katkıda bulunduğunu ve otofaji restorasyonunun ER homeostazını iyileştirdiğini göstermiştir. Son veriler ayrıca hepatosit lipid birikiminin azalmış otofajik işlevle ilişkili olduğunu ileri sürmüştür; bu da otofaji'nin hepatositlerde lipid homeostazının düzenlenmesindeki rolünü göstermektedir. Mevcut çalışmada HepG2 hücrelerinin oleik asitle muamele edilmesiyle, LC3II, Beclin 1, ATG5 ve ATG7'nin protein ekspresyonunda azalma olduğu tespit edilmiştir[44]. LC3II, otofagozomun karakterize edilmiş bileşenidir ve azalması, hepatositlerde lipid damlacıklarının birikmesine yol açabilecek olan otofagozom dönüşümünün azaldığını göstermektedir. Benzer şekilde, Beclin 1, otofaji sürecindeki anahtar gendir ve otofaji aktivitesinin miktarını temsil etmektedir[1].

Çalışmamız RA'nın etkili bir şekilde kolon kanserinde hücresel otofaji yolaklarındaki defektleri iyileştirebileceğini ve engelleyebileceğini göstermektedir. LPS hasarı oluşturduğumuz Caco-2 hücre hasarında otofajik yıkımdaki etkisine karşı RA'nın rejeneratif etkisini tespit ettik. İmmünofloresans analizinda LPS gruplarında otofajik yolaktaki Beclin 1, p62 ve LC3 proteinlerinin yıkımındaki hasar sonucu, bu proteinlerin hücrenin sitoplazmasında ve perinükleer alanlarda yoğunlaştığını belirledik. LPS+RA verilen gruplarda protein degradasyonunda yıkımın geri dönüştürebilir etkilerini tespit ettik. Ek olarak, western blot analizi, immünofloresans analizindeki sonuçlarımızı desteklemektedir ve istatistiksel olarak anlamlı farklılık bulunmuştur ($p<0,05$).

Sonuç olarak, bulgularımız RA'nın anti-kanser etkilerini güçlendirirken, kullanılan *in vitro* hücre modeli ile RA'nın otofajik yolaktaki önemli proteinlerin degradasyonunda rejeneratif etkilerini olduğunu tespit ettik. *İn vivo* ksenograft modelleri ve hasta kaynaklı organoidler

kullanılarak yapılacak gelecekteki çalışmalar, farklı ilaç kombinasyon yaklaşımının terapötik potansiyeli hakkında klinik açıdan daha anlamlı bilgiler sağlayabileceğini düşünmekteyiz.

Etik Standartlara Uygunluk
Etik onay gerekliliği bulunmamaktır.

Çıkar Çatışması
Yazarlar arasında çıkar çatışması yoktur.

Finansal Destek
Çalışmamız herhangi bir kurum ya da kuruluş tarafından maddi olarak desteklenmemiştir.

Yazar Katkısı
İÇ, EA: Çalışmanın fikrinin geliştirilmesi, hipotezin oluşturulması ve çalışma tasarımının hazırlanması; İÇ, EA: Materyal hazırlama, veri toplama ve analizinin gerçekleştirilmesi; İÇ: istatistiksel değerlendirme; İÇ: Makalenin ilk taslağının yazılması; İÇ, EA: Makalenin nihai halinin düzenlenmesi ve yayın sürecinin eleştirel değerlendirilmesi.

Kaynaklar

1. Wang Y, Wang C, Zhong R, Wang L, Sun L. Research progress of DNA methylation in colorectal cancer (Review). *Mol Med Rep*. 2024;30(3):154. doi:10.3892/mmr.2024.13278
2. Jain SM, Nagainallur Ravichandran S, Murali Kumar M, et al. Understanding the molecular mechanism responsible for developing therapeutic radiation-induced radioresistance of rectal cancer and improving the clinical outcomes of radiotherapy - A review. *Cancer Biol Ther*. 2024;25(1):2317999. doi:10.1080/15384047.2024.2317999
3. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, Laurent-Puig P, Quirke P, Yoshino T, Taieb J, Martinelli E, Arnold D; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2020;31(10):1291-1305. doi:10.1016/j.annonc.2020.06.022
4. Fasano M, Pirozzi M, Miceli CC, et al. TGF-β Modulated Pathways in Colorectal Cancer: New Potential Therapeutic Opportunities. *Int J Mol Sci*. 2024;25(13):7400. doi:10.3390/ijms25137400
5. Deshaies RJ. Proteotoxic crisis, the ubiquitin-proteasome system, and cancer therapy. *BMC Biol*. 2014;12:94. doi:10.1186/s12915-014-0094-0
6. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546-58. doi:10.1126/science.1235122
7. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009;136(5):823-37. doi:10.1016/j.cell.2009.02.024
8. Galluzzi L, Pietrocola F, Bravo-San Pedro JM, et al. Autophagy in malignant transformation and cancer progression. *EMBO J*. 2015;34(7):856-80. doi:10.15252/embj.201490784
9. Levy JM, Thorburn A. Targeting autophagy during cancer therapy to improve clinical outcomes. *Pharmacol Ther*. 2011;131(1):130-41. doi:10.1016/j.pharmthera.2011.03.009
10. Fu Q, Jiang Y, Zhang D, Liu X, Guo J, Zhao J. Valosin-containing protein (VCP) promotes the growth, invasion, and metastasis of colorectal cancer through activation of STAT3 signaling. *Mol Cell Biochem*. 2016;418(1-2):189-98. doi:10.1007/s11010-016-2746-6
11. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. *Cell Biol Int*. 2019;43(6):582-592. doi:10.1002/cbin.11137
12. Kabeya Y, Mizushima N, Ueno T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J*. 2000;19(21):5720-8. doi:10.1093/emboj/19.21.5720
13. Tanida I, Ueno T, Kominami E. LC3 conjugation system in mammalian autophagy. *Int J Biochem Cell Biol*. 2004;36(12):2503-18. doi:10.1016/j.biocel.2004.05.009
14. Cayli S, Sahin C, Sanci TO, Nakkas H. Inhibition of p97/VCP function leads to defective autophagosome maturation, cell cycle arrest and apoptosis in mouse Sertoli cells. *Theriogenology*. 2020;158:196-206. doi:10.1016/j.theriogenology.2020.09.017
15. Ji CH, Kwon YT. Crosstalk and Interplay between the Ubiquitin-Proteasome System and Autophagy. *Mol Cells*. 2017;40(7):441-449. doi:10.14348/molcells.2017.0115
16. Kwon YT, Ciechanover A. The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. *Trends Biochem Sci*. 2017;42(11):873-886. doi:10.1016/j.tibs.2017.09.002
17. Ciechanover A. The unravelling of the ubiquitin system. *Nat Rev Mol Cell Biol*. 2015;16(5):322-4. doi:10.1038/nrm3982
18. Vega-Rubín-de-Celis S. The Role of Beclin 1-Dependent Autophagy in Cancer. *Biology (Basel)*. 2019;9(1):4. doi:10.3390/biology9010004
19. Li W, Zhang L. Regulation of ATG and Autophagy Initiation. *Adv Exp Med Biol*. 2019;1206:41-65. doi:10.1007/978-981-15-0602-4_2
20. Nakkas H, Ocal BG, Kipel S, et al. Ubiquitin proteasome system and autophagy associated proteins in human testicular tumors. *Tissue Cell*. 2021;71:101513. doi:10.1016/j.tice.2021.101513
21. Tran S, Fairlie WD, Lee EF. BECLIN1: Protein Structure, Function and Regulation. *Cells*. 2021;10(6):1522. doi:10.3390/cells10061522
22. Ngo SN, Williams DB, Head RJ. Rosemary and cancer prevention: preclinical perspectives. *Crit Rev Food Sci Nutr*. 2011;51(10):946-54. doi:10.1080/10408398.2010.490883. PMID: 21955093
23. Öztürk Küp F, Koçak B, Akın AT, et al. Lipopolisakkarit'in neden olduğu bağırsak toksisitesine karşı biyosentetik gümüş nanopartiküllerin etkisi. *Türk Hijyen Ve Deneysel Biyoloji Dergisi*. 2020;77(3):333-342.
24. Liu Y, Xu X, Tang H, Pan Y, Hu B, Huang G. Rosmarinic acid inhibits cell proliferation, migration, and invasion and induces apoptosis in human glioma cells. *Int J Mol Med*. 2021;47(5):67. doi:10.3892/ijmm.2021.4900
25. Woottisin N, Sukprasert S, Kulsirirat T, Tharavanij T, Sathirakul K. Evaluation of the Intestinal Permeability of Rosmarinic Acid from Thunbergia laurifolia Leaf Water Extract in a Caco-2 Cell Model. *Molecules*. 2022;27(12):3884. doi:10.3390/molecules27123884
26. Lin TY, Fan CW, Maa MC, Leu TH. Lipopolysaccharide-promoted proliferation of Caco-2 cells is mediated by c-Src

induction and ERK activation. *Biomedicine (Taipei)*. 2015;5(1):5. doi:10.7603/s40681-015-0005-x
27. Cayli S, Alimogullari E, Piskin I, Bilginoglu A, Nakkas H. Effect of pioglitazone on the expression of ubiquitin proteasome system and autophagic proteins in rat pancreas with metabolic syndrome. *J Mol Histol*. 2021;52(5):929-942. doi:10.1007/s10735-021-10013-1
28. Liu H, Deng R, Zhu CW, et al. Rosmarinic acid in combination with ginsenoside Rg1 suppresses colon cancer metastasis via co-inhition of COX-2 and PD1/PD-L1 signaling axis. *Acta Pharmacol Sin*. 2024;45(1):193-208. doi:10.1038/s41401-023-01158-8
29. Huang JY, Hsu TW, Chen YR, Kao SH. Rosmarinic Acid Potentiates Cytotoxicity of Cisplatin against Colorectal Cancer Cells by Enhancing Apoptotic and Ferroptosis. *Life (Basel)*. 2024;14(8):1017. doi:10.3390/life14081017
30. Siegel RL, Miller KD, Sauer AG, Fedewa SA. Colorectal Cancer Statistics, 2020. 2020;70(3):145-164. doi:10.3322/caac.21601
31. Ilhan N, Bektas I, Susam S, Ozercan IH. Protective effects of rosmarinic acid against azoxymethane-induced colorectal cancer in rats. *J Biochem Mol Toxicol*. 2022;36(2):e22961. doi:10.1002/jbt.22961
32. Messeha SS, Zarmouh NO, Asiri A, Soliman KFA. Rosmarinic acid-induced apoptosis and cell cycle arrest in triple-negative breast cancer cells. *Eur J Pharmacol*. 2020;885:173419. doi:10.1016/j.ejphar.2020.173419
33. Jin BR, Chung KS, Hwang S, et al. Rosmarinic acid represses colitis-associated colon cancer: A pivotal involvement of the TLR4-mediated NF-κB-STAT3 axis. *Neoplasia*. 2021;23(6):561-573. doi:10.1016/j.neo.2021.05.002
34. Pagano K, Tomaselli S, Molinari H, Ragona L. Natural Compounds as Inhibitors of Aβ Peptide Aggregation: Chemical Requirements and Molecular Mechanisms. *Front Neurosci*. 2020;14:619667. doi:10.3389/fnins.2020.619667
35. Cao W, Mo K, Wei S, Lan X, Zhang W, Jiang W. Effects of rosmarinic acid on immunoregulatory activity and hepatocellular carcinoma cell apoptosis in H22 tumor-bearing mice. *Korean J Physiol Pharmacol*. 2019;23(6):501-508. doi:10.4196/kjpp.2019.23.6.501
36. Doğanyiğit Z, Küp FÖ, Silici S, Deniz K, Yakan B, Atayoglu T. Protective effects of propolis on female rats' histopathological, biochemical and genotoxic changes during LPS induced endotoxemia. *Phytomedicine*. 2013;20(7):632-9. doi:10.1016/j.phymed.2013.01.010
37. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Anti-Carcinogenic Effects of Carnosol-An Updated Review. *Curr Drug Discov Technol*. 2018;15(1):32-40. doi:10.2174/1570163814666170413121732
38. Andersson D, Cheng Y, Duan RD. Ursolic acid inhibits the formation of aberrant crypt foci and affects colonic sphingomyelin hydrolyzing enzymes in azoxymethane-treated rats. *J Cancer Res Clin Oncol*. 2008;134(1):101-7. doi:10.1007/s00432-007-0255-4
39. Singletary KW, Nelshoppen JM. Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumorigenesis and of in vivo formation of mammary DMBA-DNA adducts by rosemary extract. *Cancer Lett*. 1991;60(2):169-75. doi:10.1016/0304-3835(91)90224-6
40. Singletary K, MacDonald C, Wallig M. Inhibition by rosemary and carnosol of 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumorigenesis and in vivo DMBA-DNA adduct formation. *Cancer Lett*. 1996;104(1):43-8. doi:10.1016/0304-3835(96)04227-9
41. Amagase H, Sakamoto K, Segal ER, Milner JA. Dietary rosemary suppresses 7,12-dimethylbenz(a)anthracene binding to rat mammary cell DNA. *J Nutr*. 1996;126(5):1475-80. doi:10.1093/jn/126.5.1475
42. Osakabe N, Yasuda A, Natsume M, Yoshikawa T. Rosmarinic acid inhibits epidermal inflammatory responses: anticarcinogenic effect of Perilla frutescens extract in the murine two-stage skin model. *Carcinogenesis*. 2004;25(4):549-57. doi:10.1093/carcin/bgh034
43. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ*. 2005;12 Suppl 2(Suppl 2):1542-52. doi:10.1038/sj.cdd.4401765
44. Yang L, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab*. 2010;11(6):467-78. doi:10.1016/j.cmet.2010.04.005

doi: 10.53446/actamednicomedia.1590352

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

RELATIONSHIP BETWEEN ERECTILE DYSFUNCTION AND ASYMMETRIC DIMETHYL ARGININE LEVELS IN PATIENTS WITH END-STAGE RENAL DISEASE

SON DÖNEM BÖBREK HASTALIĞI OLAN HASTALARDA EREKTİL DİSFONKSİYON VE ASİMETRİK DİMETİL ARGİNİN DÜZEYLERİ ARASINDAKİ İLİŞKİ

Burak Can[1], Sibel Gökçay Bek[2*], Metin Ergül[2], Adnan Batman[3], Necmi Eren[2], Ramazan Azim Okyay[4], Betül Kalender[2], Erkan Dervişoğlu[2]

[1]Acıbadem Hospital, Department of Internal Medicine, Adana, Türkiye. [2]Kocaeli University, Faculty of Medicine, Department of Nephrology, Kocaeli, Türkiye. [3]Koç University Hospital, Department of Endocrinology, İstanbul, Türkiye. [4]Kahramanmaraş Sütçü İmam University, Department of Public Health, Kahramanmaraş, Türkiye

ABSTRACT

Objective: Asymmetric dimethylarginine is a major inhibitor of nitric oxide synthesis. Erectile dysfunction and chronic kidney disease (CKD) are associated with elevated levels of asymmetric dimethylarginine. This study aimed to examine the effects of ADMA on erectile dysfunction in patients undergoing peritoneal hemodialysis.

Methods: A total of 32 peritoneal, 32 hemodialysis patients, and 32 healthy male volunteers were included in the study. Serum asymmetric dimethylarginine levels were measured, and clinical and laboratory parameters were analyzed. The International Index of Erectile Function-5 was used to evaluate sexual function and the Pittsburgh Sleep Quality Index was used to evaluate sleep quality. Depressive symptoms were assessed using the Beck depression inventory.

Results: Asymmetric dimethylarginine levels differed significantly among the three groups ($p<0.001$). It was higher in patients undergoing hemodialysis than in those undergoing peritoneal dialysis ($p <0.002$). Erectile dysfunction was detected more frequently in the patient group than in the control group ($p<0.001$). However, correlation analysis revealed no significant relationship between asymmetric dimethylarginine levels and erectile dysfunction scores. There was a negative correlation between the erectile dysfunction score, sleep quality, and depression scale scores. Asymmetric dimethylarginine showed a significant positive correlation with treatment duration, phosphorus, calcium-phosphorous product, and parathormone. A negative correlation was observed between albumin, cholesterol, low-density lipoprotein (LDL), residual urine, and asymmetric dimethylarginine levels. Residual urine amount in the correlation analysis showed a negative correlation with asymmetric dimethylarginine, phosphorus, and calcium-phosphorus products and a positive correlation with total cholesterol and low-density lipoprotein.

Conclusion: Residual renal function and urine amount are important parameters that correlate with ADMA levels for sustainable healthy erectile function in CKD.

Keywords: ADMA, Erectile Dysfunction

ÖZ

Amaç: Asimetrik dimetilarginin, nitrik oksit sentezinin majör inhibitörüdür. Erektil disfonksiyon ve kronik böbrek hastalığı (KBH), yüksek asimetrik dimetilarginin seviyeleriyle ilişkili bulunmuştur. Bu çalışma, ADMA'nın peritoneal hemodiyaliz geçiren hastalarda erektil disfonksiyon üzerindeki etkilerini incelemeyi amaçlamıştır.

Yöntem: Çalışmaya toplam 32 peritoneal, 32 hemodiyaliz hastası ve 32 sağlıklı erkek gönüllü dahil edildi. Serum asimetrik dimetilarginin seviyeleri ölçüldü ve klinik ve laboratuvar parametreleri analiz edildi. Cinsel fonksiyonu değerlendirmek için Uluslararası Erektil Fonksiyon İndeksi-5 ve uyku kalitesini değerlendirmek için Pittsburgh Uyku Kalitesi İndeksi kullanıldı. Depresif semptomlar Beck Depresyon Envanteri Ölçeği kullanılarak değerlendirildi.

Bulgular: Asimetrik dimetilarginin seviyeleri üç grup arasında önemli ölçüde farklılık gösterdi ($p<0,001$). Hemodiyalize giren hastalarda peritoneal diyalizi yapanlara göre daha yüksekti ($p <0,002$). Hasta grubunda erektil disfonksiyon kontrol grubuna göre daha sık tespit edildi ($p<0,001$). Ancak korelasyon analizi asimetrik dimetilarginin düzeyleri ile erektil disfonksiyon skorları arasında anlamlı bir ilişki olmadığını ortaya koydu. Erektil disfonksiyon skoru, uyku kalitesi ve depresyon ölçeği skorları arasında negatif korelasyon gösterdi. Asimetrik dimetilarginin tedavi süresi, fosfor, kalsiyum-fosfor ürünü ve parathormon ile anlamlı pozitif korelasyon gösterdi. Albümin, kolesterol, düşük yoğunluklu lipoprotein (LDL), rezidüel idrar ve asimetrik dimetilarginin düzeyleri arasında negatif korelasyon gözlendi. Korelasyon analizindeki rezidüel idrar miktarı asimetrik dimetilarginin, fosfor ve kalsiyum-fosfor ürünleri ile negatif korelasyon, toplam kolesterol ve düşük yoğunluklu lipoprotein ile pozitif korelasyon gösterdi.

Sonuç: Kronik böbrek hastalığında (KBH) sürdürülebilir sağlıklı erektil fonksiyon için rezidüel böbrek fonksiyonu ve idrar miktarı, ADMA düzeyleriyle korelasyon gösteren önemli parametrelerdir.

Anahtar Kelimeler: ADMA, Erektil Disfonksiyon

***Corresponding author/İletişim kurulacak yazar:** Sibel Gökçay Bek; Kocaeli University, Faculty of Medicine, Department of Nephrology, Kocaeli, Türkiye
Phone/Telefon: +90 (506) 807 06 81, **e-mail/e-posta:** beksibel@gmail.com
Submitted/Başvuru: 25.11.2024 • **Accepted/Kabul:** 11.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Asymmetric dimethylarginine (ADMA) is a significant inhibitor of endothelial nitric oxide synthase (eNOS) production and L-arginine entry into cells.[1]ADMA has been discovered to significantly increase the uncoupling of eNOS and generate free radicals. [2,3]

Recent studies have demonstrated that ADMA plays a crucial role in predicting the likelihood of cardiovascular complications and death in individuals with chronic kidney disease (CKD) who are either pre-dialysis or dialysis-dependent.[4,5] ADMA causes vasoconstriction and inhibition of acetylcholine-induced vasorelaxation in the brain.[6] This plays a significant role in the cognitive decline in patients with CKD. ADMA has also been linked to the development of many comorbidities affecting whole human physiology.[7-14] Studies have connected ADMA to the activation of polymorphonuclear cells and expression of adhesion molecules.[15]

Erectile dysfunction (ED) is a common problem in patients with CKD and its frequency increases in patients undergoing dialysis.[16] Mental disorders, drug side effects, decreased penile vascularity, and hormonal factors are associated with its etiology. [17]

Nonetheless, there is a lack of randomized clinical research examining the possible impact of arginine on erectile function in patients with CKD. This research sought to explore the connection between ADMA and erectile dysfunction in individuals receiving dialysis treatment.

Methods

This study included patients receiving dialysis treatment at the Kocaeli University Faculty of Medicine Hospital. The inclusion criteria were as follows: age > 18 years, male sex, married or having a sexual partner, having undergone peritoneal dialysis (PD)/hemodialysis (HD) for at least three months, and volunteering to participate in the study. The exclusion criteria were female sex, diabetes mellitus, psychiatric disorders, active infection, alcohol and substance addiction, malignancy, serious neurological diseases, and cardiac and hepatic failure.

Age, marital status, and routine habits were recorded by meeting the patients one-on-one. The International Index of Erectile Function-5 (IIEF-5), Pittsburgh Sleep Quality Index (PSQI), and Beck Depression Inventory (BDI) were used to evaluate erectile function, sleep quality, and depressive symptoms, respectively. Blood samples were collected from patients undergoing HD and peritoneal dialysis to determine ADMA levels. Blood was obtained from patients undergoing hemodialysis prior to dialysis and peritoneal exchange.

Information such as the dialysis duration, body mass index, the underlying cause of end-stage kidney disease (ESKD), type of peritoneal dialysis (PD), frequency of daily exchanges, daily ultrafiltration for hemodialysis (HD) patients, weekly duration and frequency of hemodialysis, dry weight, and the volume of ultrafiltration was extracted from the medical records.

Urea, creatinine, sodium, potassium, phosphorus, calcium, alkaline phosphatase, total protein, albumin, hemoglobin, sedimentation, C-reactive protein (CRP), parathyroid hormone (PTH), iron, transferrin saturation, ferritin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), serum triglyceride (TG), uric acid, hemoglobin, mean platelet volume (MPV), platelets, FSH, LH, total testosterone, residual renal function, and weekly Kt/V values are listed in the files. For patients undergoing hemodialysis, the average Kt/V values in the last 3 months were recorded. After collecting blood samples for ADMA measurement, the serum was stored at 80°C. The Immune Diagnostic ADMA Xpress ELISA Kit (Bendheim, Germany) was used for the ADMA measurements.

Statistical Analysis

Statistical analyses were performed using SPSS 20 for Windows (SPSS Inc., Chicago, IL, USA). Made using the latest version. To evaluate the significance level of the differences between groups, a t-test was used between the two groups, and one-way ANOVA was used between the three groups for normally distributed variables. For variables that were not normally distributed, the Mann–Whitney U test was applied between two groups, and the Kruskal–Wallis test was applied between three groups.

To evaluate the relationship between ADMA and the clinical, biochemical, and quality of life scales, the Pearson correlation test was used for normally distributed variables, and the Spearman correlation test was used for normally distributed variables. Statistical significance was set at $p < 0.05$.

Results

Ninety-six male participants, including 32 patients undergoing HD, 32 undergoing PD, and 32 healthy volunteers, were included in the study. All patients undergoing PD exchanged 2000 ml four times a day. All HD patients underwent dialysis for three days-four hours per week. The mean age was 48.6 ± 11.3 in PD patients, 48.8 ± 12.1 in HD patients, and 46.6 ± 7.4 in the control group. The causes of ESKD in patients undergoing PD included hypertension in 19, glomerulonephritis in two, idiopathic in three, polycystic kidney disease in six, vesicoureteral reflux in one, and nephrolithiasis in one. The causes of ESKD in patients undergoing HD were hypertension in 14 (of patient had both hypertension and polycystic kidney disease), glomerulonephritis in 2, polycystic kidney disease in 5, amyloidosis in 1, idiopathic in 7, congenital renal hypoplasia in 1, nephrolithiasis in 2, and vesicoureteral reflux in 1. In the group of patients receiving hemodialysis, 27 individuals (84.37%) were diagnosed with erectile dysfunction. Comparatively, 20 patients (62.5%) in the peritoneal dialysis category and 8 individuals (25%) in the control group were also affected. Overall, 73.43% of those undergoing dialysis (both HD and PD) experienced erectile dysfunction.

Asymmetric dimethylarginine levels were measured in PD patients: 0.74 ± 0.42 micromol/L, in HD patients: 1.1±0.4 micromol/L, and in the control group: 0.47±0.2 micromol/L. The PSQI, BDI, and IIEF-5 values of PD, HD patients, and the control group, as well as treatment duration, residual urine amount, Kt/V values, and other laboratory data of PD and HD patients, are shown in Table 1. Upon examining the data, it was discovered that there were statistically significant discrepancies in the ADMA, BDI, PSQI, IIEF, and total testosterone levels (Table 1).

A pairwise comparison was performed between the groups to determine the group that had differences in ADMA level, IIEF-5, PSQI, and BDI scores. Bonferroni correction was used, and a p-value <0.0167 was considered significant. (Table 2)

When patients with PD and those with HD were compared, no significant difference was observed between the two groups in terms of IIEF-5, PSQI, BDI scores, and total testosterone levels. ADMA levels were significantly lower in patients undergoing PD than in HD patients [0.7±0.4 micromol/L, respectively; 1.1±0.4 micromol/L (p=0.002)]. In patients with PD, ADMA levels and PSQI scores were higher than those in the control group (p=0.002, p=0.008, respectively), and the IIEF score was significantly lower (p<0001). In patients undergoing HD, the IIEF-5 scores were lower (p<0.001) and the ADMA, BDI, and PSQI scores were higher than those in the control group (ADMA, p=0.002; PSQI, p=0.002; BDI, p<0.0001).

When the relationships between the laboratory values of HD patients and PSQI, IIEF-5, BDI scores, and ADMA were examined, a significant relationship was found between transferrin and ADMA levels. When peritoneal dialysis and HD patients were taken together as a patient group and the relationship between laboratory values, PSQI, IIEF-5, BDI scores, and ADMA were examined, a significant relationship was found in the same direction with treatment duration, phosphorus, parathormone, MPV, and ferritin, and a significant reverse relationship was found between residual urine amount and LDL.

Table 1. Comparison of variables in peritoneal dialysis and hemodialysis patient groups

	PD patients Mean ±SD	HD patients Mean ±SD	p value
Age (year)	48.6 ± 11.3	48.8 ± 12.1	0.945
Number of offspring	0.7 ± 0.4	1.1± 0.4	**0.0002**
IIEF-5	14.9 ± 6.9	14,69 ± 6.8	0.907
ADMA (µmol/L)	0.7 ± 0.4	1.1 ± 0.4	**0.0002**
PSQI	6.3 ± 4.1	7.4 ± 4.7	0.322
BDI	12.0 ± 10	16.8 ± 11.5	0.079
Total Testosterone (ng/dL)	357.5 ± 103.1	263 ± 124.8	**0.001**
Duration of Dialysis (months)	52 ± 34.7	106.1 ± 67.9	**0.0002**
Residual Urine (mL)	827.2 ± 744.3	212.5 ± 421.8	**0.0001**
UF Volume(mL)	1339.8 ± 692.6	3064.1 ± 992.2	**0.0001**
Kt/V	2.10 ± 0.9	1.4 ± 0.2	**0.0001**
T. protein (g/dL)	6.2 ± 0.8	6.7 ± 0.6	**0.006**
Albumin (g/dL)	3.5 ± 0.5	3.8 ± 0.3	**0.005**
Calcium (mg/dL)	9.0 ± 0.7	8.9 ± 0.9	0.621
Phosphorus (mg/dL)	5.1 ± 1.3	5.7 ± 1.6	0.104
Ca – P Product	46.4 ± 13	50.7 ± 13.6	0.200
PTH (pg/mL)	603.5 ± 662.4 (Median: 574.28)	683.4 ± 468.7 (Median:727.130)	0.579
Total cholesterol (mg/dL)	169.9 ± 43.3	154.6 ± 35	0,125
LDL (mg/dL)	107.6 ± 34.9	80 ± 30.3	**0.001**
BMI (kg/m^2)	26.4 ± 3.8	24.4 ± 3.5	**0.032**
Hemoglobin (g/dL)	11.5 ± 2.2	12.2 ± 2	0.187
MPV (fL)	7.3 ± 1.1	8.8 ± 1	**0.0001**
Platelet (cells/mm^3)	254.281 ± 71.026	200.312 ± 68.263	**0.002**
Iron (mcg/dL)	72.9 ± 30	66.9 ± 48.8	0.555
Transferrin saturation (%)	34.9 ± 20.9	46.8 ± 69.1	0.354
Ferritin (ng/mL)	341.5 ± 280.5 (Median:361.68)	791.6 ± 956 (Median:833.33)	**0.013**
Uric Acid (mg/dL)	5.7 ± 1	6 ± 1.7	0.392
CRP (mg/L)	1.2 ± 1.5	1.7 ± 2	0.262

Abbreviations: SD: Standard deviation, CRP: C-reactive protein, PTH: Parathormone, BMI: Body Mass Index, LDL: Low Density Lipoprotein, MPV: Mean Platelet Volume, UF: Ultrafiltration, ADMA: Asymmetric dimethylarginine, IIEF -5: International Index of erectile Function-5, PSQI: Pittsburgh Sleep Quality Index, BDI: Beck depression Inventory. T-test was used.

Table 2. Comparison of Common Variables Between Groups

	PD (n=32)	HD (n=32)	Control (n=32)	*p*
	Mean ± SD	Mean ± SD	Mean ± SD	
Age**	48.6 ± 11.3	48.8 ± 12.1	46.6 ± 7.4	0.645
Total Testosterone **(ng/dL)	357.5 ± 103.1	263 ± 124.8	321.4 ± 112.5	**0.005**
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	
ADMA *(µmol/L)	0.7 (0.4)	1.1 (0.4)	0.5 (0.2)	**< 0.0001**
PSQI *	6.3 (4.1)	7.4 (4,7)	4 (3.1)	**0.003**
BDI *	12 (10)	16.8 (11.5)	6.7 (4.3)	**< 0.0001**
IIEF-5 *	14.9 (6.9)	14.7 (6.8)	22.3 (2.8)	**< 0.0001**

Abbreviations: SD: Standard deviation, IQR: Interquartile range ADMA: Asymmetric dimethylarginine, IIEF -5: International Index of erectile Function-5, PSQI: Pittsburgh Sleep Quality Index, BDI: Beck depression Inventory. *Kruskal Wallis, **One Way ANOVA

Table 3. Association between ADMA levels and other variables in HD and PD groups.

	r	*p*
Age [b]	0.008	0.947
IIEF-5 [b]	0.068	0.595
PSQI [b]	0.006	0.961
Total testosterone [a]	-0.200	0.114
Treatment period [b]	0.251	**0.046**
Residual urine volume [b]	-0.413	**0.001**
Total protein [b]	-0.138	0.276
Albumin [b]	-0.260	**0.038**
Calcium [b]	-0.187	0.150
Phosphorus [a]	0.296	**0.018**
Calcium x phosphorus product [b]	0.217	0.086
Parathormone [b]	0.451	**0.0001**
Total cholesterol [b]	-0.229	0.069
HDL [b]	-0.234	0.062
LDL [b]	-0.250	**0.047**
Hemoglobin [b]	-0.017	0.892
Transferrin saturation [b]	0.059	0.642
Ferritin [b]	0.276	**0.027**
Uric acid [a]	0.114	0.368
CRP [b]	0.214	0.089

Abbreviations: ADMA: Asymmetric dimethylarginine, IIEF-5: International Index of Erectile Function-5, PSQI: Pittsburgh Sleep Quality Index, BDI: Beck Depression Inventory, CRP: C-reactive protein, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein

[a] Pearson correlation analysis, [b] Spearman correlation analysis

Table 4. Residual urine volume and variables associated with residual urine volume

	Residual urine volume
ADMA	**-0.413****
Total testosterone	**0.413****
Total cholesterol	**0.249***
LDL	**0.336****
Phosphorus	**-0,402****
Parathormone	**-0,329****
Calcium x Phosphorus Product	**-0,397****

Spearman correlation test was used. * p<0.05, ** p<0.01

ADMA: Asymmetric dimethylarginine, LDL: Low Density Lipoprotein

Discussion

The ADMA levels were higher in the ESKD group than in the control group. Furthermore, patients undergoing HD had significantly higher ADMA levels than those undergoing PD The results of this study are consistent with those of previous studies. A study by Zhang et al.[18] in 2010 revealed that ADMA levels were at their lowest in healthy individuals, increased in patients undergoing PD, and reached their peak in those receiving HD. ADMA is linked to an increased risk of cardiovascular issues. In patients with ESKD, the removal of high ADMA content from the body may decrease the risk of morbidity and mortality.[19] In some instances, choosing peritoneal dialysis could offer greater benefits in potentially

lowering mortality rates when compared to hemodialysis.

After evaluating patients with PD and HD individually, no correlation was observed between ADMA and IIEF-5 scores. The outcomes remained consistent when the PD and HD patients were combined into a single group. Erectile dysfunction in individuals with ESKD can occur for several reasons in addition to an increase in ADMA levels, which impedes nitric oxide synthesis. Paroni et al.[20] examined patients who were exclusively monitored in the ED. The findings revealed that both arteriogenic and non-arteriogenic ED patients exhibited higher levels of ADMA than the control subjects. ADMA levels were higher in patients with arteriogenic ED than in those without. Due to the limitations of performing penile Doppler on patients who participated in the study, it was not feasible to differentiate between arteriogenic and non-arteriogenic ED. As a result, no remarks were made regarding the relationship between IIEF score and ADMA in patients undergoing dialysis with either type of ED.

Studies have indicated that people with ED often exhibit a higher mean platelet volume, which indicates heightened platelet activity. This points to a likelihood of platelet aggregation, regardless of the root cause.[21] In this study, a connection was observed between ADMA and MPV levels. Previous studies have not documented this association or the impact of MPV on patients with CKD. [22,23,24]

IIEF scores were significantly lower in patients with PD and HD than in the control group, and erectile dysfunction, depression, and sleep disorders were more common in patients with ESKD than in the normal population. [25] When patients undergoing PD and HD were compared, no significant differences in the IIEF-5, BDI, and PSQI scores were observed. Erectile dysfunction is common in dialysis patients. Patients express sleep-related and psychological distress, but have difficulty expressing complaints about sexual dysfunction. This situation also caused difficulties in filling out the questionnaires and in daily practice. Quality of life variables and sexual dysfunction experienced by patients undergoing physical examination and laboratory tests should be thoroughly assessed, particularly those that are difficult to articulate. In this study, the incidence of depression was higher in the patient group than in the nonpatient group.

The frequency of erectile dysfunction in patients with chronic kidney disease is approximately 20%–87.7%. [26,27,28] The frequency of ED among patients included in the study was 68.8% in the PD group and 81.3% in the HD group. ED was more common in patients undergoing HD than in those undergoing PD; however, this difference was not statistically significant.

Several factors can cause ED in ESKD patients. Factors such as vascular problems, medications, hormonal changes, psychological stress, zinc deficiency, and anemia play a role in the development of erectile dysfunction. [28] In the analysis we conducted to determine the factors affecting the IIEF-5 score of the patient group, there was a negative correlation between the amount of residual urine, PSQI and BDI scores, and the IIEF score. In addition, age correlated with erectile dysfunction. No relationship was found between other laboratory markers and IIEF-5 in the hemodialysis group.

In the peritoneal dialysis group, there was a significant inverse relationship between ADMA and total protein and albumin levels, which are nutritional markers. [29] When the PD and HD groups were considered together, no significant relationship was found with total protein, whereas a significant negative correlation was found between albumin and ADMA levels. Many studies have shown a negative correlation between plasma albumin concentration and a positive correlation between high ferritin levels and morbidity in patients undergoing PD or HD. [30] Given that both are significant indicators of inflammation, it makes sense to comprehend this connection.

When we examined the relationship between total cholesterol, LDL, and ADMA in our patients, we found a negative correlation. The negative correlation between total cholesterol and LDL, which increases the risk of atherosclerosis in the normal population, and ADMA, which increases vascular endothelial damage in patients with ESKD, is consistent with previous studies and once again reveals the importance of nutritional status in patients undergoing dialysis[31,32]. Hypocholesterolemia is a strong risk factor for mortality in patients undergoing dialysis and a marker of poor nutritional status. [33] Similar to hypoalbuminemia, hypocholesterolemia is thought to be associated with inflammation.

In this study, ADMA was significantly associated with inorganic phosphorus and PTH levels. ADMA is a predictive marker of hyperparathyroidism. [34] Although it cannot be concluded that ADMA causes PTH secretion, an increase in ADMA and PTH values occurs together.[35] No significant relationship was found between ADMA and calcium and phosphorus levels, which are other factors contributing to vascular calcification in this study, probably due to the small number of patients.

The most significant data in this study were related to the amount of residual urine volume. A significant negative correlation was found between residual urine function and ADMA, phosphorus, PTH, calcium phosphorus product values, and treatment duration, whereas a positive correlation was found between total cholesterol, LDL, and total testosterone levels. A notable correlation between ADMA and total testosterone levels has been observed in patients with hypogonadotropic conditions.[36,37] However, this association has not been documented in patients with CKD.

Residual renal function is an important predictor of survival in patients [36,37] Very few large-scale studies have been conducted on patients undergoing hemodialysis. The CHOICE study showed that mortality was reduced in patients undergoing hemodialysis with residual renal function, regardless of the cause.[38] Many studies have shown that patients with residual renal function have better nutritional status than those without residual renal function[39]. Emphasizing the preservation of the remaining kidney's function may play a crucial role in

enhancing patients' overall quality of life and sexual health.[40]

Conclusion

In conclusion, this study confirmed the elevated ADMA levels in dialysis patients, which vary based on the type of dialysis they undergo. Although a direct relationship between ADMA and ED was not found, its associations with nutritional markers, mineral metabolism, and especially the impact of residual renal function on various biochemical parameters, underscore the importance of a multidimensional approach in managing this patient population. Future studies should be conducted in larger patient cohorts to understand better the role of ADMA in the pathophysiology of CKD and to evaluate the long-term effects of different dialysis modalities.

Ethical Approval

The study complied with the Declaration of Helsinki. Ethical approval for the study was obtained from the ethics committee of Kocaeli University Hospital (GOKAEK-KOU KAEK 2014/42). Informed consent was obtained from all the participants when they were enrolled.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

BC, ED: Concept-Design; BC, ME, NE, SGB, AB: Data curation; BC, SGB, ED ,RAO: Formal Analysis; NA: Funding acquisition; BC, ME, AB: Investigation; BC, ED: Methodology; ED: Project administration; BC, ME, NE, RAO, AB: Resources; ED, SGB,BK: Supervision; BC, SGB: Validation; BC: Visualization; BC, SGB, ED: Writing – original draft; BC, SGB, ED,BK: Writing – review & editing.

Financial Support

This study received no funding.

References

1 Bode-Böger SM, Scalera F, Ignarro LJ. The l-arginine paradox: Importance of the l-arginine/asymmetrical dimethylarginine ratio. *Pharmacol Ther*. 2007 Jun;114(3):295–306. doi: 10.1016/j.pharmthera.2007.03.002

2 Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol.* 2004 Jun;24(6):1023–30. doi: 10.1161/01.ATV.0000128897.54893.26

3 Au AYM, Mantik K, Bahadory F, Stathakis P, Guiney H, Erlich J, et al. Plasma arginine metabolites in health and chronic kidney disease. *Nephrol Dial Transplant*. 2023 Dec;38(12):2767–75. doi: 10.1093/ndt/gfad108

4 Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol*. 2005;16(8):2449–55. doi: 10.1681/ASN.2005010076

5 Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino LS, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. *Lancet.* 2001 Dec;358(9299):2113–7. doi: 10.1016/s0140-6736(01)07217-8

6 Bima C, Parasiliti-Caprino M, Rumbolo F, Ponzetto F, Gesmundo I, Nonnato A, et al. Asymmetric and symmetric dimethylarginine as markers of endothelial dysfunction in cerebrovascular disease: A prospective study. Nutrition, Metabolism and *Cardiovascular Diseases*. 2024 Jul;34(7):1639–48. doi: 10.1016/j.numecd.2024.03.015.

7 Wieczór R, Wieczór AM, Kulwas A, Rość D. ADMA (asymmetric dimethylarginine) and angiogenic potential in patients with type 2 diabetes and prediabetes. *Exp Biol Med.* 2021 Jan;246(2):153. doi: 10.1177/1535370220959738.

8 Gkaliagkousi E, Gavriilaki E, Triantafyllou A, Nikolaidou B, Anyfanti P, Koletsos N, et al. Asymmetric dimethylarginine levels are associated with augmentation index across naïve untreated patients with different hypertension phenotypes. *The Journal of Clinical Hypertension*. 2018 Apr;20(4):680. doi: 10.1111/jch.13237.

9 Braekke K, Ueland PM, Harsem NK, Staff AC. Asymmetric Dimethylarginine in the Maternal and Fetal Circulation in Preeclampsia. *Pediatric Research* 2009 66:4. 2009;66(4):411–5. doi:10.1203/PDR.0b013e3181b33392.

10 Zakrzewicz, Dariusz. Asymmetric dimethylarginine metabolism and its involvement in the pathogenesis of pulmonary arterial hypertension (PhD Thesis). *Poznan, Poland: Faculties of Veterinary Medicine and Medicine of the Justus Liebig University Giessen*; 2008.

11 Schrauben SJ, Sapa H, Xie D, Zhang X, Anderson AH, Shlipak MG, et al. Association of urine and plasma ADMA with atherosclerotic risk in DKD cardiovascular disease risk in diabetic kidney disease: findings from the Chronic Renal Insufficiency Cohort (CRIC) study. *Nephrol Dial Transplant*. 2023 Dec;38(12):2809–15. doi: 10.1093/ndt/gfad103.

12 Vîlcea A, Borta SM, Popețiu RO, Alexandra RL, Pilat L, Nica DV, et al. High ADMA Is Associated with Worse Health Profile in Heart Failure Patients Hospitalized for Episodes of Acute Decompensation. Medicina 2024, Vol 60, Page 813. 2024 May;60(5):813. doi: 10.3390/medicina60050813.

13 Arlt S, Schulze F, Eichenlaub M, Maas R, Wiedemann K, Böger R, et al. Asymmetrical dimethylarginine is increased in plasma and decreased in cerebrospinal fluid of patients with Alzheimer's disease. Pharmacopsychiatry. 2009 Sep;42(05):A3. doi:10.1159/000144026.

14 Shafi T, Hostetter TH, Meyer TW, Hwang S, Hai X, Melamed ML, et al. Serum Asymmetric and Symmetric Dimethylarginine and Morbidity and Mortality in Hemodialysis Patients. Am J Kidney Dis. 2017 Jul;70(1):48–58. doi: 10.1053/j.ajkd.2016.10.033.

15 Shafi T, Hostetter TH, Meyer TW, Hwang S, Hai X, Melamed ML, et al. Serum Asymmetric and Symmetric Dimethylarginine and Morbidity and Mortality in Hemodialysis Patients. Am J Kidney Dis. 2017 Jul;70(1):48–58. doi: 10.1053/j.ajkd.2016.10.033.

16 Navaneethan SD, Vecchio M, Johnson DW, Saglimbene V, Graziano G, Pellegrini F, et al. Prevalence and correlates of self-reported sexual dysfunction in CKD: A meta-analysis of observational studies. American Journal of Kidney

Diseases. 2010 Oct;56(4):670–85. doi: 10.1053/j.ajkd.2010.06.016.

17 Moriyama T. Sexual dysfunction in chronic renal failure. J Mens Health. 2011 Apr;8(SUPPL. 1):S29–32. doi: 10.1016/S1875-6867(11)60016-X.

18 Zhang DL, Liu J, Liu S, Zhang Y, Liu WH. The differences of asymmetric dimethylarginine removal by different dialysis treatments. Ren Fail. 2010 Sep;32(8):935–40. doi: 10. 3109/0886022X.2010.502281.

19 Shafi T, Hostetter TH, Meyer TW, Hwang S, Hai X, Melamed ML, et al. Serum Asymmetric and Symmetric Dimethylarginine and Morbidity and Mortality in Hemodialysis Patients. Am J Kidney Dis. 2017 Jul;70(1):48–58. doi: 10.1053/j.ajkd.2016.10.033.

20 Paroni R, Barassi A, Ciociola F, Dozio E, Finati E, Fermo I, et al. Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-arginine in patients with arteriogenic and non-arteriogenic erectile dysfunction. Int J Androl. 2012 Oct;35(5):660–7. doi: 10 .1111/j.1365-2605.2012.01272.x.

21 Yang G, Muzepper M. Platelet indices and erectile dysfunction: A systematic review and meta-analysis. *Andrologia*, 2019, 51.5: e13248. doi: 10.1111/and13248

22 Peng J, An J., Chen Y, Zhou J, Xiang B. The associations among platelet count, mean platelet volume, and erectile dysfunction: an observational and Mendelian randomization study. *Sexual Medicine*, 2024, 12.6: qfae093. doi: 10.1093/sexmed/qfae093

23. Tangal S, Ozayar A., Ener K., Gokçe MI, Haliloglu AH.. Does mean platelet volume (MPV) have a role in evaluation of erectile dysfunction and its severity? *Revista Internacional de Andrología*, 2020, 18.1: 1-6. doi: 10.1016/j.androl.2018.07.007

24. Culha MG, Atalay HA, Canat HL, Alkan I,Ozbir S, Can O, et al. The relationship between erectile dysfunction severity, mean platelet volume and vitamin D levels. *The Aging Male*, 2020. doi: 10.1080/13685538.2018.1459544

25 Arslan D, Aslan G, Sifil A, Çavdar C, Çelebi I, Gamsari T, et al. Sexual dysfunction in male patients on hemodialysis: Assessment with the International Index of Erectile Function (IIEF). Int J Impot Res. 2002 Dec;14(6):539–42. doi: 10.1038/sj.ijir.3900937.

26 Costa MR, Reis AMB, Pereira BP, Ponciano VC, Oliveira EC de. Associated factors and prevalence of erectile dysfunction in hemodialysis patients. International braz j urol. 2014 Jan;40(1):44–55. doi: 10. 1590/S1677-5538.IBJU.2014.01.07.

27 Mesquita JFP, Ramos TF, Mesquita FP, Netto JMB, Bastos MG, de Figueiredo AA. Prevalence of erectile dysfunction in chronic renal disease patients on conservative treatment. Clinics. 2012;67(2):181. doi: 10. 6061/clinics/2012(02)15.

28 Papadopoulou E, Varouktsi A, Lazaridis A, Boutari C, Doumas M. Erectile dysfunction in chronic kidney disease: From pathophysiology to management. World J Nephrol. 2015;4(3):379. doi: 10. 5527/wjn.v4.i3.379.

29 Alipoor E, Salehi S, Dehghani S, Yaseri M, Hosseinzadeh-Attar MJ. Asymmetric dimethylarginine serum concentration in normal weight and obese CKD patients treated with hemodialysis. BMC Nephrol. 2024 Dec;25(1):1–7. doi: 10.1186/s12882-024-03736-2.

30 Lowrie EG, Lew NL. Death risk in hemodialysis patients: the predictive value of commonly measured variables and an evaluation of death rate differences between facilities. Am J Kidney Dis. 1990;15(5):458–82. doi: 10.1016/s0272-6386(12)70364-5.

31 Kielstein JT, Bode-Böger SM, Frölich JC, Ritz E, Haller H, Fliser D. Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. Circulation. 2003 Apr;107(14):1891–5. doi: 10.1161/01.CIR.0000060496.23144.A7.

32 Nanayakkara PWB, Teerlink T, Stehouwer CDA, Allajar D, Spijkerman A, Schalkwijk C, et al. Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure. Kidney Int. 2005 Nov;68(5):2230–6. doi: 10.1111/j.1523-1755.2005.00680.x.

33 Iseki K, Yamazato M, Tozawa M, Takishita S. Hypocholesterolemia is a significant predictor of death in a cohort of chronic hemodialysis patients. Kidney Int. 2002;61(5):1887–93. doi: 10.1046/j.1523-1755.2002.00324.x.

34 Oliva-Damaso E, Oliva-Damaso N, Rodriguez-Esparragon F, Payan J, Marañes A, Parodis Y, et al. Asymmetric Dimethylarginine (ADMA) Levels Are Lower in Hemodialysis Patients Treated With Paricalcitol. Kidney Int Rep. 2016;2(2):165. doi: 10.1016/j.ekir.2016.10.002.

35 Gohda T, Shou I, Fukui M, Funabiki K, Horikoshi S, Shirato I, et al. Parathyroid hormone gene polymorphism and secondary hyperparathyroidism in hemodialysis patients. American Journal of Kidney Diseases. 2002;39(6):1255–60. doi: 10.1053/ajkd.2002.33399.

36 Perl J, Bargman JM. The importance of residual kidney function for patients on dialysis: a critical review. Am J Kidney Dis. 2009 Jun;53(6):1068–81. doi: 10.1053/j.ajkd.2009.02.012.

37 Okazaki M, Obi Y, Shafi T, Rhee CM, Kovesdy CP, Kalantar-Zadeh K. Residual Kidney Function and Cause-Specific Mortality Among Incident Hemodialysis Patients. Kidney Int Rep. 2023 Oct;8(10):1989–2000. doi: 10.1016/j.ekir.2023.07.020.

38 Shafi T, Jaar BG, Plantinga LC, Fink NE, Sadler JH, Parekh RS, et al. Association of residual urine output with mortality, quality of life, and inflammation in incident hemodialysis patients: the Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) Study. Am J Kidney Dis. 2010;56(2):348–58. doi: 0.1053/j.ajkd.2010.03.020.

39 Suda T, Hiroshige K, Ohta T, Watanabe Y, Iwamoto M, Kanegae K, et al. The contribution of residual renal function to overall nutritional status in chronic haemodialysis patients. Nephrol Dial Transplant. 2000;15(3):396–401. doi: 10.1093/ndt/15.3.396.

40 Merkus MP, Jager KJ, Dekker FW, Boeschoten EW, Stevens P, Krediet RT, et al. Quality of life in patients on chronic dialysis: self-assessment 3 months after the start of treatment. The Necosad Study Group. Am J Kidney Dis. 1997;29(4):584–92. doi: 0.1016/s0272-6386(97)90342-5.

doi: 10.53446/actamednicomedia.1598709

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Araştırma Makalesi | Research Article

KRONİK LENFOSİTİK LÖSEMİ HASTALARINDA NOTCH EKSPRESYON SEVİYELERİ: AKAN HÜCRE ÖLÇER İLE DEĞERLENDİRİLMESİ

NOTCH EXPRESSION LEVEL IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: EVALUATION BY FLOW CYTOMETRY

Fatma Betül Öktelik[1,2], Murat Özbalak[3], İpek Yönal Hindilerden[3], Günnur Deniz[2], Metin Yusuf Gelmez[2]

[1]İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, İstanbul, Türkiye. [2]İstanbul Üniversitesi, Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, İmmünoloji Anabilim Dalı, İstanbul, Türkiye. [3]İstanbul Üniversitesi, İstanbul Tıp Fakültesi, İç Hastalıkları Anabilim Dalı, Hematoloji Bilim Dalı, İstanbul, Türkiye.

ÖZ

Amaç: $CD5^+CD19^+$ malign B hücrelerin kemik iliği ve kanda birikmesi ile karakterize kronik lenfositik lösemi (KLL), Amerika ve Avrupa'da yetişkinlerde en sık gözlenen lösemi tipidir. CD38, ZAP70 ekspresyonları, immünoglobülin ağır zincir değişken bölge (IgVH) mutasyonları hastalığın klinik seyri hakkında bilgi sağlasa da KLL patogenezi henüz net olarak tanımlanmamıştır. Transmembran proteini olan Notch reseptörleri hücre çoğalması, farklılaşması ve apoptozis süreçlerinde immün homeostazın sağlanmasında oldukça önemlidir. Güncel çalışmalar, Notch moleküllerinin kanser gelişiminde rolü olduğunu göstemektedir. Bu çalışmada KLL tanısı almış hastalarda Notch seviyeleri analiz edilerek KLL patogenezindeki rolleri incelenmiştir.

Yöntem: KLL tanılı 15 hasta ve 9 sağlıklı bireyin periferik kanından izole edilen periferik kan mononükleer hücrelerde akan hücre ölçer ile Notch1, Notch2, Notch3 ve Notch4 seviyeleri değerlendirildi.

Bulgular: KLL hastalarında lenfosit, $CD19^+$ B ve $CD3^+$ T hücrelerde Notch1 ifadesinin arttığı, buna karşın Notch4 ifadesinin ise azaldığı gözlendi. Hasta ve sağlıklı bireyler arasında Notch2 ve Notch3 ifadeleri arasında anlamlı fark gözlenmedi.

Sonuç: KLL hastalarında yüksek *Notch1* mRNA ifadesinin kötü prognoz ile ilişkili olduğu, Notch4 aktivasyonunun ise kemoterapiye direnç oluşturduğu bildirilmektedir. Bulgularımız akan hücre ölçer ile Notch ifadelerinin hastalarda hızlı bir şekilde değerlendirilebileceğini ve değişen Notch1 ve Notch4 ifadelerinin KLL patogenezinde potansiyel belirteçler olarak öne çıkabileceğini göstermektedir.

Anahtar Kelimeler: Kronik lenfositik lösemi, Notch1, Notch2, Notch3, Notch4

ABSTRACT

Objective: Chronic lymphocytic leukemia (CLL), characterized by the accumulation of $CD5^+CD19^+$ malignant B cells in the bone marrow and blood, is the most common type of leukemia in adults in the United States and Europe. While CD38 and ZAP70 expression and immunoglobulin heavy chain variable region (IgVH) mutations provide insights into the clinical outcome of the disease, the pathogenesis of CLL has not yet been fully elucidated. Notch receptors, which are transmembrane proteins, play a critical role in regulating cell proliferation, differentiation, and apoptosis. Recent studies have demonstrated the involvement of Notch proteins in various types of cancer. In this study, the levels of Notch molecules were analyzed in CLL patients to investigate their roles in the pathogenesis of the disease.

Methods: Notch-1, Notch2, Notch3, and Notch4 levels were analyzed by flow cytometry in peripheral blood mononuclear cells isolated from heparinized blood samples of 15 CLL patients and 9 healthy individuals.

Results: Compared to healthy individuals, increased Notch1 and decreased Notch4 levels were found in total lymphocytes, B cells, and T lymphocytes in CLL patients. No significant differences were detected in Notch2 and Notch3 expression between patients and healthy individuals.

Conclusion: It has been reported that high Notch1 mRNA expression is associated with poor prognosis and Notch4 activation contributes to chemotherapy resistance in CLL patients. Our findings demonstrate that Notch expression can be rapidly analyzed in CLL patients using flow cytometry. Additionally, our findings suggest that Notch1 and Notch4 expression can be used as potential biomarkers in CLL pathogenesis.

Key words: Chronic lymphocytic leukemia, Notch1, Notch2, Notch3, Notch4

***İletişim kurulacak yazar/Corresponding author:** Metin Yusuf Gelmez; İstanbul Üniversitesi, Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, İmmünoloji Anabilim Dalı, 34098, İstanbul, Türkiye.
Telefon/Phone: +90 (212) 414 20 00 **e-posta/e-mail:** yusufmetin@istanbul.edu.tr
Başvuru/Submitted: 09.12.2024 • **Kabul/Accepted: 26**.03.2025 • **Online Yayın/Published Online:** 30.06.2025

Giriş

Batı ülkelerinde yetişkinlerde en sık gözlenen lösemi türü olan kronik lenfositik lösemi (KLL), çoğalma kapasitesi düşük, CD5 ifade eden malign B hücrelerin kanda, lenf nodunda, kemik iliğinde birikimiyle tanımlanır.[1] Malign B hücreleri CD5, CD19 ve CD23 ifade ederken, CD79b ve FMC7 ifade etmezler.[2] Diğer lösemilerden farklı olarak KLL patogenezinde *BCL-2* ve *p53* genlerinde mutasyon, immünoglobülin ağır zincir değişken bölge (IgVH) mutasyonları, trizomi 12, 11q, 13q ve 17p delesyonları gibi genetik faktörler, CD38 ve ZAP70 ekspresyon seviyeleri hastalığın klinik gidişatını gösteren önemli belirteçlerdir.[3] Hastaların önemli bir kısmında hastalık yavaş seyirlidir, tedaviye ihtiyaç duyulmamaktadır. Buna karşın hastaların bir kısmında tanı anında ya da sonrasında tedavi uygulanmaktadır.[4]

Epidermal büyüme faktörü benzeri tekrar dizileri içeren bir transmembran proteini olan Notch reseptörü ve ligandları, hücresel farklılaşma, apoptoz, organ gelişimi, doku homeostazının düzenlenmesi ve kök hücrelerin kendini yenilemesi gibi normal biyolojik süreçleri düzenleyen, evrim boyunca yüksek derecede korunmuş moleküllerdir.[5] Notch sinyal transdüksiyonunu başlatmak için genellikle reseptör-ligand etkileşimi gerekmektedir.[6] Güncel çalışmalar Notch sinyal yolunun birçok kanser türünde onkojenik ya da tümör baskılayıcı bir faktör olarak rol alabileceğini bildirmektedir.[7] Ayrıca, Notch sinyal yolunun, tümör hücrelerine kemorezistans kazandırılmasında rol oynadığı bilinmektedir.[7] Bu özellikleri Notch sinyalini hedef alan yeni tedavi uygulamalarının geliştirilmesine ışık tutmaktadır.[8]

Notch reseptör mutasyonlarının T ve B hücrelerinde fonksiyonel bozukluklara neden olabileceği ve hematolojik malignitelerdeki rolleri farklı çalışmalar ile ortaya konulmaktadır.[9] T hücre gelişiminde önemli rolü olan Notch1'in T hücreli akut lenfoblastik lösemi (T-ALL) patogenezindeki rolü t(7;9) keşfiyle ortaya çıkmıştır.[9] Marjinal zone farklılaşması ve gelişiminde Notch2'in rolü olduğu bilinmektedir.[10,11] Güncel veriler, *Notch2* genindeki mutasyonların marjinal zone lenfoması (MZL) gelişimine neden olduğunu ve hastalığın patogenezindeki kritik rolünü vurgulamaktadır.[12] KLL patogenezinde Notch1 ve Notch2 ligandları olan Jag1 ve Jag2'in sürekli olarak ifade edildiği ve bu moleküllerin apoptoza karşı malign B hücrelerine direnç kazandırdığı ifade edilmektedir.[9,13] Bu nedenle, farklı Notch sinyal yolaklarının hematolojik kanserler için potansiyel terapötik hedefler olarak değerlendirilebileceği bildirilmektedir.[6] Notch reseptörlerinin tümörler ve kanser üzerindeki rolleri iyi bilinmekte olup, viral enfeksiyonlardaki etkileri de ayrıntılı bir şekilde ortaya konulmuştur. Özellikle, Covid-19 enfeksiyonunu ağır geçiren hastalarda Notch4 reseptörünün, düzenleyici T hücrelerinde (Treg) ekspresyon seviyelerinin arttığı bilinmektedir.[14] Ayrıca, Notch1 reseptörünün multisistem enflamatuvar sendrom (MIS-C) hastalarında Treg hücreleri tarafından yüksek seviyelerde ifade edildiği ve bu hücrelerin destabilizasyonuna yol açtığı gösterilmiştir.[15] Tüm bu veriler, Notch sinyal yolunun önemli rolleri nedeniyle, bu yolu hedef alan yeni tedavi yaklaşımlarının veya biyobelirteçlerin geliştirilmesinin hastalık patogenezinde önemli yer tuttuğunu göstermektedir.

Bu çalışmada KLL tanısı almış hastalarda Notch seviyeleri akan hücre ölçer ile analiz edilerek KLL patogenezindeki rolleri incelenmiştir.

Yöntem

Hasta Grubu

İstanbul Üniversitesi, İstanbul Tıp Fakültesi, Hematoloji polikliniğinde takibi yapılan ve herhangi bir ilaç tedavisi almayan, $5x10^3/mm^3$ üzerinde lenfosit ve periferik kanında olgun görünümlü ve CD5 ifade eden CD19 hücresi olan olan; enfeksiyonu ya da sekonder bir kronik rahatsızlığı olmayan 15 (8 erkek, 7 kadın) KLL hastası çalışmaya dahil edildi. Cinsiyet ve yaş uyumlu, 9 (6 erkek, 3 kadın) sağlıklı erişkin ile kontrol grubu oluşturuldu. Sağlıklı erişkinlerin seçiminde otoimmünite ve kanser öyküsü olmaması, örnek alındığında enfeksiyon gözlenmemesine dikkat edildi. Çalışmaya katılan tüm gönüllülerden bilgilendirilmiş gönüllü onam formları alındı (İ.Ü. İTF Etik Kurulu, no:1301, tarih:13.11.2017).

Akan Hücre Ölçer Sistemi ile Notch Analizi

Olgulardan alınan periferik kan (10ml) örneklerinde ficoll yöntemi ile periferik kan mononükleer hücreler izole edildi. Hücreler ($5x10^6$ hücre/ml) üzerine anti-Notch1 FITC, -Notch4 PE, -CD5 PECY5, -CD19 APC, -CD3 AlexaFlour700, -Notch3 BV421 ve -Notch2 BV650 monoklonal antikorları eklendi. Kullanılan tüm monoklonal antikorlar BioLegend (ABD) firmasından temin edildi. Hücreler 20 dakika inkübasyon sonrasında fosfat tuzlu tamponu (PBS) ile 1800 rpm'de 10 dakika yıkanarak hücre pelleti üzerine PBS eklendi ve örnekler Novocyte (Agilent, ABD) akan hücre ölçer cihazından geçirilerek NovoExpress analiz programında değerlendirildi. Tüm örneklerde SSC/FSC grafiğinde lenfosit kapısında en az 200.000 hücre sayıldı. Lenfosit, $CD19^+$ B, $CD3^+$ T lenfosit popülasyonları içinde Notch1, Notch2, Notch3 ve Notch4 seviyeleri analiz edildi.

İstatistiksel Analizler

İstatistiksel analizler için GraphPad Prism 9.1 uygulamasından yararlanıldı. Gruplar arası karşılaştırma analizleri için veriler normalizasyon testine tabi tutuldu. Normal dağılım göstermeyen gruplara Mann-Withney-U Testi uygulandı. Spearman Testi ile korelasyon analizleri gerçekleştirildi. Verilerin istatiksel anlamlılık değerleri, $p \leq 0,05$ olarak kabul edildi.

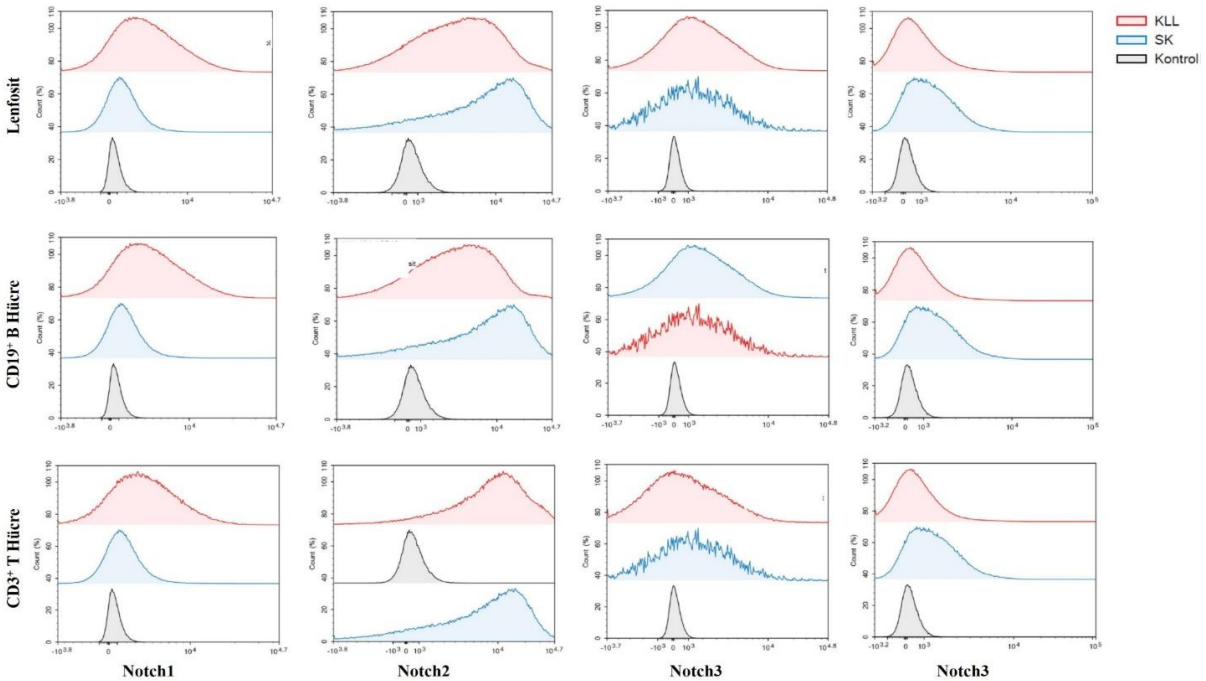
Bulgular

Çalışmada yer alan 8 erkek, 7 kadın olmak üzere toplam 15 KLL hastasının yaş ortalaması 68 (59-77) yıldır. Kadın hastaların yaş ortalaması 67 (59-73), erkek hastaların ise 71 (66-77) yıldır. Binet evreleme sistemine göre 5 hasta evre A, 10 hasta ise evre B, Rai sınıflandırmasına göre ise 4 hasta evre 0, 2 hasta evre I, 4 hasta evre II ve 5 hasta ise evre III olarak değerlendirildi. Hastaların klinik ve demografik verileri Tablo 1'de verildi.

Tablo 1. KLL hastalarına ait klinik ve demografik özellikleri

		KLL	Sağlıklı Kontrol
n		**15**	**9**
Cinsiyet	Erkek (n)	8	6
	Kadın (n)	7	3
Yaş Ortalaması (Yıl) (En düşük-En yüksek)		68 (59 - 77)	54 (49 - 60)
WBC (10^9/L) (ortalama) (En düşük-En yüksek)		47.19 (7.7 - 176)	-
LYM (10^9/L) (ortalama) (En düşük-En yüksek)		38.03 (6.3 - 159)	-
	del11q (n)	0	-
	del13q (n)	0	-
Kromozomal Anomali	del17p (n)	0	-
	tri12 (n)	0	-
CD38	Pozitif (n)	4	-
	Negatif (n)	11	-
BINET Evre	A (n)	5	-
	B (n)	10	-
	C (n)	0	-
	0 (n)	4	-
	I (n)	2	-
Rai Evre	II (n)	4	-
	III (n)	5	-
	IV (n)	-	-
Tedavi	Yok (n)	15	-
	Var (n)	0	-

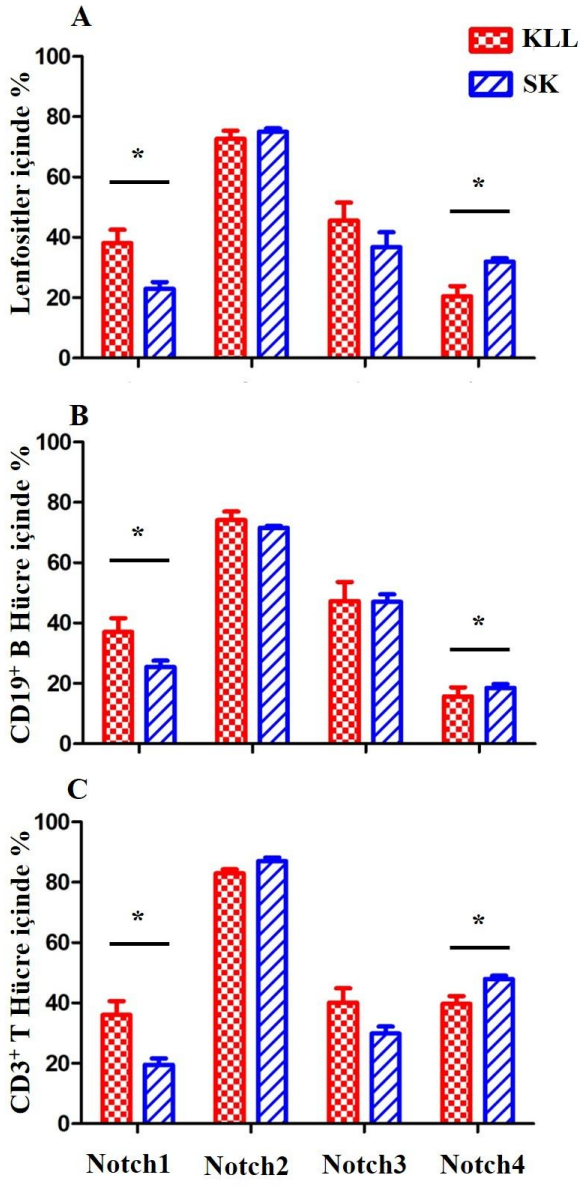
Akan hücre ölçer ile Notch seviyeleri analiz edilirken önce SSC/FSC grafiğinde lenfositler kapılandı. Lenfositler içinde SSC/CD19 grafiğinde $CD19^+$ B hücreler ve SSC/CD3 grafiğinde $CD3^+$ T hücreler kapılandı. Total lenfosit, B lenfosit ve T lenfositler içinde Notch1, Notch2, Notch3 ve Notch4 seviyeleri analiz edildi (Şekil 1).



Şekil 1. KLL (kırmızı renk), sağlıklı kontrol - SK (mavi renk) ve izotip kontrole (gri renk) ait lenfosit, $CD19^+$ B hücre ve $CD3^+$ T hücrelerde Notch1, Notch2, Notch3 ve Notch4 seviyelerini gösteren histogram görüntüleri.

Total lenfositler içinde değerlendirildiğinde Notch1seviyesinin KLL hastalarında sağlıklı bireylere göre arttığı, Notch4 seviyesinin ise azaldığı tespit edildi (sırasıyla; p=0,03 ve p=0,02) (Şekil 2A). Hasta ve sağlıklı bireyler arasında lenfositlerde Notch2 ve Notch3 seviyeleri açısından ise anlamlı bir fark gözlenmedi. Notch seviyeleri

B ve T lenfositler içinde analiz edildiğinde sağlıklı bireylere kıyasla KLL hastalarında hem B hem de T lenfositlerde yüksek Notch1seviyeleri gözlendi (sırasıyla; p=0,05 ve p=0,03). Buna karşın hastalarda Notch4 seviyelerinin ise B ve T lenfositlerde düşük olduğu saptandı (sırasıyla; p=0,05 ve p=0,05) (Şekil 2B-2C). B ve T lenfosit Notch2 ve Notch3 seviyeleri incelendiğinde hasta ve sağlıklı bireyler arasında fark olmadığı tespit edildi. Hastalarda Notch1, Notch2, Notch3 ve Notch4 seviyeleri Rai ve Binet evreleme sistemleri ve CD38 durumuna göre analiz edildiğinde hasta grupları içinde fark gözlenmedi.



Şekil 2. Lenfosit, $CD19^+$ B hücre ve $CD3^+$ T hücrelerde Notch1, Notch2, Notch3 ve Notch4 seviyelerinin KLL (kırmızı renk) ve sağlıklı kontrollerde (mavi renk) karşılaştırılması, * p<0,05, **p<0,01.

Tartışma

Batı ülkelerinde sık gözlenen KLL yavaş seyirli bir hastalıktır. Hastaların çoğunluğu tedavi almadan sadece klinik olarak takip edilirken, hastaların bir kısmında tedavi uygulanır.[2] KLL'yi diğer hematolojik hastalıklardan ayırıcı özelliklerinden biri akan hücre ölçer ile B hücrelerinde CD5 ve CD23 pozitifliği yanında, FMC7 ve CD79b ifadesinin negatif olarak gözlenmesidir.[3] *IgVH* mutasyon durumu, CD38 ve ZAP70 düzeyi ile del17p, del13q gibi kromozomal değişikliklerin hastalığın klinik gidişi ile ilişkili oldukları bilinmektedir.[16] KLL patogenezi henüz net olarak ortaya konulamamıştır ve hastalığın klinik gidişatını gösterebilecek parametrelerin belirlenmesi oldukça önemlidir.

Hücre çoğalması, hücre farklılaşması, apoptoz gibi birçok hücresel süreçte rol alan Notch sinyal yolağının birçok kanserde de önemli role sahip olduğu gösterilmektedir.[7] İlk olarak T-ALL'de t(7;9) translokasyonunun Notch1 transkriptlerini kısalttığı ve hastalığın patogenezinde rol aldığı gösterilmiştir.[17] Çalışmalar akciğer skuamöz hücreli karsinom, meme kanseri ve anaplastik büyük hücreli lenfoma gibi çeşitli kanserlerde onkojenik *Notch* gen mutasyonlarını ortaya koymaktadır.[18-20]

Notch1ve Notch2'nin KLL'li hastalarda sürekli olarak ifade edildiği ve malign B-KLL hücrelerini apoptoza dirençli kıldıkları bildirilmektedir.[9] İlaveten KLL hastaların yaklaşık %10'unda *Notch1* geninde somatik mutasyonun olduğu, refrakter hastalar ile Richter dönüşümü olan hastalarda *Notch1* mutasyon sıklığının daha yüksek olduğu bilinmektedir.[21] Ayrıca güncel çalışmalar, *Notch* mutasyonu olan KLL'li hastalarda difüz büyük B hücreli lenfomaya transformasyon riskinin daha yüksek olduğunu göstermektedir.[22] Tüm veriler, Notch sinyal yolağının ve özellikle *Notch1* mutasyonunun, KLL'nin klinik gidişini gösterebilecek bir biyobelirteç olabileceği izlenimini vermektedir.[23] Bu veriler ile uyumlu olarak çalışmamızda KLL hastalarında Notch1 seviyesinin arttığı gösterilmiştir. Bu artışın hem B hem de T lenfositlerde olduğu gözlenmiştir. Çalışmamızın önemli kısıtlamalarından biri hastalarda *Notch1* gen mutasyonu bakılmamasıdır. Buna karşın güncel çalışmalar, *Notch1* mutasyonu saptanmayan KLL hastalarında da *Notch1* mRNA ekspresyonunun yükseldiğini ve mRNA ekspresyonu açısından mutasyonu olan hastalar ile aralarında fark olmadığını bildirmektedir.[21]

Notch4, ağırlıklı olarak kan damarlarında ve kemik iliğinde mezenkimal stromal hücrelerde eksprese olur. Ayrıca *Notch4* knock-out hayvan çalışmaları, bu hayvanlarda damar gelişimde belirgin değişiklikler gözlendiğini ifade etmektedir.[24] KLL hastalarında kemik iliği stromal hücrelerinde Notch4 uyarımının kemoterapiye direnç oluşmasına neden olduğu bildirilmektedir.[9] Pre-klinik çalışmalar, B-ALL ve KLL'de, anti-Notch4 antikor uygulamasının, kemik iliği mezenkimal stromal hücreler ile tümör hücreleri arasındaki Notch4 sinyalleşmesini bloke ettiğini ve tümör hücrelerinin yaşam süresini kısalttığını göstermektedir.[25] Ayrıca, azalan Notch4 seviyesinin, Treg hücrelerinin baskılama kapasitesini artırarak tümör hücreleriyle mücadele eden T hücrelerinin inhibisyonuna ve tümör hücrelerinin lizisinin engellenmesine yol açabileceği öne sürülmektedir.[14] Çalışmamızda KLL hastalarında Notch4 seviyesinin sağlıklı kontrollere göre azaldığı gözlendi. Çalışmalar B-KLL hücrelerinin IL-10, TGF-β gibi sitokinler sekrete ettiğini ve immün yanıtları

baskılayabileceğini göstermektedir.[26,27] Bu özellikleri ile Treg hücrelere benzediği ifade edilmektedir.[27] Azalan Notch4 seviyesinin Treg fonksiyonlarını arttırıcı özelliği ve B-KLL hücrelerinin Treg'lere benzerliği dikkate alındığında, bulgularımız KLL hastalarında özellikle B hücrelerinde azalan Notch4 seviyesinin malign B hücrelerinde IL-10 gibi immün baskılayıcı sitokinlerin sekrete edilmesine neden olarak KLL'de gözlenen T hücre disfonksiyonuna neden olabileceğini düşündürmektedir. İlaveten, Notch4 seviyesinin düşük olarak gözlenmesi çalışmaya dahil edilen hastaların hem erken evre olması hem de herhangi bir tedavi uygulanmayan hastalardan oluşmasından kaynaklanmış olabileceğini düşündürmektedir. Tedavi alan ve direnç gözlenen hastalarda yapılacak çalışmalar bu konuda daha ayrıntılı sonuçlar elde edilmesini sağlayacaktır.

Sonuç olarak, özellikle artan Notch1 seviyesinin hematoloji laboratuvarlarında kullanımı her geçen gün artan akan hücre ölçer ile tespit etmek daha ucuz maliyet ile hızlı bir şekilde sonuç alınmasını sağlayabilir. Güncel çalışmalar Notch1 hedefleyen monoklonal antikor uygulaması gibi tedavi stratejilerinin agresif seyreden KLL hastaları için bir seçenek olabileceğini göstermektedir.[28] Çalışmamıza dahil edilen herhangi bir ilaç tedavisi almayan hastaların uzun dönem takipleri yapılarak Notch1 seviyesi ile ilaç tedavisi başlanması arasındaki ilişkinin ortaya konulması Notch1'in klinik bir gösterge olarak kullanılabilirliğinin test edilmesi açısından önem taşımaktadır. Ayrıca ileri çalışmalarda tedavi alan KLL hastalarında Notch1 seviyelerinin tedavi almayan hastalar ile karşılaştırılması da klinik gidiş ile Notch1 düzeyi arasındaki ilişkinin gösterilmesine katkı sunabilir.

Açıklamalar
Bu çalışmaya verdiği katkı için rahmetli Prof. Dr. Melih AKTAN'a teşekkür ederiz.

Etik Standartlara Uygunluk
İstanbul Tıp Fakültesi Klinik Araştırmalar Etik Kurulu onayı alınmıştır (No:1301, tarih:13.11.2017/25). Tüm prosedürler, kurumsal ve/veya ulusal araştırma komitesinin etik standartlarına ve Helsinki Bildirgesi'ne uygun olarak gerçekleştirilmiştir.

Çıkar Çatışması
Yazarlar arasında çıkar çatışması bulunmamaktadır.

Finansal Destek
Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi (Proje No: TDP-2019-32394) tarafından desteklenmiştir.

Yazar Katkısı
FBO, GD, MYG: Çalışmanın tasarımı, veri toplanması ve analizi, kaynak taraması ve makale yazımı; MO, IYH: Çalışmanın tasarımı, veri toplanması ve makale yazımı

Kaynaklar

1. Kikushige Y. Pathogenesis of chronic lymphocytic leukemia and the development of novel therapeutic strategies. J Clin Exp Hematop. Dec 15 2020;60(4):146-158. doi:10.3960/jslrt.20036
2. Braish J, Cerchione C, Ferrajoli A. An overview of prognostic markers in patients with CLL. Front Oncol. 2024;14:1371057. doi:10.3389/fonc.2024.1371057
3. Koehrer S, Burger JA. Chronic Lymphocytic Leukemia: Disease Biology. Acta Haematol. 2024;147(1):8-21. doi:10.1159/000533610
4. Brown JR. Clinical Risks for Chronic Lymphocytic Leukemia. J Natl Compr Canc Netw. Apr 2024;22(3)doi:10.6004/jnccn.2024.7020
5. Sachan N, Sharma V, Mutsuddi M, Mukherjee A. Notch signalling: multifaceted role in development and disease. FEBS J. Jul 2024;291(14):3030-3059. doi:10.1111/febs.16815
6. Cai J, Qiao Y, Chen L, Lu Y, Zheng D. Regulation of the Notch signaling pathway by natural products for cancer therapy. J Nutr Biochem. Jan 2024;123:109483. doi:10.1016/j.jnutbio.2023.109483
7. Shi Q, Xue C, Zeng Y, et al. Notch signaling pathway in cancer: from mechanistic insights to targeted therapies. Signal Transduct Target Ther. May 27 2024;9(1):128. doi:10.1038/s41392-024-01828-x
8. Feng M, Santhanam RK, Xing H, Zhou M, Jia H. Inhibition of gamma-secretase/Notch pathway as a potential therapy for reversing cancer drug resistance. Biochem Pharmacol. Feb 2024;220:115991. doi:10.1016/j.bcp.2023.115991
9. Gragnani L, Lorini S, Marri S, Zignego AL. Role of Notch Receptors in Hematologic Malignancies. Cells. Dec 24 2020;10(1)doi:10.3390/cells10010016
10. Meurette O, Mehlen P. Notch Signaling in the Tumor Microenvironment. Cancer Cell. Oct 8 2018;34(4):536-548. doi:10.1016/j.ccell.2018.07.009
11. Saito T, Chiba S, Ichikawa M, et al. Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. Immunity. May 2003;18(5):675-85. doi:10.1016/s1074-7613(03)00111-0
12. Alderuccio JP, Lossos IS. NOTCH signaling in the pathogenesis of splenic marginal zone lymphoma-opportunities for therapy. Leuk Lymphoma. Feb 2022;63(2):279-290. doi:10.1080/10428194.2021.1984452
13. Rosati E, Sabatini R, Rampino G, et al. Constitutively activated Notch signaling is involved in survival and apoptosis resistance of B-CLL cells. Blood. Jan 22 2009;113(4):856-65. doi:10.1182/blood-2008-02-139725
14. Harb H, Benamar M, Lai PS, et al. Notch4 signaling limits regulatory T-cell-mediated tissue repair and promotes severe lung inflammation in viral infections. Immunity. Jun 8 2021;54(6):1186-1199 e7. doi:10.1016/j.immuni.2021.04.002
15. Benamar M, Chen Q, Chou J, et al. The Notch1/CD22 signaling axis disrupts Treg function in SARS-CoV-2-associated multisystem inflammatory syndrome in children. J Clin Invest. Jan 3 2023;133(1)doi:10.1172/JCI163235
16. Hallek M, Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol. Dec 1 2021;96(12):1679-1705. doi:10.1002/ajh.26367
17. Ferrando AA. The role of NOTCH1 signaling in T-ALL. Hematology Am Soc Hematol Educ Program. 2009:353-61. doi:10.1182/asheducation-2009.1.353
18. Kontomanolis EN, Kalagasidou S, Pouliliou S, et al. The Notch Pathway in Breast Cancer Progression.

ScientificWorldJournal. 2018;2018:2415489. doi:10.1155/2018/2415489
19. Sun J, Dong M, Xiang X, Zhang S, Wen D. Notch signaling and targeted therapy in non-small cell lung cancer. Cancer Lett. Mar 31 2024;585:216647. doi:10.1016/j.canlet.2024.216647
20. Larose H, Prokoph N, Matthews JD, et al. Whole Exome Sequencing reveals NOTCH1 mutations in anaplastic large cell lymphoma and points to Notch both as a key pathway and a potential therapeutic target. Haematologica. Jun 1 2021;106(6):1693-1704. doi:10.3324/haematol.2019.238766
21. Isık S, Gunden G, Davutoglu NO, et al. Kronik Lenfositik Lösemi Olgularında NOTCH1 Gen Amplifikasyonu. Osmangazi Journal of Medicine. 2023;46(1):104-109. doi:10.20515/otd.1329205
22. Zou Y, Fan L, Xia Y, et al. NOTCH1 mutation and its prognostic significance in Chinese chronic lymphocytic leukemia: a retrospective study of 317 cases. Cancer Med. May 2018;7(5):1689-1696. doi:10.1002/cam4.1396
23. Rossi D, Rasi S, Fabbri G, et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood. Jan 12 2012;119(2):521-9. doi:10.1182/blood-2011-09-379966
24. Lopez-Lopez S, Romero de Avila MJ, Hernandez de Leon NC, et al. NOTCH4 Exhibits Anti-Inflammatory Activity in Activated Macrophages by Interfering With Interferon-gamma and TLR4 Signaling. Front Immunol. 2021;12:734966. doi:10.3389/fimmu.2021.734966
25. Nwabo Kamdje AH, Mosna F, Bifari F, et al. Notch-3 and Notch-4 signaling rescue from apoptosis human B-ALL cells in contact with human bone marrow-derived mesenchymal stromal cells. Blood. Jul 14 2011;118(2):380-9. doi:10.1182/blood-2010-12-326694
26. Rivas JR, Liu Y, Alhakeem SS, et al. Interleukin-10 suppression enhances T-cell antitumor immunity and responses to checkpoint blockade in chronic lymphocytic leukemia. Leukemia. Nov 2021;35(11):3188-3200. doi:10.1038/s41375-021-01217-1
27. Ozden O, Gelmez MY, Çınar S, Deniz G, Aktan M. Intracellular Levels of IL-10 and STAT3 in Patients with Chronic Lymphocytic Leukemia. Clinical and Experimental Health Sciences. 2023;13(1):99-104. doi:10.33808/clinexphealthsci.1056727
28. Lopez-Guerra M, Xargay-Torrent S, Fuentes P, et al. Specific NOTCH1 antibody targets DLL4-induced proliferation, migration, and angiogenesis in NOTCH1-mutated CLL cells. Oncogene. Feb 2020;39(6):1185-1197. doi:10.1038/s41388-019-1053-6Cree BAC, Arnold DL, Chataway J, et al. Secondary progressive multiple sclerosis: new insights. *Neurology*. 2021;97(8):378-388. doi:10.1212/WNL.0000000000012323

doi: 10.53446/actamednicomedia.1599482

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

ISCHEMIA-MODIFIED ALBUMIN MAY NOT BE A RELIABLE BIOMARKER IN CHILDREN WITH ACUTE RHEUMATIC FEVER

AKUT ROMATİZMAL ATEŞİ OLAN ÇOCUKLARDA İSKEMİ MODİFİYE ALBÜMİN GÜVENİLİR BİR BİYOBELİRTEÇ OLMAYABİLİR

Yasemin Nuran Dönmez [1*], Mehmet Ramoğlu[1], Zerrin Epçaçan[2] Mustafa Orhan Bulut[3] Serdar Epçaçan[1]

[1]Van Training and Research Hospital, Department of Pediatric Cardiology, Van, Türkiye. [2]Van Training and Research Hospital, Department of Pediatrics, Van, Türkiye. [3]Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, Department of Pediatric Cardiology, İstanbul Türkiye.

ABSTRACT

Objective: Acute rheumatic fever (ARF) poses significant health challenges in low- and middle- income countries, particularly due to its potential to cause severe cardiac complications. This study investigated the role of ischemia-modified albumin (IMA) as a biomarker for inflammation in children diagnosed with ARF.

Methods: The study included 25 ARF patients and a control group of 25 healthy children and was conducted between January 2019 and May 2020 at a regional hospital. Clinical assessments and echocardiographic evaluations were performed, in addition to serum IMA level measurements at diagnosis and post-treatment.

Results: The findings indicated that while acute phase reactants such as C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) were elevated in the ARF patients, the IMA levels were not significantly different from those in the control group, both at diagnosis and post-treatment. Specifically, the IMA levels were lower at diagnosis (0.62 ± 0.73 ng/mL) compared to post-treatment (1.18 ± 0.94 ng/mL), with no consistent correlation with the CRP, although a negative correlation with the ESR was observed.

Conclusion: These findings suggest that IMA may not be a reliable biomarker for diagnosing and monitoring ARF in children, challenging previous claims about its utility in this patient group. Further research is necessary to explore the effects of other factors on IMA levels in this population.

Keywords: Acute rheumatic fever, ischemia-modified albumin, enzyme-linked immunosorbent assay

ÖZ

Amaç: Akut romatizmal ateş (ARA), gelişmekte olan ülkelerde sık görülen ve ciddi kardiyak komplikasyonlara yol açabilen önemli bir sağlık problemidir . Bu çalışmada, ARA tanısı alan çocuklarda inflamasyon belirteci olarak iskemi modifiye albüminin (IMA) rolü araştırılmıştır.

Yöntem: Çalışmaya 25 ARA hastası ve 25 sağlıklı çocuktan oluşan kontrol grubu dahil edilmiştir. Ocak 2019 ile Mayıs 2020 tarihleri arasında bölgesel bir hastanede yürütülmüştür. Klinik değerlendirmeler, ekokardiyografik incelemeler yapılmış; tanı anında ve tedavi sonrası serum IMA düzeyleri ölçülmüştür.

Bulgular: Bulgular, ARA hastalarında C-reaktif protein (CRP) ve eritrosit sedimantasyon hızı (ESR) gibi akut faz reaktanlarının yüksek olduğunu göstermiştir. Ancak, IMA düzeyleri hem tanı anında hem de tedavi sonrasında kontrol grubu ile anlamlı farklılık göstermemiştir. Tanı anındaki IMA düzeyleri (0.62 ± 0.73 ng/mL), tedavi sonrasına göre (1.18 ± 0.94 ng/mL) daha düşük olmakla birlikte CRP ile anlamlı bir korelasyon göstermemiştir; ancak ESR ile negatif korelasyon izlenmiştir.

Sonuç: Elde edilen bulgular, IMA'nın çocukluk çağı ARA tanı ve izleminde güvenilir bir biyobelirteç olmayabileceğini göstermekte; bu parametrenin bu hasta grubundaki kullanımına ilişkin önceki bulguları sorgulatmaktadır. IMA düzeylerini etkileyebilecek diğer faktörlerin ortaya konması için ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Akut romatizmal ateş, iskemi modifiye albümin, enzim bağlantılı immünosorbent test

***Corresponding author/İletişim kurulacak yazar:** Yasemin Nuran Dönmez; Van Training and Research Hospital, Department of Pediatric Cardiology, Van, Türkiye
Phone/Telefon: +90 (533) 646 93 90, **e-mail/e-posta:** yaseminnurandonmez@gmail.com
Submitted/Başvuru: 10.12.2024 • **Accepted/Kabul:** 18.04.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Acute rheumatic fever (ARF) is one of the most prevalent and serious cardiac diseases in developing countries. It is characterized by damage to the heart, joints, brain, and skin, and results due to an autoimmune response in susceptible individuals following group A streptococcal (GAS) pharyngitis. Cardiac involvement, primarily manifesting as pancarditis, predominantly presents with valvulitis. The prognosis for ARF depends on the occurrence of recurrence and the severity of cardiac involvement. Chronic rheumatic heart disease is a potential consequence of cardiac involvement, and accounts for 25%–45% of cardiovascular diseases in adults and remains a leading cause of heart failure in developing countries [1].

Detecting inflammation markers is important for early diagnosis, preventing missed cases, and distinguishing true GAS pharyngitis from carriers in high-risk populations. Consequently, biomarkers indicative of inflammation may facilitate early diagnosis and potentially improve long-term clinical outcomes.

The role of albumin in the human body has been well established for decades. However, it was later discovered that albumin undergoes conformational changes under various conditions such as hypoxia, acidosis, ischemia, and oxidative stress [2]. Ischemia-modified albumin (IMA) is a variant of albumin produced due to the degradation of its N-terminal region caused by tissue ischemia, hypoxia, or oxidative stress, leading to a diminished metal-binding capacity. Elevated circulating levels of IMA have been identified in ischemia, hypoxia, acidosis, and oxidative stress [3]. Despite some research conducted on IMA in ARF [4,5], its application in routine diagnosis and disease monitoring remains unclear. Hence, the aim of this study was to assess the potential role of IMA as an inflammation biomarker in children with ARF.

Methods

The study was conducted at Ministry of Health Van Regional Education and Research Hospital between January 2019 and May 2020. Patients and their parents were informed about the study and the additional blood sample collection. The local ethics committee approved the study, and informed consent was obtained from all the participants.

Children diagnosed with ARF in the pediatric cardiology department of our institution were included in the ARF group, while healthy children who presented to the pediatric cardiology department with symptoms like murmur, palpitation, and non-specific chest pain constituted the control group. Children with known congenital or acquired cardiac disease, any chronic diseases, and morbidity, and those receiving specific medication were excluded from the study.

Demographic information and a detailed history were obtained from all the participants. Following a thorough physical examination, each participant underwent electrocardiography and echocardiography. An erythrocyte sedimentation rate (ESR) above 30 mm/h and a CRP level exceeding 3 mg/dL were considered as elevated [6]. Anti-streptolysin O (ASO) levels were deemed high if they exceeded the age-specific upper limit, and serum albumin values below 35 g/L were categorized low [7–9].

Two experienced pediatric cardiologists performed a detailed echocardiographic evaluation using a Vivid 7 Pro echocardiography device (GE Vingmed Ultrasound AS, Horten, Norway) with a 3–6 M Hz transducer. Doppler and morphological findings were used in the diagnosis of rheumatic valvulitis in accordance with the 2015 Revised Jones Criteria [6].

Carditis was described as mild in the presence of trivial-to-mild valvar disease; moderate when there were valve lesions without signs or symptoms of heart failure and normal left ventricular function; and severe when there was severe valvar disease or moderate-to-severe valvar lesion with signs of heart failure [10]. Patients with mild carditis were treated with naproxen sodium, while those with moderate-to-severe carditis received oral prednisolone [11].

In addition to routine laboratory tests used for diagnosing and managing ARF, extra blood samples for IMA analysis were collected from all the ARF group participants at the time of diagnosis and post-treatment. Blood samples for IMA analysis were also collected from the control group. To ensure unbiased analysis, a nurse assigned a code to each blood sample, and neither the pediatric cardiologists nor the biochemists were aware of whether the sample belonged to the study or control group.

Venous blood samples for the IMA analysis were centrifuged for 15 min at 1000 × *g* at 2–8 °C after clotting at room temperature for 2 h. The serum was collected and stored at −80 °C until the day that it would be assayed. The concentration of serum IMA was analyzed via micro enzyme-linked immunosorbent assay (ELISA) using commercial kits (Wuhan Elabscience Biotechnology Co. Ltd., Wuhan, Hubei, China; Lot No. CRQXD8EW17 and MF4LTGELGA, respectively) and in accordance with the manufacturer's instructions.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). The mean ± standard deviation (SD) and frequency were used to express the descriptive statistics. The Independent Sample's t test was used for comparison of the groups. The Paired Sample's T test was used to compare the pre- and post-treatment findings in the patient group. One-way analysis of variance was used for comparison of the subgroups. Pearson's correlation analysis was used for the correlation analysis. The confidence interval was given as 95% and statistical significance was set at $p < 0.05$.

Results

The study included 50 participants, comprising 25 children diagnosed with ARF in the ARF group, and a control group of 25 healthy children. The mean ages of the ARF and control groups were 9.8 ± 2.3 and 10.6 ± 2.6 years, respectively (p = 0.167). In the ARF group, 11 (44%) patients were female compared to 14 (56%) in the control group, with no significant difference in the sex distribution between the groups (p = 0.572).

The clinical and laboratory findings of the ARF group based on the diagnostic criteria are summarized in Table 1. None of the patients had Sydenham chorea, subcutaneous nodules, or erythema nodosum.

Table 1. Findings of the ARF group based on the diagnostic criteria

	n (%)
Carditis	21 (84%)
Mild	12 (48%)
Moderate	7 (28%)
Severe	2 (8%)
Arthritis	20 (80%)
Fever	18 (72%)
Elevated ESR and/or CRP	25 (100%)
Elevated ASO titers	17 (68%)
Positive throat culture for group A β-hemolytic streptococcus	11 (44%)
Low albumin level	9 (36%)
First degree AV block	14 (56%)

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, ASO: anti-streptolysin O

The mean ESR and CRP levels in the ARF group were significantly higher at the time of diagnosis compared to the post-treatment level (p = 0.000). In the ARF group, the albumin concentration was 36.5 ± 3.2 g/L, compared to 43 ± 1.99 g/dL in the control group. There was a statistically significant difference in albumin levels between the ARF and control groups (p < 0.001).

The mean IMA level in the ARF group at the time of diagnosis (0.62 ± 0.73 ng/mL) was significantly lower than the mean post-treatment level (1.18 ± 0.94 ng/mL) (p = 0.010). When compared with the control group, the mean IMA levels of both groups were similar at the time of diagnosis and post-treatment.

Additionally, the IMA level was found to be negatively correlated with the ESR at the time of diagnosis (p = 0.014, correlation coefficient = -0.487), whereas no correlation was observed between the IMA and CRP levels.

Table 2. Comparison of the mean serum IMA levels of the ARF and control groups

	ARF Group (n = 25)	Control Group (n = 25)	p-value
IMA			
Pre-treatment (mean ± SD)	0.62 ± 0.73	1.15 ± 1.21	0.063
Post-treatment (mean ± SD)	1.18 ± 0.94	1.15 ± 1.21	0.930
P-value	**0.010**	-	

T test, IMA is expressed as ng/mL

Discussion

This study examined the role of IMA as an inflammatory marker in the diagnosis and monitoring of ARF. According to the findings, while acute phase reactants such as CRP and ESR are typically elevated, IMA levels may not be significantly increased in children with ARF. This suggests that IMA may not serve as a reliable biomarker for diagnosing or monitoring ARF in the pediatric population.

The initial manifestation of ARF typically occurs between the ages of 5 and 14 years, and the current investigation included patients within this age group [12]. The findings herein align with previous research on this population. ARF occurs with similar prevalence in both males and females [12]. In the present analysis, while there was a slightly higher prevalence among the males, the difference between the sexes was not statistically significant. Consistent with prior findings, the prevalence of carditis, fever, and arthritis/polyarthralgia was 100%, 72%, and 80%, respectively [6,13].

IMA is a biomarker that has been extensively studied in cardiovascular diseases, particularly in ischemic heart diseases [14,15]. Ischemic conditions may lead to the generation of free radicals, and the release of free metal ions and acidosis. These free radicals result in albumin with reduced metal-binding capacity. Beyond cardiovascular disease, IMA has also been investigated in chronic non-cardiac diseases that may lead to increased oxidative stress or chronic inflammation [16].

Some studies have shown a positive correlation between IMA and acute phase reactants [5,4,17]. The IMA values reported in these studies on ARF are given in Table 3. Other research has found significantly higher IMA levels in patients with ARF compared to the control groups. In the current study, in contrast, it was found to be lower compared to the control group. Nevertheless, upon looking at the values, there was no substantial difference in the IMA levels compared to previous studies.

In the present study, the IMA levels were measured using ELISA. Unlike the technique for measuring cobalt-binding capacity in the albumin cobalt binding (ACB) test, the results were obtained using specific antibody in ELISA. Çalışkan et al. reported that the measurement of IMA levels using ELISA was not a reliable method for diagnosing mesenteric ischemia [18]. In the study of Sbarouni, which evaluated IMA levels using the ACB test, no statistically significant increase was found in patients

with dilated cardiomyopathy compared to the healthy group [19]. Roy et al. reported that IMA levels decreased after an exercise test in patients with peripheral vascular disease [20]. It has been suggested that this decline may be due to the interference of metabolites released from skeletal muscle during exercise with IMA measurement. Furthermore, molecules and complexes generated due to a compromised immune response in ARF may affect the result of IMA levels, potentially impacting its reliability as a biomarker in this condition.

Table 3. Comparison of the IMA values of children with ARF in different studies

	IMA Values
Dawn et al [(17)*]	0.42 ± 0.05 ABSU
Toker et al [(4)*]	0.55 (0.44 - 1.13) ABSU
Karataş et al [(5)*]	0.54 ± 0.12 ABSU
The current study	0.62 ± 0.73 ng/mL

*ACB test, ABSU- absorbance units,

Toker et al. found that IMA levels were elevated during the acute phase before therapy and subsequently diminished following treatment. Conversely, according to the findings of the current study, the level of IMA, which was reduced pre-treatment, increased post-treatment. It may be speculated that this may have resulted from the confounding influence of the administered medications. There is a need for further research on how medications influence IMA levels, as no studies have been conducted on this topic to date.

Moreover, the albumin concentration serves as the primary predictor of IMA levels [22]. Variations in albumin concentrations may explain the differences in IMA levels observed across studies. Additionally, extremely high or low albumin levels could have led to falsely low IMA readings [21]. Supporting this, the study of Gaze et al. demonstrated a negative correlation between IMA levels and albumin levels below 34 g/L [22].

The current study had several limitations that should be considered. First, it was conducted at a single center. Furthermore, there was a relatively small sample size, which may have affected the generalizability of the findings. However, despite this limitation, this is the only study to have reported lower IMA levels in ARF, as previous studies have demonstrated elevated levels. This emphasizes the necessity for further studies with larger cohorts to better understand this variation and its therapeutic implications. Moreover, serial measurements of IMA rather than measurements at the time of diagnosis and post-treatment would yield more reliable data. Additionally, it is necessary to acknowledge that IMA levels may produce different findings based on the measuring technique employed, such as ELISA or the ACB test, which may possibly account for the differences reported among the research. Therefore, more research that directly compares these two methods is required in order to assess their reliability.

In conclusion, this study aimed to investigate the role of IMA in pediatric patients with ARF. The findings suggest a negative correlation between the IMA level and ARF. However, to better assess the reliability of IMA in the diagnosis and monitoring of ARF and elucidate the mechanisms influencing IMA levels, additional multicenter studies with larger sample sizes and different techniques are needed.

Ethical Approval

The study was approved by the ethics committee of Ministry of Health Van Regional Education and Research Hospital (2018/14).

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

YND: Writing – Original draft; MR: Writing; ZE: Investigation; MOB: Methodology; SE: Supervision

Financial Support

This research received no specific grant from any funding agency, commercial or not-for-profit sector.

References

1. Libby P. Braunwald's heart disease-E-book: a textbook of cardiovascular medicine: Elsevier Health Sciences, 2021.
2. Mou H, Shao J, Zhang J, Yang J, Yu S, Wang H. Ischemia-modified Albumin to Evaluate Short-term Prognostic of Patients with Acute Coronary Syndrome. *J Coll Physicians Surg Pak* 2021;30:841-845.
3. Erenler AK, Yardan T, Kati C, Altuntaş M, Türedi S. Role of ischemia-modified albumin in clinical practice. *Lab Med.* 2015;39:241-247.
4. Toker A, Karatas Z, Altin H, Karaarslan S, Cicekler H, Alp H. Evaluation of serum ischemia modified albumin levels in acute rheumatic fever before and after therapy. *Indian J Pediatr* 2014;81:120-5.
5. Karatas Z, Baysal T, Sap F, Alp H, Mehmetoglu I. Increased ischaemia-modified albumin is associated with inflammation in acute rheumatic fever. *Cardiol Young* 2014;24:430-6.
6. Gewitz MH, Baltimore RS, Tani LY et al. Revision of the Jones Criteria for the diagnosis of acute rheumatic fever in the era of Doppler echocardiography: a scientific statement from the American Heart Association. *Circulation* 2015;131:1806-18.
7. Kaplan EL, Rothermel CD, Johnson DR. Antistreptolysin O and anti-deoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States. *Pediatrics* 1998;101:86-8.
8. Steer AC, Vidmar S, Ritika R et al. Normal ranges of streptococcal antibody titers are similar whether streptococci are endemic to the setting or not. *Clin Vaccine Immunol* 2009;16:172-5.
9. Chen CB, Hammo B, Barry J, Radhakrishnan K. Overview of Albumin Physiology and its Role in Pediatric Diseases. *Curr Gastroenterol Rep* 2021;23:11.
10. Cannon J, Roberts K, Milne C, Carapetis JR. Rheumatic Heart Disease Severity, Progression and Outcomes: A Multi-State Model. *J Am Heart Assoc* 2017;6.

11. Parnaby MG, Carapetis JR. Rheumatic fever in indigenous Australian children. *J Paediatr Child Health* 2010;46:527-33.
12. Carapetis JR, Beaton A, Cunningham MW et al. Acute rheumatic fever and rheumatic heart disease. *Nat Rev Dis Primers* 2016;2:15084.
13. Kumar RK, Tandon R. Rheumatic fever & rheumatic heart disease: the last 50 years. *Indian J Med Res* 2013;137:643-58.
14. Gok M, Kundi H, Kiziltunc E, Topcuoglu C, Ornek E. The relationship between ischaemia-modified albumin and good coronary collateral circulation. *Kardiol Pol* 2018;76:370-375.
15. Pipikos T, Kapelouzou A, Tsilimigras DI et al. Stronger correlation with myocardial ischemia of high-sensitivity troponin T than other biomarkers. *J Nucl Cardiol* 2019;26:1674-1683.
16. Mangoni AA, Zinellu A. Ischemia-modified albumin in rheumatic diseases: A systematic review and meta-analysis. *Immun Inflamm Dis* 2024;12:e1324.
17. Dawn I, Biswas G. Assessment of serum Ischemia Modified Albumin (IMA) Levels in Acute Rheumatic Fever. *Int J Hum and Health Sci* (IJHHS);5:222-225.
18. Caliskan A, Yavuz C, Karahan O et al. Serum ischaemia-modified albumin level is an irrelevant predictive factor for ischaemic duration in mesenteric ischaemia. *Perfusion* 2014;29:226-30.
19. Sbarouni E, Georgiadou P, Koutelou M, Sklavainas I, Panagiotakos D, Voudris V. Ischaemia-modified albumin in dilated cardiomyopathy. *Ann Clin Biochem* 2009;46:241-3.
20. Roy D, Quiles J, Sharma R et al. Ischemia-modified albumin concentrations in patients with peripheral vascular disease and exercise-induced skeletal muscle ischemia. *Clin Chem* 2004;50:1656-60.
21. Demir H, Topkaya BÇ, Erbay A, Doğan M, Yücel D. Ischaemia-modified albumin elevation after percutaneous coronary intervention reflects albumin concentration rather than ischaemia. *Ann. Clin. Biochem*. 2009;46:327-331.
22. Gaze DC, Crompton L, Collinson P. Ischemia-modified albumin concentrations should be interpreted with caution in patients with low serum albumin concentrations. *Med Princ Pract* 2006;15:322-4.

doi: 10.53446/actamednicomedia.1621640

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

INVESTIGATION OF *IFIT3* AND *KCNS3* GENE EXPRESSION PATTERNS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CRYPTOGENIC EPILEPSY

KRİPTOJENİK EPİLEPSİ HASTALARININ PERİFERİK KANINDA IFIT3 VE KCNS3 GEN EKSPRESYON PATERNLERİNİN İNCELENMESİ

Gulsima Ozcan [1], Nur Damla Korkmaz [2,3,4], Seda Susgun [4], Emrah Yucesan [5*], Ferda Ilgen Uslu [6]

[1]Bezmialem Vakıf University, Faculty of Medicine, Istanbul, Türkiye . [2] Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Neuroscience, Istanbul, Türkiye . [3] Istanbul University, Graduate School of Health Sciences, Istanbul, Türkiye . [4] Bezmialem Vakıf University, Faculty of Medicine, Department of Medical Biology, Istanbul, Türkiye. [5] Istanbul University- Cerrahpasa, Institute of Neurological Sciences, Department of Neurogenetics, Istanbul, Türkiye. [6] Bezmialem Vakıf University Medical School, Department of Neurology, Istanbul, Türkiye.

ABSTRACT

Objective: Cryptogenic epilepsy is a subtype of epilepsy marked by the absence of identifiable structural or metabolic causes, making both diagnosis and treatment particularly challenging. Molecular approaches, such as gene expression profiling, may offer insights into underlying mechanisms and help refine clinical strategies. This study aimed to evaluate the peripheral blood expression profiles of *IFIT3* and *KCNS3* genes in individuals diagnosed with cryptogenic epilepsy.

Methods: Peripheral blood samples were obtained from 20 patients with cryptogenic epilepsy and 20 age- and sex-matched healthy individuals. Total RNA was extracted and subsequently reverse-transcribed into complementary DNA (cDNA). Quantitative real-time PCR (RT-qPCR) was utilized to determine the relative expression levels of *IFIT3* and *KCNS3*, with *ACTB* serving as the internal control gene.

Results: Analysis revealed a significant upregulation of *KCNS3* expression in the patient group compared to healthy controls ($p < 0.0001$). In contrast, no statistically significant difference was observed in *IFIT3* expression between the two groups

Conclusion: The elevated expression of *KCNS3,* which encodes a voltage-gated potassium channel subunit, suggests a potential involvement of ion channel dysregulation in cryptogenic epilepsy pathophysiology. The lack of significant change in *IFIT3,* an interferon-stimulated gene, may imply that immune-related pathways are less central in this context, reinforcing the hypothesis that channelopathy plays a key role in this patient population.

Keywords: Cryptogenic epilepsy, channelopathy, gene expression, protein interaction

ÖZ

Amaç: Kriptojenik epilepsi, yapısal veya metabolik olarak tanımlanabilir bir nedenin bulunmadığı epilepsi alt türlerinden biridir ve bu durum hem tanı hem de tedavi sürecini zorlaştırmaktadır. Gen ekspresyonu gibi moleküler yaklaşımlar, hastalığın altında yatan mekanizmaları aydınlatma ve klinik stratejileri geliştirme potansiyeli taşımaktadır. Bu çalışmada, kriptojenik epilepsi tanısı almış bireylerin periferik kan örneklerinde *IFIT3* ve *KCNS3* genlerinin ekspresyon düzeyleri incelenmiştir.

Yöntem: Çalışmaya kriptojenik epilepsili 20 hasta ve yaş ve cinsiyet açısından eşleştirilmiş 20 sağlıklı birey dahil edilmiştir. Katılımcıların periferik kan örneklerinden toplam RNA izole edilerek tamamlayıcı DNA (cDNA) sentezlenmiştir. *IFIT3* ve *KCNS3* genlerinin göreli ekspresyon düzeylerini belirlemek amacıyla Gerçek Zamanlı Kantitatif PCR (RT-qPCR) uygulanmıştır. *ACTB* geni ise referans (housekeeping) gen olarak kullanılmıştır.

Bulgular: *KCNS3* geninin ekspresyon düzeyinin hasta grubunda sağlıklı kontrollere kıyasla anlamlı düzeyde yüksek olduğu bulunmuştur ($p < 0.0001$). *IFIT3* gen ekspresyonu açısından ise gruplar arasında istatistiksel olarak anlamlı bir fark gözlenmemiştir.

Sonuç: Potasyum kanalı alt birimini kodlayan *KCNS3* geninin artmış ekspresyonu, kriptojenik epilepside iyon kanalı bozukluklarının (kanalopatilerin) patofizyolojide rol oynayabileceğini düşündürmektedir. *IFIT3* geninde anlamlı bir değişiklik olmaması ise bağışıklıkla ilişkili yolların bu hasta grubunda daha az etkili olabileceğini ve kanalopati hipotezinin daha ön planda olduğunu desteklemektedir.

Anahtar Kelimeler: Kriptojenik epilepsi, kanalopati, gen ekspresyonu, protein etkileşimi

***Corresponding author/İletişim kurulacak yazar:** Emrah Yucesan; Istanbul University -Cerrahpasa, Institute of Neurological Sciences, Department of Neurogenetics, Istanbul, Türkiye
Phone/Telefon: +90 (535) 286 67 89, **e-mail/e-posta:** emrah.yucesan@iuc.edu.tr
Submitted/Başvuru: 16.01.2025● **Accepted/Kabul:** 03.06.2025 ● **Published Online/Online Yayın:** 30.06.2025

Introduction

Epilepsy is a chronic neurological disease with heterogeneous presentations, the most common of which are recurrent seizures. Even with improved health care and reduced mortality, it remains a significant burden, affecting millions of people of all ages[1]. Diagnosis of epilepsy consists of evaluating the patient's medical history, including a report from an eyewitness, electroencephalography (EEG), neuroimaging studies, and laboratory tests. The lack of a distinct element suggesting the disease and the broad spectrum of manifestations both create a more challenging process[2].

Identifying the underlying cause of epilepsy is crucial in clinical settings, as it significantly impacts treatment, prognosis, and the disease's progression[3]. The International League Against Epilepsy (ILAE) has categorized epilepsies into six etiological groups: 1) genetic, 2) structural, 3) infectious, 4) metabolic, 5) immune, and 6) unknown causes[4]. Cryptogenic epilepsy is typically classified under the group of unknown etiologies in the most recent ILAE system. However, in earlier classifications, it referred to cases where there were no signs of previous brain injury or clear etiology[5]. While presumed to be symptomatic, the actual cause remains elusive. Cryptogenic epilepsy accounts for approximately 40% of adult-onset epilepsy cases, making it a significant contributor[6].

A deeper understanding of the disease's underlying mechanisms is crucial for improving diagnosis and management. Recent research has explored various contributing factors such as neurodegeneration, inflammation, brain trauma, and channelopathies[7]. Genetic studies on patients with epilepsy can enhance clinical decision-making by facilitating more accurate diagnoses, identifying risk factors, and informing prognoses. These studies can also offer valuable insights into the biological mechanisms of epilepsy[8].

The aim of this study was to establish whether cryptogenic epilepsy patients have a difference in terms of genetic expression profiles in comparison to healthy individuals. Through this approach, we targeted for a better understanding of the underlying causes of the disease and a better sight into the mechanisms leading to the disease. In our study, we investigated genes involved in pathways (channelopathy and inflammation) that play significant roles in the molecular pathogenesis of epilepsy, particularly cryptogenic epilepsy[8-10]. To explore the potential etiology of channelopathy, we studied the *KCNS3* gene, which encodes a subunit of the Potassium Voltage-Gated Channel Modifier Subfamily S Member 3. Potassium channels play a critical role in regulating neuronal excitability, and their dysfunction has been associated with various forms of epilepsy, including cryptogenic epilepsy [11]. As for the inflammation pathway, we studied the *IFIT3* gene, encoding Interferon-Induced Protein with Tetratricopeptide Repeats 3. Protein interactions of *IFIT3* and *KCNS3* gene products obtained from the STRING database are demonstrated in Figure 1.

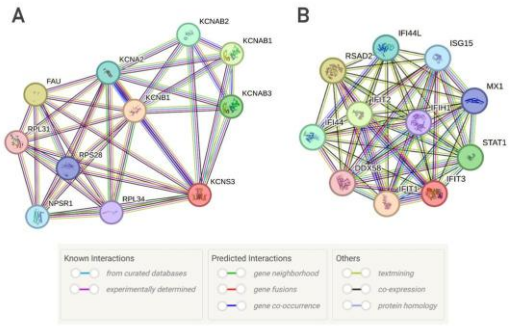


Figure 1. Protein interactions of A. KCNS3, B. IFIT3 obtained from STRING database (v12.0)

Methods

Patient Recruitment

The study consists of epilepsy patients without obvious etiology, who are long-term patients of Bezmialem Vakıf University, Faculty of Medicine, Neurology Department outpatient clinics, and healthy controls. The patients were screened in terms of; age, gender, time of onset of seizures, types of seizures, patient history, neurological examination, family history, 1.5 Tesla cranial MRI (Siemens Avanto, Erlangen, Germany), EEG findings, and the number of antiepileptics used. Patients over the age of eighteen, who were followed up by a clinician for at least a year, with no defined underlying cause for epilepsy, were included in the study. Pediatric patients (<18 years), patients with a known underlying factor leading to epilepsy (tumor, stroke, etc.), and patients with another systemic or neurological disease (diabetes, hypertension, neurodegenerative diseases, etc.) were excluded. The control group consisted of 20 healthy volunteers (11 males and 9 females) without any known neurological or systemic disorders. They were selected to be age- and sex-matched to the patient group to minimize demographic bias. Participants were informed about the study, and those who had given verbal and written consent were included in the study. According to power analysis, the optimal numbers of participants for patients (n1) and healthy controls (n2) were calculated as n1=n2=20 with a total of n=40, to obtain odds ratios of 1 and 2.16, with a power of 80%, a significance level of 0.05 and a 95% confidence interval.

RNA Isolation & Complementary DNA (cDNA) Conversion

From patients and controls who agreed to participate in the study, ten milliliters of peripheral blood samples were drawn into EDTA tubes. The collected samples were then transported to the laboratory on ice and were studied immediately. Total RNAs from the blood samples were isolated using the QIAamp RNA Blood Mini Kit (QIAGEN, Helden, Germany) according to the manufacturer's protocol. The quantitative assessment of

RNA purity was carried out using the Multiskan GO (Thermo Fisher Scientific, Boston, MA, USA) device. The RNA samples achieving an A260/A280 absorbance ratio between 1.9-2.1 were confirmed and stored at -80 °C until the next step. RNA samples were reverse transcribed to complementary DNA (cDNA), using the SensiFAST™ cDNA Synthesis Kit (Meridian Bioscience Inc., Cincinnati, OH, USA). cDNA samples were stored at -20 °C until further processing.

Quantitative Real-Time Polymerase Chain Reaction (qRT- PCR)

For the evaluation of messenger RNA (mRNA) expressions, the *ACTB* gene was designated as the housekeeping gene by the GeNorm program. Primers for *ACTB*, *KCNS3* and *IFIT3* genes were designed with the Primer3 v4.1.0 program. Primers for the *ACTB* (NM_001101.5) gene (Pf: CATCCGCAAAGACCTGTACG,Pr:CCTGCTTGCTGATCCACATC), *IFIT3* (NM_001549.6) gene (Pf:CACTTGGGCAGACTCTCAGA,Pr:AAACACACCTTCGCCCTTTC), and *KCNS3* (NM_002252) gene (Pf:AATCGCTACCAGGAACGCAA,Pr:CGATCTCCACTCCTTCCAGC). Following the optimization of annealing temperatures, RT- PCR was conducted for three genes (*ACTB, IFIT3, KCNS3*) for both patient samples and healthy controls. All samples were studied in duplicates. The SensiFAST™ SYBR No-ROX Kit P (Meridian Bioscience Inc., Cincinnati, OH, USA) was used and the experiments were conducted according to the manufacturer's instructors.

Statistical Analysis

All statistical analysis was performed using the Ct data retained from the qRT-PCR. The changes in expression levels were calculated using the delta-delta Ct method. Normalized expression levels ($2^{-\Delta Ct}$) were compared between the patient and control groups using appropriate statistical tests. Fold-change analysis using the $2^{-\Delta\Delta Ct}$ method was not applied in this study[12]. The data distribution was tested for normality with the Shapiro-Wilk test. Subsequently, a two-tailed Mann-Whitney U test was performed on independent samples to compare the expression levels between the patient and healthy control groups, using GraphPad Prism 8.0 (GraphPad Software, Inc., CA, USA). Results with a p-value of < 0.05 were considered statistically significant. Receiver operating characteristic (ROC) curves were demonstrated and area under the curve (AUC) values were calculated in order to evaluate the diagnostic potential of target genes.

Results

Clinical Assessments

The study consisted of 20 cryptogenic epilepsy patients and 20 healthy patients, who were matched to the sex distribution of patients (p <0.05). The patients who participated were between the ages of 19 to 62, with a mean of 29.2 ±10.14. Eleven were male and nine were female. Time of onset of seizures ranged from 2 to 40 years, with a mean of 18.25 ±10.45. Eighteen patients were suffering from focal seizures and two patients had focal seizures with secondary generalization. There were no findings in any of the patients' personal medical history, including their birth history or neurodevelopmental history. Two patients had consanguineous parents, but none of them had a family history of epilepsy, including those two patients. None of the patients had any findings on their 1.5 Tesla Cranial MRI (data not shown). Fourteen patients were using only one medication to control the seizure. The EEG recordings were normal in eleven of the patients. The recordings demonstrated unilateral temporal epileptiform abnormalities in five patients (Figure 2A), unilateral frontotemporal epileptiform abnormalities in two patients, unilateral frontal epileptiform abnormality in one patient and multifocal abnormalities (bilateral temporal and frontal abnormalities) in one patient (Figure 2B). Clinical characteristics of cryptogenic epilepsy patients included in the study are shown in Table 1.

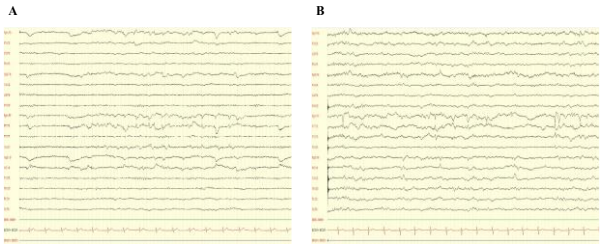


Figure 2. Demonstrated EEG findings in patients A. left temporal epileptiform anomaly, B. bilateral temporal and right frontal (multifocal) epileptiform anomaly

mRNA Expression Levels

The mean values of the cycle threshold (Ct) data obtained by the qRT-PCR study are presented in Table 2. Accordingly, the mRNA expression levels of the *KCNS3* gene were statistically higher in the patient group (p<0.0001) (Figure 3). However, levels of the *IFIT3* mRNA expression didn't show a statistical difference (Figure 4). The AUC value of *KCNS3* gene expression was determined as 0.9569 at the 95% confidence level by a ROC curve analysis (p<0.0001, Figure 5).

Table 2. The average expression levels of *KCNS3* and *IFIT3* genes

	***KCNS3* Ct**	***ACTB* Ct**	**ΔCt**	**$2^{-\Delta Ct}$**
Patients	34.25	21.68	12.57	0.00016
Controls	34.6	20.45	14.15	0.00005
	***IFIT3* Ct**	***ACTB* Ct**	**ΔCt**	**$2^{-\Delta Ct}$**
Patients	32.85	21.68	11.17	0.00062
Controls	31.63	20.45	11.18	0.00058

ΔCt values were calculated using *ACTB* as the reference gene. *KCNS3* expression was higher in patients, whereas *IFIT3* levels were comparable between groups.

Table 1. Patient Characteristics

Characteristic	Value
Age	
Range	19-62 years
Mean (SD)	29.2 (10.14)
Sex *(n of patients)*	
Male	11
Female	9
Time of onset of seizures *(average± SD)*	
Range	2-40 years
Mean (SD)	18.25 (10.45)
Types of seizures *(n of patients)*	
Focal Seizures	15
Tonic-Clonic Seizures	2
Unclassified	3
Patient history	
Medical history	No findings
Birth history	No findings
Neurodevelopmental history	No findings
Family history	
History of epilepsy	No findings
Consanguineous parents	2
Number of antiepileptics used *(n of patients)*	
One medication	14
Multiple medications	6
Cranial MRI findings (1.5 Tesla)	No findings
EEG findings *(n of patients)*	
Normal EEG	11
Unilateral temporal epileptic abnormalities	5
Unilateral frontotemporal epileptic abnormalities	2
Unilateral frontal epileptic abnormalities	1
Multifocal epileptic abnormalities	1 (bilateral temporal + unilateral frontal findings)

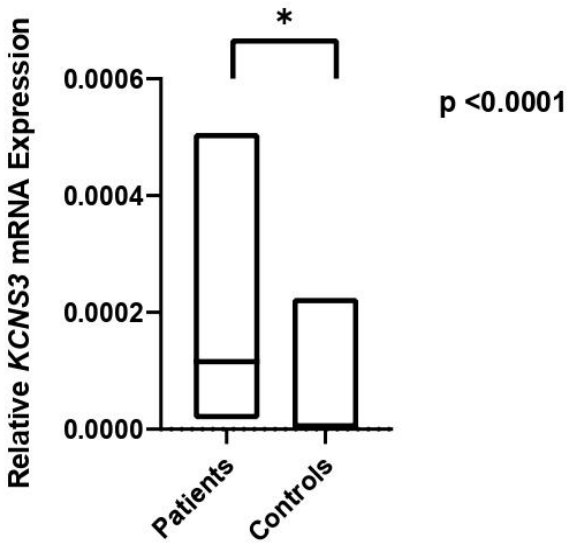


Figure 3. Comparison of relative mRNA expression levels of target gene *KCNS3*

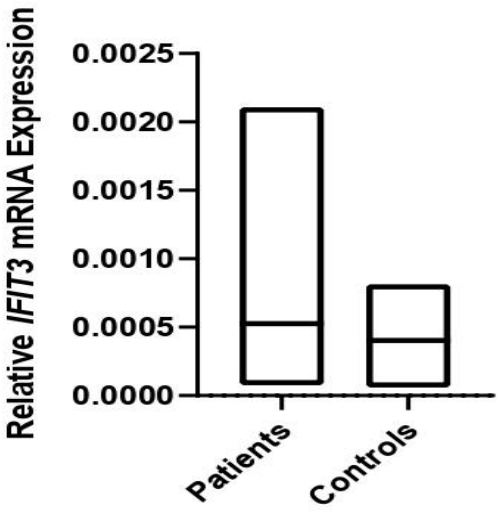


Figure 4. Comparison of relative mRNA expression levels of target gene *IFIT3*

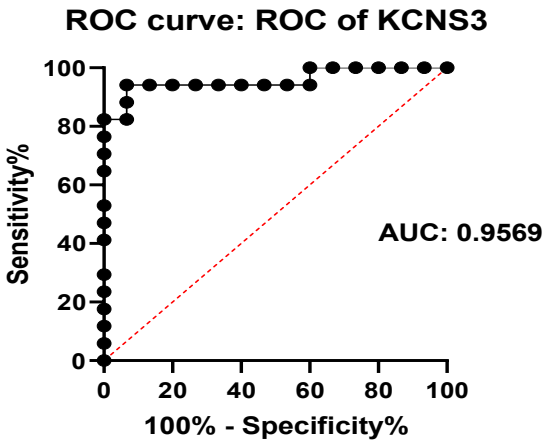


Figure 5. ROC curve analysis results reveal the diagnostic performance of *KCNS3* gene

Discussion

Epilepsy is the second most prevalent neurological disorder, following stroke[13]. It is estimated that around 0.8% of the population experiences some form of epilepsy. In approximately 30% of cases, epilepsy is linked to a specific brain injury that has caused the condition, known as symptomatic epilepsy[14]. Another 30% of patients are considered to have presumed symptomatic epilepsy, termed 'cryptogenic epilepsy,' where it is suspected that an underlying brain pathology exists but has not yet been detected with current methods[15].

Epileptogenesis refers to the sequence of molecular and cellular alterations triggered by an initial brain injury, eventually leading to spontaneous seizures. These changes include neurodegeneration, neurogenesis (the creation of new neurons), axonal growth and damage, dendritic restructuring, gliosis, the infiltration of inflammatory cells, angiogenesis (new blood vessel formation), modifications to the extracellular matrix, and the development of acquired channelopathies[16,17]. Various ion channels play a role in the regulation of current flow. For example, voltage-gated ion channels undergo conformational shifts, alternating between open and closed states. These shifts are driven by changes in membrane electrical potentials, controlling the selective ion movement across membranes. Therefore, it is evident that dysfunction in these ion channels can result in brain hyperexcitability[18,19]. Mutations in genes such as *SCN1A, SCN2A, SCN8A* (which encode voltage-gated sodium channels), and *CACNA1A* (which encodes a voltage-gated calcium channel) are associated with various forms of epilepsy. For example, loss-of-function mutations in *SCN1A* can lead to hyperexcitability in conditions like Dravet syndrome, while pathogenic variants in *CACNA1A* have been linked to childhood absence epilepsy. These genetic alterations highlight the role of channelopathies in the pathogenesis

of epilepsy[20,21]. Voltage gated potassium channels (Kv) are activated by membrane depolarization, allowing potassium to exit the cell, which helps return the membrane to its resting state[22]. These channels, found in axons, play a crucial role in delaying axonal action potentials[23]. The *KCNT1* gene encodes the KCa4.1 subunit, a sodium-activated potassium channel, associated with up to 50% of cases of epilepsy of infancy with migrating focal seizures (EIMFS)[24]. *De novo* pathogenic variants in the *KCNQ2* gene are associated with self-limited neonatal epilepsy (SeLNE) and may also be inherited in an autosomal dominant manner, leading to self-limited familial neonatal epilepsy (SeLFNE)[25].

Among these voltage-gated potassium channels, the epilepsy-related gene we examined in this study is *KCNS3*, which encodes the Potassium Voltage-Gated Channel Modifier Subfamily S Member 3. *KCNS3* operates within the same network as the *CACNA1* calcium channel protein gene and the *KCNB1* gene. While KCNS3 does not form functional channels on its own, it can create functional heterotetrameric channels by pairing with KCNB1. This interaction modulates the activation and deactivation rates of the delayed rectifier voltage-gated potassium channel, KCNB1[26]. Additionally, according to KEGG pathway analysis, the gene is associated with the serotonergic pathway via *SLC6A4* and has been reported in the literature to influence neuronal excitability through serotonin signaling[27]. A wide range of mechanisms have been studied to identify the causes of epilepsy. Among the previously explored mechanisms, our study aimed to further investigate potential genetic disturbances in these patients, with a particular focus on inflammation and ion channel disorders. Accordingly, our study found that *KCNS3* gene expression is elevated in the cryptogenic epilepsy group compared to the control group. This suggests that the altered expression of *KCNS3* could influence neuronal excitability through the regulation of potassium channel proteins and may play a role in the pathogenesis of cryptogenic epilepsy by impacting the integration of energy metabolism. Additionally, the change in *KCNS3* expression might affect its interacting partners, *KCNB1* and *SLC6A4*, which are involved in potassium channel functionality and serotonin signaling, respectively. Therefore, we propose that the altered expression of *KCNS3* may contribute to epilepsy through these mechanisms.

To this end, we also examined the levels of the *IFIT3* gene in patients with cryptogenic epilepsy and in controls. *IFIT3*, known as the interferon-inducible protein with tetratricopeptide repeats 3, is a key member of both the IFIT family and the interferon-stimulated genes family. It shares typical features of the IFIT family in terms of gene and protein structures and can be activated through the classical PRRs-IFN-JAK/STAT pathway[28]. According to the literature, via the JAK-STAT signaling pathway, *IFIT3* gene expression is found to be elevated in viral infections[29]. In a previous study comparing the differential genetic expression profiles of epilepsies with different origins, *IFIT3* expression levels were found to be higher in cryptogenic epilepsy patients compared to those with symptomatic or idiopathic epilepsies[30]. However, in terms of differentiating these patients from those of the healthy population, despite the observed difference of distribution between patients and healthy individuals, a statistically significant distinction of *IFIT3* expression levels could not be identified.

Additionally, in our study investigating the contribution of inflammation and channelopathy to the etiology of epilepsy, we found that the levels of the *KCNS3* gene are elevated in patients with cryptogenic epilepsy. Various K-channel Various potassium channel (K^+ channel) subunit genes have been reported to be altered in epileptic patients in the literature[31]. However, to our knowledge, no prior studies have examined the levels of the *KCNS3* gene, which we identified as being higher in the cryptogenic epilepsy group. We suggest that this gene may play a role in the pathogenesis of the disease and could serve as a potential biomarker for cryptogenic epilepsy in future research. As known, ROC curve analysis calculates the sensitivity and specificity of target molecules and is a useful graphical tool for assessing the diagnostic value of biomarkers[32]. The current study examined *KCNS3* as a diagnostic biomarker by calculating the AUC value using ROC curve analysis. Consequently, *KCNS3* may be a helpful diagnostic biomarker for cryptogenic epilepsy.

Moreover, our findings suggest that potassium (K^+) related channelopathy may be a more plausible underlying mechanism in these patients, rather than an inflammatory process. A larger cohort study is needed to better elucidate this result though.

Conclusion

In conclusion, our study highlights the potential role of the *KCNS3* gene in the pathogenesis of cryptogenic epilepsy, suggesting that alterations in potassium channel function, rather than inflammatory processes, may contribute to the disease mechanism. The observed elevation in *KCNS3* gene expression in cryptogenic epilepsy patients underscores its potential as a biomarker, warranting further investigation in larger cohorts. While the *IFIT3* gene showed differential expression patterns, its role in distinguishing cryptogenic epilepsy from other forms of epilepsy and from healthy individuals remains inconclusive. Future studies focusing on the *KCNS3* gene and its network, as well as additional research into the genetic profiles of cryptogenic epilepsy patients, will be essential to better understand the underlying mechanisms and to develop targeted therapeutic strategies.

Ethical Approval

All procedures involving human participants were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent amendments, as well as the relevant guidelines set forth by national and institutional research

ethics boards. The study protocol was reviewed and approved by the Ethics Committee of Bezmialem Vakıf University (Approval No: 9/3, Date: 05/11/2022).

Conflict of Interest

The authors have no conflict of interest to declare.

Author Contributions

E.Y. conceptualized the research idea, designed the study, conducted data analysis, and interpreted the findings. G.O. and F.I.U. were responsible for data acquisition. The initial draft of the manuscript was prepared by G.O. and N.D.K. Critical revision for important intellectual content was carried out by G.O., N.D.K., S.S., and E.Y. All authors reviewed and approved the final version of the manuscript to be published.

Financial Support

This study was funded by Bezmialem Vakıf University Scientific Research Projects Unit (Grant number: 20220601E).

References

1. Collaborators GBDE. Global, regional, and national burden of epilepsy, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. Apr 2019;18(4):357-375. doi:10.1016/S1474-4422(18)30454-X
2. Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. *Lancet*. Feb 16 2019;393(10172):689-701. doi:10.1016/S0140-6736(18)32596-0
3. Shorvon SD. The etiologic classification of epilepsy. *Epilepsia*. Jun 2011;52(6):1052-7. doi:10.1111/j.1528-1167.2011.03041.x
4. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia*. Apr 2017;58(4):512-521. doi:10.1111/epi.13709
5. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia*. Jul-Aug 1989;30(4):389-99. doi:10.1111/j.1528-1157.1989.tb05316.x
6. Chow JSW, Poon TL. *Emerging Trends in the Management of Cryptogenic Epilepsy*. IntechOpen; 2022.
7. Dwivedi R, Kaushik M, Tripathi M, Dada R, Tiwari P. Unraveling the genetic basis of epilepsy: Recent advances and implications for diagnosis and treatment. *Brain Res*. Nov 15 2024;1843:149120. doi:10.1016/j.brainres.2024.149120
8. Yu Y, Sun FJ. Research progress on the role of inflammatory mediators in the pathogenesis of epilepsy. *Ibrain*. Spring 2025;11(1):44-58. doi:10.1002/ibra.12162
9. Ng AC-H, Chahine M, Scantlebury MH, Appendino JP. Channelopathies in epilepsy: an overview of clinical presentations, pathogenic mechanisms, and therapeutic insights. *Journal of Neurology*. 2024;271(6):3063-3094.
10. Stommel EW, Seguin R, Thadani VM, et al. Cryptogenic epilepsy: an infectious etiology? *Epilepsia*. 2001;42(3):436-438.
11. Khan R, Chaturvedi P, Sahu P, et al. Role of potassium ion channels in epilepsy: Focus on current therapeutic strategies. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*. 2024;23(1):67-87.
12. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*. May 1 2001;29(9):e45. doi:10.1093/nar/29.9.e45
13. Richens A. Clinical pharmacology and medical treatment. *A textbook of epilepsy*. 1993:524-527.
14. Engel J, Pedley TA. Epilepsy: a comprehensive textbook. *(No Title)*. 1998;
15. Engel J, Jr. Report of the ILAE classification core group. *Epilepsia*. Sep 2006;47(9):1558-68. doi:10.1111/j.1528-1167.2006.00215.x
16. Pitkanen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav*. Jan 2009;14 Suppl 1:16-25. doi:10.1016/j.yebeh.2008.09.023
17. Ellerkmann RK, Remy S, Chen J, et al. Molecular and functional changes in voltage-dependent Na(+) channels following pilocarpine-induced status epilepticus in rat dentate granule cells. *Neuroscience*. 2003;119(2):323-33. doi:10.1016/s0306-4522(03)00168-4
18. Bezanilla F, Perozo E. The voltage sensor and the gate in ion channels. *Adv Protein Chem*. 2003;63:211-41. doi:10.1016/s0065-3233(03)63009-3
19. Stafstrom CE. Pump-Opathies: Mutations in Na(+)-K(+)-ATPase Genes Produce Severe Developmental Epileptic Encephalopathies. *Epilepsy Curr*. Jan-Feb 2022;22(1):72-74. doi:10.1177/15357597211057356
20. Brunklaus A, Ellis R, Stewart H, et al. Homozygous mutations in the SCN1A gene associated with genetic epilepsy with febrile seizures plus and Dravet syndrome in 2 families. *Eur J Paediatr Neurol*. Jul 2015;19(4):484-8. doi:10.1016/j.ejpn.2015.02.001
21. Catterall WA, Kalume F, Oakley JC. NaV1.1 channels and epilepsy. *J Physiol*. Jun 1 2010;588(Pt 11):1849-59. doi:10.1113/jphysiol.2010.187484
22. Grizel A, Glukhov G, Sokolova O. Mechanisms of activation of voltage-gated potassium channels. *Acta Naturae (англоязычная версия)*. 2014;6(4 (23)):10-26.
23. Köhling R, Wolfart J. Potassium channels in epilepsy. *Cold Spring Harbor perspectives in medicine*. 2016;6(5):a022871.
24. Ohba C, Kato M, Takahashi N, et al. De novo KCNT1 mutations in early-onset epileptic encephalopathy. *Epilepsia*. Sep 2015;56(9):e121-8. doi:10.1111/epi.13072
25. Sands TT, Balestri M, Bellini G, et al. Rapid and safe response to low-dose carbamazepine in neonatal epilepsy. *Epilepsia*. Dec 2016;57(12):2019-2030. doi:10.1111/epi.13596
26. Shepard AR, Rae JL. Electrically silent potassium channel subunits from human lens epithelium. *Am J Physiol*. Sep 1999;277(3):C412-24. doi:10.1152/ajpcell.1999.277.3.C412
27. Barghaan J, Bahring R. Dynamic coupling of voltage sensor and gate involved in closed-state inactivation of kv4.2 channels. *J Gen Physiol*. Feb 2009;133(2):205-24. doi:10.1085/jgp.200810073
28. Zhang W, Li Y, Xin S, et al. The emerging roles of IFIT3 in antiviral innate immunity and cellular biology. *J Med Virol*. Jan 2023;95(1):e28259. doi:10.1002/jmv.28259
29. Xu S, Huang J, Xun Z, et al. IFIT3 Is Increased in Serum from Patients with Chronic Hepatitis B Virus (HBV) Infection and Promotes the Anti-HBV Effect of Interferon Alpha via JAK-STAT2 In Vitro. *Microbiol Spectr*. Dec 21 2022;10(6):e0155722. doi:10.1128/spectrum.01557-22
30. Rawat C, Kushwaha S, Srivastava AK, Kukreti R. Peripheral blood gene expression signatures associated with

epilepsy and its etiologic classification. *Genomics*. Jan 2020;112(1):218-224. doi:10.1016/j.ygeno.2019.01.017
31. Wang J, Lin ZJ, Liu L, et al. Epilepsy-associated genes. *Seizure*. Jan 2017;44:11-20. doi:10.1016/j.seizure.2016.11.030
32. Hsu MJ, Chang YC, Hsueh HM. Biomarker selection for medical diagnosis using the partial area under the ROC curve. *BMC Res Notes*. Jan 10 2014;7:25. doi:10.1186/1756-0500-7-25

doi: 10.53446/actamednicomedia.1623663

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Araştırma Makalesi | Research Article

JUVENİL İDİYOPATİK ARTRİTLİ HASTALARDA HEPATİT AŞILARINA KARŞI BAĞIŞIKLIK YANITI

IMMUNE RESPONSE TO HEPATITIS VACCINES IN JUVENILE IDIOPATHIC ARTHRITIS PATIENTS

Yunus Emre Bayrak[1*], Selim Öncel[2], Ali Öksel[3], Nihal Şahin[1], Hafize Emine Sönmez[1]

[1]Kocaeli Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Çocuk Romatoloji Bilim Dalı, Kocaeli, Türkiye. [2]Kocaeli Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Çocuk Enfeksiyon Bilim Dalı, Kocaeli, Türkiye. [3]VM Medical Park Kocaeli Hastanesi, Çocuk Sağlığı ve Hastalıkları Bölümü, Kocaeli, Türkiye.

ÖZ

Amaç: Juvenil idiyopatik artrit (JİA), çocukluk çağının en sık görülen kronik artrit türüdür. On altı yaşından önce başlayan, altı haftadan uzun süren ve diğer nedenlerin dışlandığı artrit vakalarının tanısıdır. Uzun süreli immünomodülatör/immünsupresif tedavi alan bu hastalarda enfeksiyonlara yatkınlık artar; bu nedenle, enfeksiyonları önlemeye yönelik koruyucu sağlık hizmetleri, özellikle aşılama, JİA hastalarının yönetiminde kritik önem taşır. Bu çalışmanın amacı, JİA tanılı çocuk hastaların tanı anında ve izlem süresince hepatit A ve hepatit B aşılarına karşı geliştirdikleri bağışıklık yanıtlarını değerlendirmektir.

Yöntem: Çalışma, Ağustos 2020-Haziran 2024 aylarında Kocaeli Üniversitesi Tıp Fakültesi Çocuk Romatoloji Bilim Dalı'nda takip edilen JİA hastalarını kapsamaktadır. Hastaların demografik ve klinik verileri, aşı geçmişleri, hepatit A ve B serolojileri geriye dönük olarak incelenmiştir. Hepatit B yüzey antikoru (anti-HBs) titresinin 10 IU/L ve üzerinde olması anlamlı kabul edilmiştir. Eksik bağışıklığı olanlara üç doz hepatit B virüsü (HBV) ve iki doz hepatit A virüsü (HAV) aşısı uygulanmıştır.

Bulgular: Toplamda 190 (98 kız, 92 erkek) hastanın hepatit serolojisi incelendi. Hastaların ortanca semptom başlama yaşı 111 (6-217) ay, tanı alma yaşı 113 (6-218) ay ve değerlendirme sırasındaki yaşları 150 (9-232) aydı. Hastaların %50,5'inde anti-HBs pozitifti ve HAV immünoglobulin (Ig) G ise %51,1'inde pozitifti. Anti-HBs negatif olan 94 hastanın %47,8'i yeniden aşılandı ve bu grubun %33,3'ünde seropozitivite sağlandı. Anti-HAV IgG negatif olan 93 hastanın 24'üne (%25,8) rapel aşı uygulandı ve 10'unda (%41,6) aşılama sonrası seropozitif yanıt sağlandı.

Sonuç: JİA hastalarında seropozitivite oranı normal popülasyona göre daha düşük olduğu gözlemlenmiştir. Bu bulgular, bu hasta grubunda aşılama stratejilerinin daha etkili hale getirilmesi gerektiğini ortaya koymaktadır.

Anahtar Kelimeler: Romatoloji, hepatit, aşılama

ABSTRACT

Objective: Juvenile idiopathic arthritis (JIA) is the most prevalent chronic arthritis in childhood, manifesting as arthritis that commences before the age of 16, persists for more than six weeks, and excludes other potential etiologies. Patients receiving long-term immunomodulatory/immunosuppressive therapies exhibit an augmented susceptibility to infections. Consequently, preventive healthcare services, particularly vaccination, assume a pivotal role in the management of JIA patients. The aim of this study is to evaluate the immune responses to hepatitis A and hepatitis B vaccines at the time of diagnosis and during follow-up in children diagnosed with JIA.

Method: This study included patients with JIA, who were followed between August 2020 and June 2024 at the Department of Pediatric Rheumatology. Patients' demographic and clinical data, vaccination history, and hepatitis A and B serologies were retrospectively reviewed. An anti- hepatitis B surface antibody (anti-Hbs) titer of ≥10 IU/L was considered significant. Patients with inadequate immunity were received three doses of the hepatitis B virus (HBV) vaccine and two doses of hepatitis A virus (HAV) vaccine.

Results: The hepatitis serology of a total of 190 patients (98 girls, 92 boys) was analyzed. The median age at symptom onset was 111 months (6-217), the median age at diagnosis was 113 months (6-218), and the median age at the time of evaluation was 150 months (9-232). The results indicated that 50.5% of the patients were positive for anti-HBs, while 51.1% were positive for hepatitis A virus immunoglobulin G antibody (anti-HAV IgG). Among the 94 patients who were negative for anti-HBs, 47.8% had received vaccination, and 33.3% of this group subsequently became seropositive. Of the 93 patients who were anti-HAV IgG negative, 24 (25.8%) received a booster vaccine, and 10 of them (41.6%) developed a seropositive response after vaccination.

Conclusion: It has been observed that the seropositivity rate is lower in JIA patients compared to healthy population. These findings suggest that vaccination strategies need to be strengthened for this patient group.

Keywords: Rheumatology, hepatitis, vaccination

*** İletişim kurulacak yazar/Corresponding author:** Yunus Emre Bayrak; Kocaeli Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Çocuk Romatoloji Bilim Dalı, Kabaoğlu Mahallesi, 41001 Kocaeli, Türkiye.
Telefon/Phone: +90 (537) 573 84 01, **e-mail/e-posta:** yeb6141@gmail.com
Başvuru/Submitted: 20.01.2025 • **Kabul/Accepted:** 27.06.2025 • **Online Yayın/Published Online:** 30.06.2025

Giriş

Juvenil idiyopatik artrit (JİA), çocukluk çağında en sık karşılaşılan kronik artrit nedenidir. Tanı, 16 yaşından küçük bireylerde, en az altı hafta süren artritin diğer olası nedenler dışlanarak değerlendirilmesiyle koyulur. Uluslararası Romatoloji Dernekleri Birliği (ILAR) sınıflamasına göre JİA, yedi alt gruba ayrılmaktadır: Sistemik, romatoid faktör (RF) pozitif poliartiküler, RF negatif poliartiküler, oligoartiküler form, psöriyatik artrit, entezit ilişkili artrit ve sınıflandırılamayan artrit.[1] Türkiye'de yapılan bir çalışmada, JİA alt tiplerinin dağılımı şu şekilde bildirilmiştir: %14,5 sistemik, %40 oligoartiküler, %3,2 RF pozitif poliartiküler, %20,3 RF negatif poliartiküler, %18,9 entezit ilişkili artrit ve %2,1 psöriatik artrit.[2] Aynı çalışmada üveit sıklığı %15,7 olarak saptanmıştır.[2] JİA'nın prevalansı dünya genelinde 100.000 çocukta 16 ile 150 arasında değişmektedir.[3]

JİA hastalarının tedavisinde hastalık modifiye eden anti-romatizmal ilaçlar (DMARD), kortikosteroidler ve biyolojik ajanlar (BA) gibi immunmodulatör/immunsupresif özelliklere sahip ilaçlar kullanılabilmektedir.[4] Bu ilaçlar bağışıklık sistemi etkileyerek JİA'lı hastaların enfeksiyonlara duyarlığını artırabilirler. Hepatit B virüsü (HBV), hepatotropik bir virüs olup, insanlarda akut ve kronik hepatite neden olabilir. HBV'nin tüm dünya nüfusunun üçte birini (2 milyar) enfekte ettiği bilinmektedir. Bu vakaların yaklaşık 400 milyonunda kronik enfeksiyon sonucunda hepatosellüler karsinom, siroz ve karaciğer yetmezliği gelişmiştir.[5]

Aşı ve standart tedavi ile hepatit B enfeksiyonu insidansı ve ölüm oranı, enfekte kişilerde önemli oranda azalmıştır.[6] Türkiye'de de ulusal aşılama programı ile HBV enfeksiyonu insidansı çarpıcı biçimde azalmıştır.[7,8] Çocukluk çağında uygulanan aşı programı sayesinde, 2009'dan bu yana %95 civarında yüksek aşı kapsamı sağlanmış ve bunun sonucu olarak 1990-2019 döneminde HBV insidans yıllık %1,81, Türkiye'de prevalans ise %2,52 yıllık azalma göstermiştir.[9] İmmünomodülatör tedavi gören veya bağışıklık yanıtını etkileyen bir hastalığı olan bireylerin aşıya verdikleri yanıt, sağlıklı popülasyona kıyasla genellikle daha düşüktür.[10,11] Bu hastalarda, rapel doz uygulamasına rağmen aşıya karşı yeterli immün yanıt oluşmayabilir.[12] İmmünomodülatör tedaviye başlamadan önce, klinisyenlerin hastaların aşılanma durumunu dikkatlice değerlendirmesi önemlidir.

Juvenil idiopatik artrit tanılı hastalar, hastalığın seyri, bozulmuş bağışıklık yanıtı, bağışıklık baskılayıcı tedaviler, sık hastaneye yatışlar ve çeşitli invaziv terapötik ve teşhis prosedürleri (intravenöz tedaviler, periferik kateterizasyon vb.) nedeniyle enfeksiyon riski altındadırlar.[13] Juvenil idiopatik artrit tanılı hastalar, sağlıklı yaşıtlarına göre enfeksiyonlara daha yatkın olduklarından aşılanmaları büyük önem taşır; ancak hastalığın kendisi ve kullanılan ilaçlar, hastaların aşı yanıtını ideal olmaktan uzaklaştırabilir. Bu çalışmanın amacı, JİA tanısı almış hastalarda tanı anında ve izlem sürecinde uygulanan hepatit A ve hepatit B aşılarına karşı gelişen bağışıklık yanıtlarını retrospektif olarak değerlendirmektir.

Yöntem

Çalışmaya Ağustos 2020-Haziran 2024 tarihleri arasında Kocaeli Üniversitesi Çocuk Romatoloji Bilim Dalı'nda takip edilen JİA tanılı hastalar dahil edildi. JİA tanısı ILAR kriterlerine göre belirlendi.[1] Tanı anında, Sağlık Bakanlığı tarafından belirlenen ulusal aşılama programına (primer aşılama) uygun olarak ilk aşılarını tamamlamamış olan hastalar çalışma dışı bırakıldı.

Hasta verileri elektronik dosyalardan geriye dönük olarak tarandı. Hastaların demografik verileri ve klinik bulguları, aşı geçmişleri ve laboratuvar bulguları değerlendirildi. Hastaların tanı anında, hepatit B yüzey antikoru (anti-HBs) durumu ve titresi, hepatit B yüzey antijeni (HbsAg) düzeyleri, ayrıca anti-hepatit A virüs (HAV) immünoglobulin (Ig) M ve anti- HAV IgG düzeyleri kaydedildi. Anti-HBs titresi ≥ 10 IU/L olan hastaların yeterli bağışıklığa sahip oldukları kabul edildi. Klinik rutinde Anti-HBs titresi <10 IU/L olan hastalara üç ek doz HBV aşısı uygulanmaktadır ve bu koşulu sağlayan hastaların verileri de ek olarak kaydedildi. Anti-HAV IgG titresinin sinyal-kesim (S/CO) değeri ≥ 1.0 olan hastalar pozitif olarak kabul edildi. Aynı şekilde klinik pratikte Anti-HAV IgG titresi negatif olan hastalara da iki ek doz HAV aşısı uygulanmaktadır, bu koşulu sağlayan hastaların verileri de not edildi. Romatoid faktör (RF) için cut-off değeri <14 IU/ml olarak kabul edildi.

Çalışmaya başlamadan önce etik kurul onayı alındı (Onay numarası ve tarihi:2022/279-17.10.2022).

İstatistiksel Yöntem

SPSS yazılımı kullanılarak veri tabanı oluşturuldu. Değişkenlerin normal dağılıma uygunluğu görsel (histogram ve olasılık grafikleri) ve analitik yöntemlerle (Kolmogorov- Smirnov/Shapiro-Wilks) incelendi. Tanımlayıcı analizlerde, normal dağılıma uymayan sayısal değişkenler için ortanca (minimum-maksimum) değer kullanıldı.

Bulgular

Çalışmada, 336 hasta dosyası geriye dönük olarak incelendi ve 190 hastanın hepatit serolojisi sonuçlarına ulaşıldı. Analiz, bu 190 hasta üzerinden yapıldı. Bu hastaların 98'i (%51,6) kız, 92'si (%48,4) erkekti. Hastaların ortanca semptom başlama yaşları, tanı yaşları ve şu anki yaşları sırasıyla 111 (6-217), 113 (6-218) ve 150 (9-232) aydı.

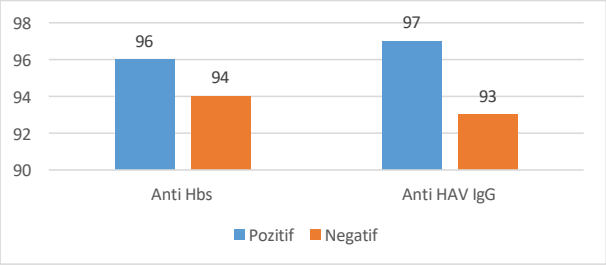
ILAR sınıflanmasına göre; hastaların 82'si (%43,2) oligoartiküler JİA, 64'ü (%33,7) entezit ilişkili artrit, 20'si (%10,5) RF negatif poliartiküler JİA, 11'i (%5,8) psoriatik artrit, 8'i (%4,2) sistemik JİA, 2'si (%1,1) RF pozitif poliartiküler JİA olarak sınıflamaktaydı, kalan 3 (%1,5) hasta ise sınıflanamayan JİA grubuna dahil edildi. Hastaların 154'ü (%81,1) DMARD, 89'u (%46,8) BA ile tedavi edilmekteydi.

Hastaların hepatit serolojileri incelendiğinde, 96 hastada (%50,5) Anti-HBs pozitif olarak bulundu. Hastaların

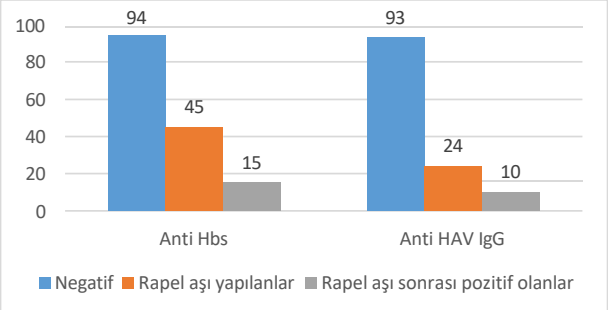
sadece birinde HBsAg pozitifti ve bu hastaya kronik hepatit B enfeksiyonu tanısı koyularak antiviral tedaviye başlandı. Doksan yedi (%51,1) hastada Anti-HAV IgG, pozitifti (Şekil 1). Elli yedi hastada ise hem anti-HBs hem de anti-HAV IgG pozitifti. Anti-HBs negatif olan 94 hastanın 45'i (%47,8) yeniden aşılandı. Rapel aşı olan hastaların 36'sı DMARD, 24'ü BA ile tedavi edilmekteydi. Aşılanan 45 hastadan 15'inde (%33,3) kontrolde Anti-HBs titresi 10'un üzerinde saptandı. Anti-HAV IgG negatif olan 93 hastadan 24'ü (%25,8) yeniden aşılandı ve bu hastaların 10'unda (%41,6) kontrolde Anti-HAV IgG pozitif bulundu (Şekil 2).

Hastaların 164'ünün 63'ünde (%33,2) ANA pozitif bulundu. Ayrıca, RF düzeyi ölçülen 156 hastanın 8'inde (%4,2) hastada RF pozitif olarak saptandı. ANA pozitif olan 31 hastanın anti-HBs değeri pozitifken, 35'inde ise anti-HAV IgG pozitifti. ANA negatif olan grup ile aralarında anlamlı bir fark bulunmadı (sırasıyla, p=0.816 ve p=0.129). RF pozitif hastaların beşinde Anti-HBs değeri pozitifken dördünde anti-HAV IgG pozitifti. RF negatif olan grup ile aralarında anlamlı bir fark bulunmadı (sırasıyla, p=0.583 ve p=0.778).

Anti-HBs titrelerinin ortancası 11 (0-1000) IU/L iken, yaş gruplarına göre değişkenlik göstermekteydi. Anti-HBs titrelerinin ortanca değeri beş yaş altındaki hastalarda 36,3 (0,5- 1000) IU/L, 5-10 yaş arası hastalarda 12,4 (0-1000) IU/L ve 10 yaş üzeri hastalarda 18,2 (0- 1000) IU/L idi (p<0,001).



Şekil 1. Hastaların tanı anındaki aşı yanıtları.



Şekil 2. Hastaların rapel doz aşılama sonrası aşı yanıtları.

Tartışma

Bu çalışmada JİA tanılı 190 hastanın hepatit serolojisi incelenmiş olup, hastaların %50,5'inde anti-HBs pozitifliği, %51,1'inde ise HAV IgG pozitifliği saptanmıştır. Anti-HBs negatif olan 94 hastanın %47,8'i yeniden aşılanmış ve bu grubun %33,3'ünde seropozitivite sağlanmıştır. Anti-HAV IgG negatif olan 93 hastanın 24'üne (%25,8) rapel aşı uygulanmış ve bunların 10'unda (%41,6) aşılama sonrası seropozitif yanıt elde edilmiştir.

Romatizmal hastalığı bulunan veya immünosüpresif tedavi gören çocuklarda tedavi öncesi aşı durumunun belirlenmesi ve buna göre aşılama yapılması, HBV gibi enfeksiyonları önlemek açısından kritik bir öneme sahiptir; çünkü bu enfeksiyonlar romatizma hastalarında, normal popülasyona göre daha ağır klinik bulguların gelişmesine ve romatizmal hastalıkların alevlenmesine neden olabilir. Bu nedenle, JİA'lı çocukların hepatit seropozitifliği durumlarının bilinmesi ve aşı yanıtı negatif olan hastaların yeniden aşılanması, potansiyel enfeksiyonlara karşı bağışıklık kazanmalarını sağlayarak yaşam kalitelerini artıracaktır. Böylece enfeksiyonların neden olduğu mortalite ve morbiditedeki azalmanın yanı sıra sağlık harcamalarında da azalma sağlanması beklenmektedir. Bu nedenle, romatizmal hastalığı olan bireylerde tedavi öncesi hepatit markerlarının taranmasını önerilmektedir.

Maritsi ve ark.[14] tarafından yapılan bir çalışmada, JİA'lı 89 çocuğun HBV'ye karşı bağışıklık durumu değerlendirilmiş ve HBV seropozitiflik oranı sağlıklı kontrollere kıyasla daha düşük bulunmuştur (%55'e karşı %92, p<0.001). Türkiye'de yapılan bir çalışmada, tedavi başlanmamış 262 JİA tanılı hasta ile 276 sağlıklı akranının hepatit B serolojileri karşılaştırılmıştır. JİA grubunda seropozitiflik oranı (%59,1), kontrol grubuna göre (%72,9) anlamlı şekilde düşük bulunmuştur (p=0,002). Ayrıca, hasta grubunda anti-HBs titresi 14 (0- 1000) IU/L, kontrol grubunda ise 43,3 (0-1000) IU/L olarak ölçülmüş ve bu fark istatistiksel olarak anlamlı saptanmıştır (p=0,01).[15] Bizim çalışmamızda, JİA tanılı 190 hastanın hepatit serolojisi incelenmiş ve Türkiye'de yapılan diğer bir çalışmaya benzer şekilde, bu hastaların %50,5'inde anti-HBs pozitif olarak bulunmuştur. Anti-HBs titresi ortancası 11 (0-1000) IU/L iken, beş yaş altındaki hastalarda anti-HBs titresi 36,3 (0,5-1000) IU/L, 5-10 yaş arası hastalarda 12,4 (0-1000) IU/L ve 10 yaş üzeri hastalarda ise 18,2 (0-1000) IU/L olarak tespit edilmiştir (p<0,001). Çakmak ve ark.[15] çalışmasında ise, beş yaş altındaki hastalarda anti-HBs titresi 122,5 (0,8-1000) IU/L, 5-10 yaş arası hastalarda 25,4 (0-1000) IU/L, 10 yaş üzerindeki hastalarda ise 33,6 (0-1000) IU/L olarak bulunmuştur.

HBV aşısının JİA hastalarında güvenli ve etkili olduğu gösterilmiştir.[16] Nerome ve ark.[17], aşısız 25 JİA hastasının 19'unda (%76) aşılama sonrası anti-HBV yanıtı geliştirdiğini rapor etmişlerdir. Bu hastaların 18'inin biyolojik tedavi aldığı ve anti-HBs titresinin biyolojik tedavi alan hastalarda tek başına DMARD tedavisi alan hastalardakine kıyasla daha düşük olduğu da gösterilmiştir. Çakmak ve ark.[15] 18 hastaya güçlendirici aşı uyguladıktan sonra, dört hasta (%22,2) seronegatif kalmıştır. Bizim çalışmamızda ise, anti-HBs negatif olan 45 hasta yeniden aşılanmış ve bu 45 hastadan 15'inde (%33,3) anti-HBs titresinin 10 IU/L'nin üzerine çıktığı saptanmıştır.

Maritsi ve ark.[18], JİA hastalarındaki hepatit A seropozitifliğine yönelik yaptıkları bir çalışmada, JİA tanılı 83 hasta ile 76 sağlıklı kişiden oluşan kontrol grubunu karşılaştırmış ve seroproteksiyon oranlarının, JİA'lı

çocuklarda sağlıklı çocuklara kıyasla anlamlı derecede daha düşük olduğunu belirlemişlerdir (%48,2'ye karşı %65; p=0,05). Ayrıca, anti-HAV-IgG titreleri, birinci, yedinci ve onsekinci aylarda JİA'lı çocuklarda sağlıklı kontrol grubuna kıyasla belirgin şekilde daha düşük bulunmuştur (p<0,001). Çalışmamızda anti-HAV IgG, Maritsi ve ark.'nın çalışmasına benzer şekilde hastalarda %51,1 pozitif bulunmuştur.

Pediatrik romatolojide daha iyi klinik sonuçlar elde etmek amacıyla hastaların erken ve yoğun bir şekilde tedavi edilmesi, giderek yaygınlaşan bir yaklaşım haline gelmiştir. Bu kapsamda, inflamasyonu hızlı bir şekilde kontrol altına almak için biyolojik ajanların kullanımı oldukça yaygınlaşmıştır; ancak biyolojik tedavi alan hastalarda HBV reaktivasyonu riskinin arttığına dair literatürde çeşitli raporlar bulunmaktadır.[6,8,15] Bu durum, hastaların aşı yanıtlarının değerlendirilmesi ve tanı sırasında eksik aşıların tamamlanması ya da rapel dozların uygulanmasının, olası enfeksiyonlardan korunmada etkili bir strateji olabileceğini ortaya koymaktadır.[19-25] Avrupa Romatizma Karşıtı Birliği'nin (EULAR), romatizmal hastalığı olan bireylerde aşılama ile ilgili önerileri, kronik romatizmal hastalığa sahip bireylerin aşılama durumunun ve ek aşı gereksinimlerinin romatoloji ekipleri tarafından yıllık olarak değerlendirilmesi gerektiğini vurgulamaktadır.[26]

Çalışmamızın en önemli kısıtlılığı, tek merkezli olması ve verilerin geriye dönük olarak toplanmasıydı. Ayrıca, çalışmaya dahil edilen tüm seronegatif hastalara güçlendirici aşı yapılmamış olması, diğer önemli bir kısıtlılık olarak öne çıkmaktadır. Çalışmamızın bir diğer önemli kısıtlılığı sağlıklı kontrol grubunun olmamasıydı. Doğu Anadolu bölgesinde 0-16 yaş arasındaki çocuklarda anti-HBs pozitiflik oranı %71,3 olarak bildirilirken, İstanbul'da 1-6 yaş grubundaki aşılanmış çocuklarda bu oran %89,3'e kadar yükselmektedir.[27,28] Bu oranlarla karşılaştırıldığında JİA hastalarının seropozitivite oranlarının normal popülasyona göre daha düşük olduğu gözlemlenmiştir.

Gelecekteki çalışmalar, pediatrik romatoloji alanında aşılama konusunda farkındalığı artırmak ve bilgi eksikliğini gidermek için uluslararası iş birlikleriyle daha kapsamlı ve iyi planlanmış şekilde yürütülmelidir.

Etik Standartlara Uygunluk

Çalışmaya başlamadan önce Kocaeli Üniversitesi Etik Kurulu'ndan onay alınmıştır (Onay numarası ve tarihi: 2022/279-17.10.2022).

Çıkar Çatışması

Yazarların konuyla ve/veya herhangi başka bir yazar ile ilgili maddi veya manevi bir çıkar çatışması yoktur.

Finansal Destek

Yoktur.

Yazar Katkısı

YEB, SÖ, AÖ, NŞ, HES: Çalışmanın tasarımı, veri toplanması ve analizi, kaynak taraması ve makale yazımı esnasında ortak çalışmıştır.

Bütün yazarlar yazının son halini okumuştur ve onay vermiştir.

Kaynaklar

1. Fink CW. Proposal for the development of classification criteria for idiopathic arthritides of childhood. *J Rheumatol*. 1995;22(8):1566-1569.
2. Demirkaya E, Ozen S, Bilginer Y, et al. The distribution of juvenile idiopathic arthritis in the eastern Mediterranean: results from the registry of the Turkish Paediatric Rheumatology Association. *Clin Exp Rheumatol*. 2011;29(1):111-116.
3. Ravelli A, Martini A. Juvenile idiopathic arthritis. *Lancet*. 2007;369(9563):767-778.
4. Petty RE, Southwood TR, Manners P, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol*. 2004;31(2):390-392.
5. McMahon BJ. Epidemiology and natural history of hepatitis B. *Semin Liver Dis*. 2005;25(1):3-8.
6. Baymakova MP, Karcheva M. Trends in the acute hepatitis B and acute hepatitis C in Bulgaria. *Folia Med*. 2019;61(2):197-203.
7. Igde FA, Taskin H, Igde M, Yazici Z, Atilla A. Where we are in the fight against hepatitis B infection; trends in hepatitis B virus seroprevalence in the Black Sea Region of Turkey. *Niger J Clin Pract*. 2018;21(1):87-92.
8. Acikgoz A, Cimrin D, Kizildag S, et al. Hepatitis A, B, and C seropositivity among first-year healthcare students in western Turkey: a seroprevalence study. *BMC Infect Dis*. 2020;20:529.
9. Derin O. Temporal dynamics of hepatitis B infection and relation of childhood vaccination program in Türkiye: a longitudinal study. *Infect Dis Clin Microbiol*. 2024;6:195-205.
10. Okay G, Biberci Keskin E, Akkoyunlu Y, et al. Evaluation of hepatitis B vaccine efficacy and factors affecting vaccine nonresponse in patients receiving anti-tumor necrosis factor agents. *Eur J Gastroenterol Hepatol*. 2020. doi:10.1097/MEG.0000000000001849.
11. Ozisik L, Tanriover MD, Calik Basaran N, Oz SG, Unal S. Missed opportunities for hepatitis B vaccination among diabetic patients. *Hum Vaccin Immunother*. 2015;11(11):2806-2810.
12. Haykir Solay A, Eser F. High-dose hepatitis B vaccine is not effective in patients using immunomodulatory drugs: a pilot study. *Hum Vaccin Immunother*. 2019;15(6):1177-1182.
13. Beukelman T, Xie F, Chen L, et al. Rates of hospitalized bacterial infection associated with juvenile idiopathic arthritis and its treatment. *Arthritis Rheum*. 2012;64(9):2773-2780.
14. Maritsi D, Vartzelis G, Soldatou A, et al. Markedly decreased antibody titers against hepatitis B in previously immunized children presenting with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2013;31(6):969-973.
15. Çakmak F, Çakan M, Demir F, et al. Hepatitis B vaccination response of treatment-naive patients with juvenile idiopathic arthritis. *Rheumatol Int*. 2022;42(7):1199-1205. doi:10.1007/s00296-021-04833-3.
16. Silva CA, Aikawa NE, Bonfa E. Vaccinations in juvenile chronic inflammatory diseases: an update. *Nat Rev Rheumatol*. 2013;9(9):532-543.
17. Nerome Y, Akaike H, Nonaka Y, et al. The safety and effectiveness of HBV vaccination in patients with juvenile

idiopathic arthritis controlled by treatment. *Mod Rheumatol*. 2016;26(3):368-371.
18. Maritsi DN, Coffin SE, Argyri I, et al. Immunogenicity and safety of the inactivated hepatitis A vaccine in children with juvenile idiopathic arthritis on methotrexate treatment: a matched case-control study. *Clin Exp Rheumatol*. 2017;35(4):711-715.
19. Aygun D, Sahin S, Adrovic A, et al. The frequency of infections in patients with juvenile idiopathic arthritis on biologic agents: 1-year prospective study. *Clin Rheumatol*. 2019;38(5):1025-1030.
20. Beukelman T, Xie F, Baddley JW, et al. Brief report: incidence of selected opportunistic infections among children with juvenile idiopathic arthritis. *Arthritis Rheum.* 2013;65(5):1384-1389.
21. Swart J, Giancane G, Horneff G, et al. Pharmacovigilance in juvenile idiopathic arthritis patients treated with biologic or synthetic drugs: combined data of more than 15,000 patients from Pharmachild and national registries. *Arthritis Res Ther*. 2018;20(1):285.
22. Leuvenink R, Aeschlimann F, Baer W, et al. Clinical course and therapeutic approach to varicella zoster virus infection in children with rheumatic autoimmune diseases under immunosuppression. *Pediatr Rheumatol*. 2016;14:34.
23. Bizjak M, Blazina S, Zajc Avramovic M, et al. Vaccination coverage in children with rheumatic diseases. *Clin Exp Rheumatol*. 2020;38(2):164-170.
24. Minden K, Horneff G, Niewerth M, et al. Time of disease-modifying antirheumatic drug start in juvenile idiopathic arthritis and the likelihood of a drug-free remission in young adulthood. *Arthritis Care Res (Hoboken).* 2019;71(4):471-481.
25. Carroll MB. The impact of biologic response modifiers on hepatitis B virus infection. *Expert Opin Biol Ther.* 2011;11(4):533-544.
26. Furer V, Rondaan C, Heijstek MW, et al. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis.* 2020;79(1):39-52.
27. Erbey F, Acar MN, Güven A, Kaya A, Okur M. Van ili ve çevresinde 0-18 yaşları arasındaki çocuklarda hepatit a seropozitifliği. *Duzce Med J*. 2011;13:6-9.
28. Topal E, Hatipoğlu N, Türel Ö, Aydoğmuş Ç, Hatipoğlu H, Erkal S. İstanbul ilinde 1-6 yaş arası çocuklarda hepatit B seroprevalansı, aşı yaptırma ve seroproteksiyon oranı. *Turkiye Klinikleri J Pediatr*. 2011;20(3).

doi: 10.53446/actamednicomedia.1640000

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

CETUXIMAB-RELATED SKIN TOXICITY AS A PREDICTIVE MARKER FOR TREATMENT RESPONSE AND PROGNOSIS IN RECURRENT/METASTATIC HEAD AND NECK CANCER PATIENTS TREATED WITH CETUXIMAB AND CHEMOTHERAPY COMBINATION

SETÜKSİMAB VE EŞZAMANLI KEMOTERAPİ İLE TEDAVİ EDİLEN REKÜRREN/METASTATİK BAŞ VE BOYUN KANSERLİ HASTALARDA TEDAVİ YANITI VE PROGNOZ İÇİN PREDİKTİF BİR BELİRTEÇ OLARAK SETÜKSİMAB İLİŞKİLİ CİLT TOKSİSİTESİ

Ilkay Citakkul[1*], Kazim Uygun[1], Yasemin Bakkal Temi[1], Ercan Ozden[2], Umut Kefeli[1], Devrim Cabuk[1], Elif Sahin[3]

[1]Kocaeli University, Faculty of Medicine, Department of Internal Medicine and Medical Oncology, Kocaeli, Türkiye. [2]Pendik Medical Park, Department of Internal Medicine and Medical Oncology, Istanbul, Türkiye. [3]Kocaeli City Hospital, Department of Internal Medicine and Medical Oncology, Kocaeli, Türkiye.

ABSTRACT

Objective: Cetuximab (Cmab),an EGFR inhibitor, is commonly associated with skin toxicity in the treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). We aim if skin toxicity can be used as a prognostic sign for Cmab therapy in patients with R/M SCCHN.

Methods: A retrospective review was conducted on demographic data, prognostic features, treatment responses, Cmab-related skin toxicity, and dates of diagnosis, treatment initiation, disease progression, and death for r/mSCCHN patients treated with Cmab at Kocaeli University Medical Oncology Department between 2010 and 2019.The significance of the results has been evaluated by using SPSS (20.0 SPSS Inc., Chicago, IL, USA.) statistical program.

Results: A total of 77 patients were enrolled.A significant association was found between Cmab-related skin toxicity and longer survival in patients with R/M SCCHN. Patients with grade 3 skin toxicity demonstrated prolonged overall survival (OS) and markedly improved progression-free survival (PFS) compared to those without skin toxicity.Additionally, compared to patients without skin toxicity, those with grade 1 or grade 2 skin toxicity had a noticeably prolonged PFS.No significant OS difference was observed between patients with grade 1 or grade 2 toxicity and those without skin toxicity.

Conclusion: Grade 3 skin toxicity correlates with enhanced prognosis, resulting in prolonged OS and PFS. Grade 1 and Grade 2 skin toxicity are associated with improved progression-free survival relative to the absence of toxicity. The data indicate that preventive measures for managing Cmab-related skin toxicity, particularly grade 2 and grade 3, may improve patient outcomes.

Keywords: Cetuximab, head and neck cancer, skin toxicity

ÖZ

Amaç: Rekürren veya metastatik baş ve boyun kanserinde(r/mSCCHN) Setuximab (Cmab) ile ilişkili cilt toksisitesi, tedavide sık görülen bir yan etkidir. Cilt toksisitesinin prognostik bir ölçüt olarak kullanılıp kullanılamayacağını değerlendirmek istedik.

Yöntem: 2010-2019 yılları arasında Kocaeli Üniversitesi Tıbbi Onkoloji Bölümü'nde Cmab ile tedavi edilen r/mSCCHN hastalarının demografik verileri, prognostik özellikleri, tedavi yanıtları, Cmab ile ilişkili cilt toksisitesi, tanı zamanı, tedavi başlama zamanı, progresyon ve ölüm tarihleri retrospektif olarak incelenmiş ve sonuçların anlamlılığı SPSS (20.0 SPSS Inc., Chicago, IL, USA.) istatistik programı kullanılarak değerlendirilmiştir.

Bulgular: Toplam 77 hasta çalışmaya dahil edilmiştir.R/M SCCHN hastalarında Cmab ile ilişkili cilt toksisitesi ile daha uzun sağkalım arasında anlamlı bir ilişki bulunmuştur. Cilt toksisitesi olmayan hastalarda, grad 1 ve grad 2 cilt toksisitesi olanlara kıyasla daha kısa progresyonsuz sağkalım (PFS) görülmüştür. Özellikle, grad 3 cilt toksisitesi olan hastalar, cilt toksisitesi olmayanların yanı sıra grad 1 veya grad 2 toksisitesi olanlara göre daha uzun genel sağkalım (OS) ve daha iyi PFS sergilemiştir. Grad 1 veya grad 2 toksisitesi olan hastalar ile cilt toksisitesi olmayan hastalar arasında anlamlı bir OS farkı gözlenmemiştir.

Sonuç: Özellikle grad 3 cilt toksisitesi , daha uzun OS ve PFS ile yani daha iyi prognoz ile ilişkilidir. Grad 1 ve grad 2 cilt toksisitesi, cilt toksisitesi olmayanlara kıyasla daha iyi PFS ile bağlantılıdır. Bu bulgular, Cmab ile ilişkili cilt toksisitesini, özellikle de grad 2 ve grad 3 cilt toksisitesini yönetmeye yönelik önleyici stratejilerin hasta sonuçlarını iyileştirebileceğini göstermektedir.

Anahtar Kelimeler: Setüksimab, cilt toksisitesi, baş boyun kanseri

***Corresponding author/İletişim kurulacak yazar:** Ilkay Citakkul; Kocaeli University, Faculty of Medicine, Umuttepe Campus, 41001, İzmit/Kocaeli, Türkiye.
Phone/Telefon: +90 (262) 303 75 75, **e-mail/e-posta:** citakkulilkay@gmail.com
Submitted/Başvuru: 14.02.2025 • **Accepted/Kabul:** 08.05.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Squamous cell carcinomas (SCC), which constitute the majority of head and neck cancers, originate from various areas such as the oral cavity, oropharynx, hypopharynx, larynx, and nasopharynx.[1] The incidence of recurrent or metastatic SCC of the head and neck (R/M SCCHN) varies widely across different geographical areas, reflecting regional differences in risk factor exposure. Worldwide, these cancers contribute to over 400,000 deaths annually, with nearly 900,000 new cases diagnosed each year.[2]

Cetuximab, an inhibitor of the Epidermal Growth Factor Receptor (EGFR), has become a key therapeutic option in the treatment of R/M SCCHN. EGFR plays a significant role in promoting cellular growth and maintaining the skin's balance, making cetuximab a critical drug for managing these malignancies.

In treating R/M SCCHN, clinical trials have shown that combination chemotherapy regimens are more effective than single-agent therapies, leading to better survival outcomes. Consequently, combination treatments are the preferred first-line therapy for this patient group.[3-6]

The results of the Phase III EXTREME trial demonstrated that adding cetuximab to a platinum-based chemotherapy regimen (platinum/5-FU) significantly improved both overall survival (OS) and progression-free survival (PFS) when compared to chemotherapy alone, establishing this combination as the standard treatment approach for R/M SCCHN.[7,8]

Despite cetuximab's proven efficacy in R/M SCCHN, most research has focused on its effects in metastatic colorectal cancer, and there is a lack of studies exploring how cetuximab-induced skin toxicity might correlate with prognosis in patients with head and neck cancerThis study seeks to explore the possible link between cetuximab-induced skin toxicity and clinical outcomes, including OS and PFS, in patients with R/M SCCHN at our clinic.

Methods

This retrospective study included patients with R/M SCCHN treated between January 2010 and October 2019, selected from the Oncology Clinic archive at Kocaeli University Faculty of Medicine. Patients who were diagnosed at our hospital but continued treatment at other centers or had inaccessible medical records were excluded.

We analyzed patient data collected from hospital records and from the information system, specifically focusing on those who received Cetuximab, when administered in conjunction with chemotherapy (5-FU/cisplatin or carboplatin/Cmab). The study examinedcomprehensive patient characteristics, including demographics, primary tumor sites, and treatment details.

We also examined the occurrence of skin toxicity associated with cetuximab and the relationship between the severity of skin toxicity and both overall survival (OS) and progression-free survival (PFS). The tumor's site of origin, the initial treatment provided, and the radiological response to the first-line treatment (complete, partial, or stable response) were also assessed.

Inclusion criteria for this study were: (1) a confirmed diagnosis of R/M SCCHN; (2) treatment with cetuximab in combination with chemotherapy at Kocaeli University Faculty of Medicine; and (3) complete availability of clinical and treatment data. Patients were excluded if they: (1) discontinued treatment at our center; (2) had incomplete or inaccessible medical records; or (3) were lost to follow-up before receiving cetuximab treatment.

Skin toxicity was evaluated by both dermatologists and oncologists at Kocaeli University Faculty of Medicine Hospital, using the NCI-CTCAE (Common Toxicity Criteria for Adverse Events, version 4.0) criteria.[9]

Statistical Analysis

The study assessed the correlation between the severity of skin toxicity and extended OS and PFS, without incorporating time-specific criteria. Statistical significance was determined using the SPSS (20.0). Normal distribution was verified through the Kolmogorov-Smirnov and Shapiro-Wilk tests. Numerical data were expressed as mean ± standard deviation, and categorical data were presented as frequency (percentage). Independent sample t-tests were used for group comparisons, while Chi-square analysis assessed categorical variable relationships. Survival analysis was conducted using the log-rank test and the Kaplan-Meier method. A p-value of <0.05 was considered statistically significant.

Ethics Approval

This study received ethical approval from the Institutional Review Board of Kocaeli University (Approval Code: KOÜ GOKAEK-2019/16/09, Project Identifier: 2019/269). All procedures were conducted in compliance with the principles outlined in the Declaration of Helsinki.

Results

This investigation comprised 77 patients, with a median age of 62 years (range: 53-67). Of the patients who experienced recurrence following the initial treatment, 65 (84.4%) presented with metastatic disease, while 12 (15.6%) exhibited locally advanced cancer. Among the metastatic cases, 47 (61%) demonstrated lung metastasis, six (7.8%) exhibited liver metastasis, 15 (19.5%) presented with bone metastasis, and 33 (42.9%) showed mediastinal lymph node metastasis. Patient demographics, including sex, tumor location, disease stage at diagnosis, and initial treatment, were all considered (Table 1).

The distribution of skin toxicity severity was as follows: 32 patients (41.6%) developed grade 3 toxicity, 11 (11.4%) developed grade 2, 11 (11.4%) developed grade 1, and 23 patients (29.9%) exhibited no skin toxicity. In

the progression-free survival analysis, patients with grade 3 skin toxicity had markedly improved survival compared to those without any skin toxicity (log-rank test, P < 0.001), as well as compared to those with grade 1 toxicity (P = 0.003) or grade 2 toxicity (P = 0.001).

Table 1. Patient Characteristics

Patient Characteristics	Number (Number Of Person)	Percentage
Sex		
Male	63	81.8%
Female	14	18.2%
Primary Site		
Oral Cavity	28	36.4%
Nasopharynx	3	3.9%
Oropharynx	2	2.6%
Hypopharynx	9	11.7%
Larenyx	27	35.1%
Sinus	4	5.2%
External Auditory Canal	1	1.3%
Parotid Gland	1	1.3%
Mandibular	1	1.3%
Primary Site Unknown	1	1.3%
At The Time Of Diagnosis		
Local	47	61%
Local Advanced	20	26%
Metastatic	10	13%
The First Treatment Patients Received		
Surgery And Adjuvan RT	38	49.4%
Chemoradiation	20	26%
De Novo Metastatic	10	13%
Surgery	2	9.1%
RT	7	2.6%
Treatment Regimen		
5FU+Cis+Cmab	65	84.4%
5FU+Carbo+Cmab	12	15.6%
Total	77	100%

Both grade 1 and grade 2 skin toxicity were linked to considerably improved PFS when compared to patients who did not exhibit skin toxicity. (Figure 1).

When analyzing the impact of skin toxicity on overall survival, it was clear that patients with grade 3 skin toxicity had significantly longer survival compared to those with no skin issues (hazard ratio, 0.36; 95% CI, 0.19 to 0.66, P < 0.001). In contrast, there was no significant difference in OS between patients with grade 1 or grade 2 skin toxicity and those with no skin reactions. (Figure 2)

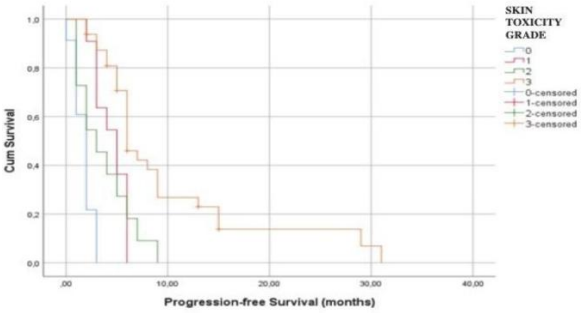


Figure 1. Kaplan-Meier survival curve for progression-free survival in relation to cutaneous toxicity.

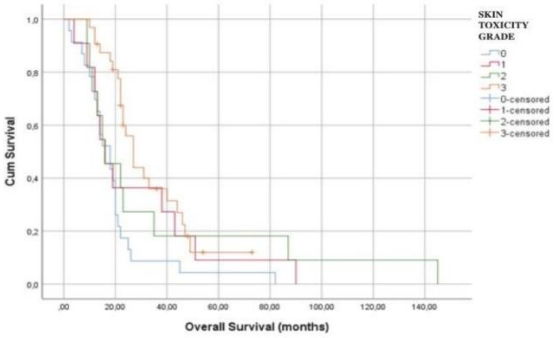


Figure 2. Kaplan-Meier survival curve for overall survival in relation to cutaneous toxicity.

Upon retrospective examination, marked differences in both OS and PFS were detected between patients who developed severe skin reactions (grade 3 toxicity) and those who did not experience any skin complications. Additionally, those with grade 1 or grade 2 skin toxicity had better progression-free survival rates than those who remained free of skin toxicity.

In addition to skin toxicity, several other factors have been evaluated for their potential impact on the prognosis. The analysis revealed that patients with lung metastasis exhibited a mortality risk 2.5 times higher than those without lung involvement (P = 0.18). Age and sex were not significantly associated with mortality (P = 0.73). Furthermore, cachexia was observed to increase the mortality risk by 2.5 times, altough this result was not statistically significant (P = 0.2).

Discussion

Cetuximab plays a significant role in the treatment of R/M SCCHN. Our investigation demonstrated that skin toxicities in cetuximab-treated R/M SCCHN patients may serve as a prognostic indicator of patient survival outcomes. Patients who developed grade 3 skin toxicity exhibited significantly improved overall survival (hazard ratio, 0.36; 95% CI, 0.19-0.66; P < 0.001) and progression-free survival compared to individuals without skin toxicity (log-rank test, P < 0.001), grade 1 (P = 0.003), or grade 2 toxicity (P = 0.001). A study conducted in Japan with 105 patients observed that grade 3 skin toxicity, which developed within 90 days of cetuximab administration, was associated with enhanced survival outcomes[10]. Our findings are consistent with this observation, indicating

that patients who experienced grade 3 skin toxicity demonstrated improved OS and PFS. Although the distribution of skin toxicity was relatively similar in both studies, our cohort had a slightly higher incidence of grade 3 toxicity. These results suggest that skin reactions are not only common treatment-related adverse effects but may also serve as prognostic indicators associated with improved clinical outcomes.

While cetuximab-induced skin toxicity has been extensively investigated in the context of metastatic colorectal cancer, there remains a dearth of data examining its prognostic implications in patients with R/M SCCHN. By demonstrating a correlation between grade 3 skin toxicity and enhanced OS and PFS, our findings suggest that cetuximab-induced dermatologic reactions may reflect not only treatment efficacy, but also serve as a clinically relevant indicator of favorable prognosis in R/M SCCHN.

Cetuximab is generally well tolerated; but cutaneous eruptions, predominantly observed on the facial region, cervical area, scalp, and superior dorsal surface, remain among the most prevalent adverse events. Acneiform rash was the most frequently documented cutaneous reaction in a Japanese study, manifesting in 87% of subjects[10]. Given the established correlation between cutaneous reactions and improved survival outcomes, it is imperative not to prematurely discontinue cetuximab administration in the presence of these adverse effects unless the reactions are of severe intensity. Efforts are underway to develop methods for preventing or minimizing these dermatologic reactions to avoid interrupting treatment. These approaches include recommending mild, hypoallergenic skincare products, using emollients, and protecting patients from sun exposure with high-SPF sunscreen.[11] Nutritional status also plays a key role in managing the side effects of cetuximab, as patients with inadequate nutrition may be more susceptible to complications.[12,13]

Despite advancements in immunotherapy anticipated to confer future survival benefits, current evidence comparing cetuximab-based regimens with immunotherapy for recurrent or metastatic head and neck cancers has yet to demonstrate a clear survival advantage. Cetuximab remains fundamental in the treatment of head and neck tumors owing to its established efficacy and tolerability. Severe cutaneous toxicity associated with cetuximab is a common adverse event and predictive marker for treatment response, providing valuable insights into patient prognosis. Given the prognostic value of skin toxicity in patients receiving cetuximab, earlier consideration of personalized and novel treatment modalities is warranted.

Although our study is constrained by its limited sample size and single-center design, the results imply that cetuximab-induced skin toxicity could serve as a potential indicator of a favorable prognosis in patients with R/M SCCHN. Further multicenter studies involving a larger number of patients are necessary to confirm these findings and investigate their potential clinical significance.

In conclusion, this study provides compelling evidence that cetuximab-induced skin toxicity, particularly grade 3, may serve as a valuable prognostic indicator for patients with R/M SCCHN. The findings demonstrated a positive correlation between severe skin toxicity and improved OS and PFS outcomes. These results are consistent with previous research and underscore the potential dual role of cutaneous reactions as treatment-related adverse effects and indicators of therapeutic efficacy.

Compliance with Ethical Standards

The Institutional Review Board of Kocaeli University granted ethical permission for this investigation (Approval Code: KOÜ GOKAEK-2019, Project Identifier: 2019/269). All procedures were conducted in compliance with the principles outlined in the Declaration of Helsinki.

Conflict of Interest

In this study, there is no conflict of interest with any individual or institution.

Author Contributions

All the authors equally contributed to this work.

Financial Disclosure

None.

References

1. Marur S, Forastiere AA. Head and Neck Cancer: Changing Epidemiology, Diagnosis, and Treatment. *Mayo Clin Proc*. 2008;83(4):489-501. doi:10.4065/83.4.489
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5). doi:10.1002/ijc.29210
3. Clavel M, Vermorken JB, Cognetti F, et al. Randomized comparison of cisplatin, methotrexate, bleomycin and vincristine (CABO) versus cisplatin and 5-fluorouracil (CF) versus cisplatin (C) in recurrent or metastatic squamous cell carcinoma of the head and neck. A phase III study of the EORTC Head and Neck Cancer Cooperative Group. *Ann Oncol*. 1994;5(6):521-526. doi:10.1093/OXFORDJOURNALS.ANNONC.A058906
4. Gibson MK, Li Y, Murphy B, et al. Randomized phase III evaluation of cisplatin plus fluorouracil versus cisplatin plus paclitaxel in advanced head and neck cancer (E1395): an intergroup trial of the Eastern Cooperative Oncology Group. *J Clin Oncol*. 2005;23(15):3562-3567. doi:10.1200/JCO.2005.01.057
5. Jacobs C, Lyman G, Velez-García E, et al. A phase III randomized study comparing cisplatin and fluorouracil as single agents and in combination for advanced squamous cell carcinoma of the head and neck. *J Clin Oncol*. 1992;10(2):257-263. doi:10.1200/JCO.1992.10.2.257
6. Forastiere AA, Metch B, Schuller DE, et al. Randomized comparison of cisplatin plus fluorouracil and carboplatin plus fluorouracil versus methotrexate in advanced squamous-cell carcinoma of the head and neck: a Southwest Oncology Group study. *J Clin Oncol*. 1992;10(8):1245-1251. doi:10.1200/JCO.1992.10.8.1245
7. Guigay J, Aupérin A, Fayette J, et al. Cetuximab, docetaxel, and cisplatin versus platinum, fluorouracil, and cetuximab

as first-line treatment in patients with recurrent or metastatic head and neck squamous-cell carcinoma (GORTEC 2014-01 TPExtreme): a multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol*. 2021;22(4):463-475. doi:10.1016/S1470-2045(20)30755-5

8. Vermorken JB, Mesia R, Rivera F, et al. Platinum-Based Chemotherapy plus Cetuximab in Head and Neck Cancer. *New England Journal of Medicine*. 2008;359(11):1116-1127. doi:10.1056/NEJMOA0802656/ASSET/A666E058-0210-4F26-B697-A012FB7B4135/ASSETS/IMAGES/LARGE/NEJMOA0802656_T3.JPG
9. Common Terminology Criteria for Adverse Events (CTCAE) | Protocol Development | CTEP. Accessed January 6, 2025. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_40
10. Uozumi S, Enokida T, Suzuki S, et al. Predictive Value of Cetuximab-Induced Skin Toxicity in Recurrent or Metastatic Squamous Cell Carcinoma of the Head and NECK. *Front Oncol*. 2018;8. doi:10.3389/fonc.2018.00616
11. Pinto C, Barone CA, Girolomoni G, et al. Management of Skin Toxicity Associated with Cetuximab Treatment in Combination with Chemotherapy or Radiotherapy. *Oncologist*. 2011;16(2):228-238. doi:10.1634/theoncologist.2010-0298
12. Salas S, Baumstarck-Barrau K, Alfonsi M, et al. Impact of the prophylactic gastrostomy for unresectable squamous cell head and neck carcinomas treated with radio-chemotherapy on quality of life: Prospective randomized trial. *Radiother Oncol*. 2009;93(3):503-509. doi:10.1016/J.RADONC.2009.05.016
13. Lee JH, Machtay M, Unger LD, et al. Prophylactic gastrostomy tubes in patients undergoing intensive irradiation for cancer of the head and neck. *Arch Otolaryngol Head Neck Surg*. 1998;124(8):871-875. doi:10.1001/ARCHOTOL.124.8.871

doi: 10.53446/actamednicomedia.1644183

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

HYPEROSTOSIS FRONTALIS INTERNA AND ITS CLINICAL SIGNIFICANCE

HIPEROSTOZIS FRONTALIS INTERNA VE KLİNİK ÖNEMİ

Hurriyet Cetinok[1*]

[1]Istanbul Atlas University Faculty of Medicine, Department of Anatomy, Istanbul, Türkiye.

ABSTRACT

Objective: The metabolic, endocrinological, neurological, and psychological causes of heterotopic ossification in the frontal bone have become increasingly important. Overgrowth of the frontal bone, called hyperostosis frontalis interna (HFI), can cause headaches and, rarely, seizures. HFI is nine times more common in women and is called Morgagni-Stewart-Morel syndrome when it occurs with virilization, obesity, and neuropsychiatric problems. Long-term estrogen exposure, advanced age, female gender, testosterone suppression removal, male-type hypogonadism, genetics, environmental factors, obesity, diet, Diabetes mellitus, some metabolic diseases, autoimmunity (ANA+), endocrine imbalance, and LEPTIN cause HFI. About 20% of HFI patients experience headaches, obesity, vertigo/dizziness, cognitive decline, and depression.

Methods: Our study was conducted over four years, from 2016 to 2019, at the Anatomy Department of Albert Einstein College of Medicine, New York, USA. We utilized formalin- fixed course cadavers from the department to assess heterotopic ossification. The cadavers exhibiting HFI+ were particularly recognized. The gender and age of the cases were considered. 74 donors, ranging in age from 42 to 103, were assessed.

Results: The study indicates that the frequency of HFI is 41.89%, with a prevalence of 9.45% among men in the population. This represents 22.58% of all HFI cases. The incidence among women is recorded at 32.43% within the population, representing 77.42% of total HFI cases.

Conclusion: Our study sample had 9.45% male HFI, which is remarkable. Although estrogen has been the main driver in HFI etiopathogenesis, the reported rate in males will illuminate fresh research and conclusions, allowing a full study of alternative variables.

Keywords: Hyperostosis frontalis interna, acromegaly, postmenopausal women, calvarial growth, heterotopic ossification

ÖZ

Amaç: Frontal kemikteki heterotopik ossifikasyonun metabolik, endokrinolojik, nörolojik ve psikolojik nedenleri giderek önem kazanmaktadır. Hiperostozis frontalis interna (HFI) adı verilen ön kemiğin aşırı büyümesi baş ağrısına ve nadiren nöbetlere neden olabilir. HFI kadınlarda dokuz kat daha sık görülür ve virilizasyon, obezite ve nöropsikiyatrik problemlerle ortaya çıktığında Morgagni-Stewart-Morel sendromu olarak adlandırılır. Uzun süreli östrojen maruziyeti, ileri yaş, kadın cinsiyet, testosteron baskılanmasının ortadan kalkması, erkek tipi hipogonadizm, genetik, çevresel faktörler, obezite, diyet, Diabetes Mellitus, bazı metabolik hastalıklar, otoimmünite (ANA+), endokrin dengesizliği ve LEPTIN HFI'ye neden olur. HFI hastalarının yaklaşık %20'sinde baş ağrısı, obezite, vertigo/baş dönmesi, bilişsel gerileme ve depresyon görülür.

Yöntem: Çalışmamız ABD'nin New-York Albert Einstein College of Medicine'de, Anatomi Laboratuvarında 2016-2019 yılları arasında dört yıl boyunca gerçekleştirildi. Heterotopik ossifikasyonu değerlendirmek için bölümden alınan formalinle sabitlenmiş kadavralardan yararlandık. HFI+ sergileyen kadavralar özellikle tanındı. Olguların cinsiyeti ve yaşı dikkate alındı. Yaşları 42 ila 103 arasında değişen 74 donör değerlendirildi.

Bulgular: Çalışma, HFI sıklığının %41,89 olduğunu, toplumdaki erkeklerde görülme sıklığının ise %9,45 olduğunu göstermektedir. Bu, tüm HFI vakalarının %22,58'ini temsil etmektedir. Kadınlar arasındaki insidans, popülasyonda %32,43 olarak kaydedilmiş olup, toplam HFI vakalarının %77,42'sini temsil etmektedir.

Sonuç: Bulgular, HFI araştırması ve etiyolojisinde östrojen maruziyetinin diğer faktörlerle birlikte dikkate alınması gerektiğini göstermektedir. Genetik veya epigenetik faktörler bazı kliniklerde daha sık görülen HFI'yi tetikleyebilir. Bu korelasyonu doğrulamak için daha büyük bir popülasyon çalışmasına ihtiyaç vardır.

Anahtar Kelimeler: Hiperostozis frontalis interna, akromegali, postmenopozal kadın, calvarial büyüme, heterotopik ossifikasyon

***Corresponding author/İletişim kurulacak yazar:** Hurriyet Cetinok; Istanbul Atlas University, Faculty of Medicine, Department of Anatomy, Anadolu Street No: 40 Kagithane/Istanbul, Türkiye.
Phone/Telefon: +90 (532) 641 18 98, **e-mail/e-posta:** hurriyet.cetinok@atlas.edu.tr
Submitted/Başvuru: 25.02.2025 • **Accepted/Kabul:** 18.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Heterotopic ossification in the calvaria, while present in other cranial bones, is particularly prevalent in the frontal bone and has garnered significance in recent years regarding its metabolic, endocrinological, neurological, and psychiatric etiopathogenesis. Hyperostosis Frontalis Interna (HFI) refers to anomalies in the inner surface of the calvaria, characterized by widespread, nodular development and thickening of the bone tissue from the lamina interna of the frontal bone to the cranial cavity. This action transpires within the spongy bone tissue. The frontal bone is intact in the midline and is distinctly bounded by the middle meningeal artery. In radiological imaging, "butterfly-like density" is characteristic. Initially delineated by Morgagni in 1719[1], HFI was thoroughly analyzed and categorized by Moore in 1955[2], and further subtyped in 1999 by Herskovitz et al.[3] For classification, it is essential to ascertain whether it pertains to morphology, distribution, localization, size, or adjacent structures of the frontal bone. Estrogen, parathormone, calcium ATPase, and neuropeptides contribute to the pathophysiology of HFI. The majority of instances involve postmenopausal women. Chronic exposure to estrogen, advanced age, female sex, cessation of testosterone's suppressive effects, male-type hypogonadism, genetic predisposition, environmental influences, obesity, dietary factors, diabetes mellitus, certain metabolic disorders, autoimmunity (ANA+), endocrine dysregulation, and leptin are implicated in the etiology. Klippel-Trenaunay-Weber Syndrome, Frolich Syndrome, Morgagni Syndrome, Stewart-Moral Syndrome, Troell-Junet Syndrome, and Morgagni-Stewart-Morel Syndrome have all been linked to HFI. Frontal pain, psychoneuroses, obesity, Parkinsonism, depression, frontal cortex managerial dysfunctions, epilepsy, and hypertrichosis may accompany HFI. Using osteoarchaeological evidence and unintentional cadaver autopsy images, HFI can be diagnosed. In the differential diagnosis, it is crucial to consider localized malignancies (endosteal osteoma, osteosarcoma), Paget's Disease, fibrous dysplasia, Leontiasis ossea, pregnancy osteophytes, and metabolic craniopathy.

Moore comprehensively analyzed roentgenograms to identify four distinct forms of hyperostosis cranii.[4-6] HFI was utilized solely for description. Instances where only the frontal bone is involved; nebula frontalis (NF) refers to a consistent, uniform thickening of the frontal bone; hyperostosis calvaria diffusa (HCD) denotes a condition affecting all flat bones of the calvaria. Perou expanded the existing terminology to incorporate hyperostosis cranii interna (HCI) for cases of HFI where remodeling extends beyond the frontal bone to include the parietal, temporal, or sphenoid bones (Perou, 1964)[7]. Therefore, the term HCI encompasses all instances of endostosis, irrespective of their location and extent.

A further classification has been developed by Herskovitz et al.[3] Observation or extension of hyperostosis in bones other than the frontal bone, classification based on; morphology, form, type of border, location of lesion;

*Type A: Singular or multiple isolated osseous elevations of less than 10 mm in diameter situated on the endocranial surface of the frontal bone.

*Type B: Nodular osseous formations that comprise less than 25% of the frontal bone.

*Type C: Nodular osseous formations include up to 50% of the frontal bone.

*Type D: Nodular osseous formations situated on the endocranial surface of the frontal bone, exceeding 50% and exhibiting continuity.

*Type E: Severe hyperostosis frontalis interna characterized by soft tissue proliferation and expansion.

Hyperostosis frontalis interna (HFI) develops when the frontal bone grows abnormally behind its internal table. According to She et al., 5%-12% of people could develop HFI.[8] Clinicians consider the pathology benign mostly because a significant number of patients are asymptomatic. When compared to the general population of the same age, those with HFI who do experience symptoms most often report headaches and depression.[9]

This condition should be seen as a metabolic bone disease because it is associated with HFI, diabetes, hirsutism, and acromegaly. Dementia and seizures are two other disorders that are not as strongly linked to HFI. Hormones seem to be the likely culprit, according to the research. For example, according to Murphy et al. (2018), the primary population of HFI consists of elderly, obese, diabetic, hyperandrogenic, or nulliparous postmenopausal women.[9] According to Morita et al. (2021), one theory is that estrogen dysregulation can promote bone formation by stabilizing the networks of meningeal microvasculature.[10] Despite Hershkovitz's first classification scheme for HFI in cadavers, neither the living population nor cadaveric case reports consistently use it. [11] Patients coping with HFI do not have access to a similarly reliable classification scheme. Furthermore, historical records show that HFI has been a problem for humans for a long time.[12]

The pathogenesis of HFI remains little elucidated. The 'global model' of HFI, presented by Hershkovitz et al., identifies vascularization from the dura as a crucial element in the pathophysiology of HFI.[3] Talarico et al. found that in the woman with HFI, the inner table displayed significant remodeling, comprised predominantly of big sinuses, and extended to the external periosteal layer of the dura.[1]

Larger pores may arise from the infiltration of blood vessels from the dura, ultimately resulting in the diploidization of the inner table.[13]

Estrogen receptors are predominantly situated on the vascular tissue of the dura, and estrogen is recognized for its significant influence on meningeal vascularity.[14] Angiogenesis stimulated by estrogen has been extensively studied.[15,16] Estrogen is proposed to trigger the hypoxia-inducible factor-a (HIFa) signaling pathway, resulting in the activation of proangiogenic genes, chiefly vascular endothelial growth factor (VEGF).[15,17] Estrogen can thereby promote angiogenesis. Numerous studies

indicate that bone turnover is associated with bone vasculature.[18,19,20]

Methods

Our study was conducted for 4 years between the years of 2016 to 2019 at Albert Einstein College of Medicine, Anatomy Department, New York – U.S.A., utilizing cadavers of the department, which were examined and evaluated for heterotopic ossification. This study examined heterotopic ossifications in the calvaria of 74 formalin-embalmed cadavers (44 males, 30 females, aged 42-103) donated to the Department of Anatomy at Albert Einstein College of Medicine (AECOM). All donors permitted for donation and utilization in clinical trials were accepted in accordance with New York's Anatomical Gift Law. This study does not require ethical approval as it utilizes course cadavers from the Albert Einstein College of Medicine C&DA Department, in accordance with the exemption categories outlined in Einstein-IRB-citation104(d).

Excision of Calvaria and Cerebrum

The scalp was retracted from the cranium while in the supine posture. The temporalis fascia was severed. The temporalis muscle was reflected inferiorly, and the bones were meticulously cleaned. The external lamina and diploë were circumferentially incised with a Stryker saw along a line commencing 1.5 cm above the supraorbital edge and extending to 2 cm superior to the external occipital protuberance. Meticulous precautions were implemented to avoid harming the underlying dura mater and brain. A Verchow Skull Breaker and a chisel were employed to penetrate the endocranium. The calvaria was excised by carefully separating it from the dura mater through blunt dissection. The cadaver was thereafter positioned prone, and the tentorium cerebelli was incised bilaterally, extending the incision posteriorly to the superior border of the petrous bone. The spinal cord, along with the vertebral arteries and cranial nerves IV, V, VII to XI, as well as the hypoglossal nerve bundle, were severed. Subsequently, while in the supine posture, gentle pressure was exerted on the frontal poles of the cerebral hemispheres, the falx cerebri was incised near the crista galli, and the brain was retracted superiorly and posteriorly, displacing the olfactory bulbs and tracts from the cribriform plates. The brain was elevated, and the infundibulum, internal carotid arteries, and other cranial nerves were transected.

The brain was carefully retracted posteriorly, extracted from the cerebral cavity, and immersed in 10% neutral buffered formalin.

Comprehensive Assessment of the Calvaria and Cerebrum

The calvaria was examined, and images were captured using a Canon PowerShot SD1100 IS Digital ELPH 8.0-megapixel camera, which was utilized to document the extent and borders of hyperostosis, as well as the dimensions of hyperostotic nodules. The brain, with intact meninges, was analyzed and photographed.

The meninges were meticulously dissected to prevent injury to the underlying tissue, and the brain was thoroughly examined and photographed for potential indications of topographical anomalies resulting from hyperostotic bone or bony nodules.

Results

HFI+ was detected in 31 cases in total among 74 donors. In contrast to the expected number of HFI cases, 7 of the 31 cases were observed in the male gender. In addition, another important feature of this study is its evaluation in terms of HFI in the American population observed during these 4 years. Our study reveals that the frequency of HFI stands at 41.89%, with a prevalence of 9.45% among men in the population. This accounts for 22.58% of all HFI cases. In contrast, the incidence in women is observed at a rate of 32.43% within the society, constituting 77.42% of total HFI cases.

In the male cases of HFI, one case was identified in the 54-year age range, four cases were identified in the 70-80 year age range, and two cases were identified in the 80-90 year age range. Among female cases of HFI, 91.6% were aged over 60 years.

Figure 1, visually illustrates the incidence of HFI cases detected in males and females separately, as well as the overall cases.

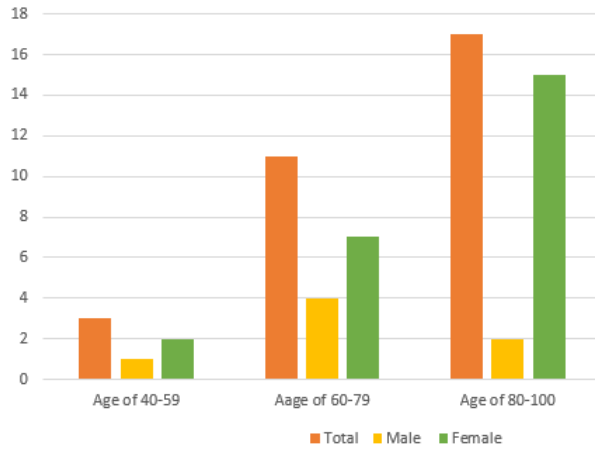


Figure 1. Displays graphically the overall case count, the number of cases found in males and females individually, and the incidence of HFI.

Macroscopic Analysis of the Frontal Lobe and the Calvaria

In Figure 2a, in a 78-year-old male case, a butterfly-shaped location in the Os frontale, bilaterally on the right and left, not exceeding the distribution area of the a.meningea media, is observed, which is the typical definition of HFI.

In Figure 2b, illustrates the impressions created by protrusions from heterotopic ossifications on both the right and left brain lobes, corresponding to the regions where impressions from hyperostosis frontalis interna are observed, as indicated by the areas encircled in red.

In Figure 3a, regarding the 64-year-old female instance, the os frontale is more prominent on the right, encompassing 50% of the frontal bone area up to the bifurcation of the a. meningea media on the left. On the left, it is less prominent than on the right, heterotopic ossifications are noted reaching to the branches of the a. meningea media.
In Figure 3b, HFI, observable in the right os frontale region, also significantly impacted the corresponding brain lobe due to compression.
In instances where HFI was identified, the dimensions of the osseous protrusions in the frontal bone correlated with observable compression-induced impressions in the neighboring dura mater and frontal cerebral lobes. The primary feature that captures our attention is heterotopic ossifications, located in the frontal bone and adjacent to the parietal bone, attributable to their morphological characteristics. It did not extend to the bifurcation of the meningea media and did not occupy the midline. In other words, the midline exhibited no involvement of HFI.

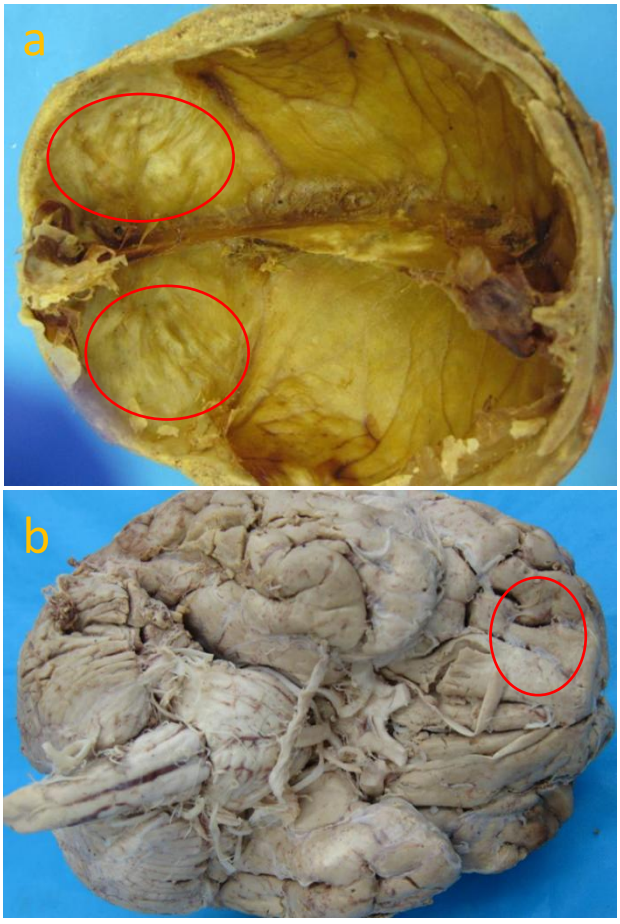


Figure 2a-2b. A typical definition of HFI is noted in a 78-year-old male case: a butterfly-shaped position in the Os frontale, bilaterally on the right and left, not surpassing the distribution area of the a.meningea media.

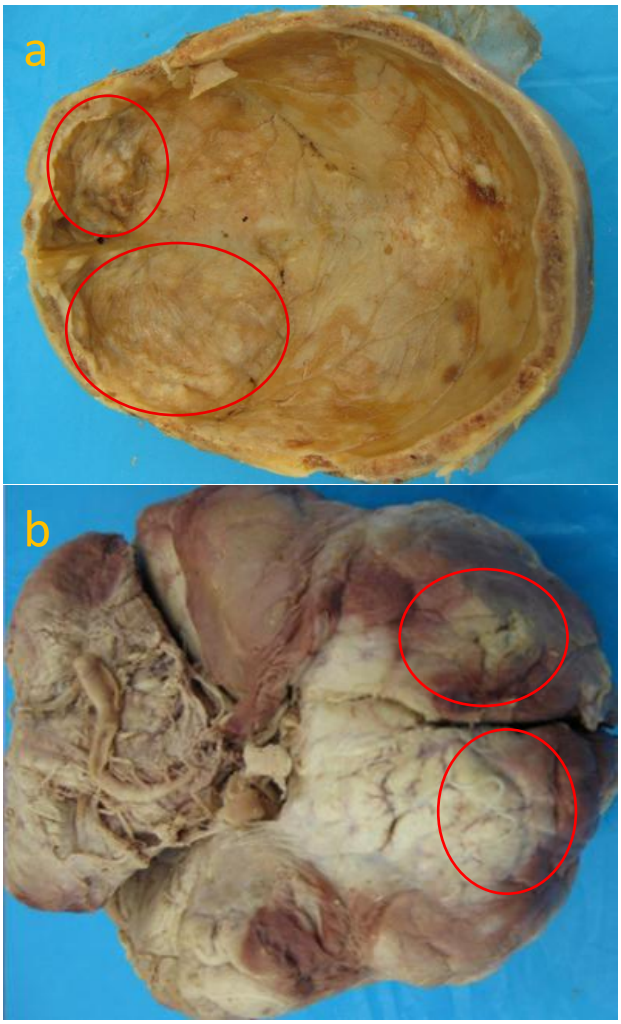


Figure 3a-3b. In the case of the 64-year-old female, the os frontale exhibits greater prominence on the right side, covering 50% of the frontal bone area up to the bifurcation of the a. meningea media on the left. Heterotopic ossifications are observed on the left, where they are less prominent compared to the right, extending to the branches of the a. meningea media. **3b.** HFI, which is visible in the right os frontale area, compressed the corresponding brain lobe and had a substantial impact on it.

Discussion

Our work was significant in identifying HFI in males through the examination of 74 cases. Nevertheless, no documentation existed concerning the backgrounds of the cases. The existing research indicates that the replacement of estrogen effects by testosterone dominance plays a crucial role in the pathogenesis of HFI; notably, we observed considerable cases of HFI in males. Nonetheless, it is recognized that HFI induces not only morphological alterations in bone. Simultaneously, it may coexist with other metabolic, neuroendocrine, genetic, and psychiatric disorders.
Symptoms occurring in over 20% of HFI patients included headaches, obesity, vertigo/dizziness, cognitive decline, and depression. Symptoms of moderate frequency observed in 5% to 20% of HFI patients included unspecified psychological diagnoses, extremity weakness

or gait abnormalities, giddiness, vision dysfunctions, diabetes, and hypertension.

Symptoms occurring at low frequency in fewer than 5% of HFI patients included a history of head trauma, energy decrease or fatigue, frontal lobe compression, cerebral atrophy, epilepsy, cystic ovaries, endocrine dysfunctions (excluding diabetes), speech dysfunctions, and increased intracranial pressure.[42]

Roybal et.al. suggested that Msx genes play a dual role in calvarial development by facilitating the differentiation and proliferation of osteogenic cells in rudiments, while also inhibiting an osteogenic program in the surrounding cell layer where the rudiments develop. The inactivation of this repressive activity may contribute to the formation of Wormian bones, which are ectopic bones associated with various pathological conditions that compromise calvarial bone development.[43]

The absence of Fgfr1 in neural crest cells results in heterotopic chondrogenesis and osteogenesis during the development of the frontal bone.[44]

Klippel-Trenaunay-Weber Syndrome, Frolich Syndrome, Morgagni Syndrome, Stewart-Moral Syndrome, Troell-Junet Syndrome, and Morgagn-iStewart-Morel Syndrome have all been linked to HFI. Frontal pain, psychoneuroses, obesity, parkinsonism, depression, frontal cortex managerial dysfunctions, epilepsy, and hypertrichosis may accompany HFI. In the differential diagnosis, it is crucial to consider localized malignancies (endosteal osteoma, osteosarcoma), Paget's Disease, fibrous dysplasia, Leontiasis ossea, pregnancy osteophytes, and metabolic craniopathy.[1]

Klippel-Trenaunay-Weber Syndrome is associated with varicose veins, port wine stains, and bone and soft tissue enlargement.

Troell-Junet Syndrome, accompanied by acromegaly, toxic goiter, diabetes mellitus.

Frolich Syndrome is associated with, pituitary anterior lobe atrophy-pituitary hypocrinism, obesity, growth retardation, port wine stain, and pituitary gonadotropic hormone deficiency.

The coexistence of metabolic, endocrine, and neuropsychiatric disorders with HFI is referred to as Morgagni-Stewart-Morel (MSM) syndrome. This instance was first recorded in 1719 by Giovanni Battista Morgagni, who noted a relationship between hirsutism and obesity, along with a thickening of the frontal bone.[38] Surprisingly, very little is known about this disease, even after three centuries of research. Stewart and Morel documented chronic headaches and neuropsychiatric symptoms in the early 1930s. [39,40] Recent studies have established a connection between MSM syndrome and neuropsychiatric disorders, metabolic and hormonal problems (including hirsutism, diabetes, and obesity), and other health issues. The reported clinical pattern is rarely consistent because the illness can have a wide array of symptoms and varied degrees of severity. Many medical professionals dispute the syndrome's validity because its related diseases can appear in older women on their own.[8,3] Although the exact cause of HFI and MSM syndrome is still a mystery, prominent hypotheses point to malfunctions in estrogen, obesity, and leptin, in addition to genetic components.[33]

Conditions and cases associated with HFI encompass frontal headache, intracranial hypertension, cognitive impairment, behavioral disorders, psychoneuroses, obesity, Alzheimer's disease, Parkinsonism, depression, executive dysfunction of the frontal lobe, epilepsy, and hypertrichosis.

Frontal lobe compression due to significant HFI may lead to psychiatric illnesses and cognitive deficits, especially impacting executive processes. In instances of concurrent degenerative dementia, the existence of HFI may signify a negative prognosis for the swift advancement of cognitive deterioration.[41]

A case presented by Brodoehl et al. illustrates transcortical motor aphasia, marked by reduced spontaneous speech, naming, and writing capabilities, whereas repetition and comprehension are preserved. The patient presents with moderate dementia and right-sided parkinsonism, characterized by increased muscle tone, a slight hand tremor, and supranuclear vertical gaze palsy. Neuroimaging demonstrated notable bifrontal hyperostosis. Hyperostosis frontalis interna (HFI) is characterized by a continuous proliferation of the frontal bone, commonly linked to chronic headaches and, less frequently, to seizures. HFI is observed to occur nine times more frequently in women and, when associated with virilisation, obesity, and neuropsychiatric issues, is classified as Morgagni-Stewart-Morel syndrome.[45]

HFI is associated with increased thickness of all calvarial bones and a reduction in intracranial volume.[46]

A considerable proportion of female specimens displaying heightened severity of HFI demonstrated a range of neuropsychiatric abnormalities, such as dementia, depression, Parkinson’s disease, and Alzheimer’s disease. Alzheimer's disease correlates with bone density. The severity of Alzheimer's disease in a patient may increase the risk of low bone mineral density.[47] This indicates that the body's reaction to the reduction of brain matter and the increase in skull volume is to promote bone deposition, which serves to reduce brain movement and stabilize it within the cranial cavity.[48]

Postmenopausal women, people with long-term estrogen exposure, overweight people, diabetics, and the elderly are at increased risk for HFI.

Hershkovitz indicates that the least severe variant of HFI manifests in females as early as 21 years of age, while advanced HFI does not emerge prior to 40 years of age. The initial psychological symptoms linked to HFI generally manifest after the age of 35.[49]

HFI occurs more frequently in females, particularly in postmenopausal women (40-60%).

Patients are typically asymptomatic or exhibit non-specific symptoms, including headache, neurological, or mental issues. The characteristic observation on [99mTc] HDP-bone scintigraphy and [18F] FDG-PET/CT is symmetrical and bilateral uptake in the frontal bones, resembling a butterfly pattern. [99mTc] HMPAO-labeled white blood cell scintigraphy demonstrates elevated

uptake, as the thickening results from higher bone marrow activity.[50]

This work is significant for elucidating clinically linked disorders by analyzing the incidence of HFI cases across both genders in American cadavers during a four-year period, as well as referencing reported instances in the literature. In Clinical Anatomy, it is crucial to recognize that HFI is not merely a physical structure seen in the calvaria, but is linked to various clinical fields, particularly neuroendocrinology, genetics, and neuropsychiatry.

Conclusion

The prevalence of male individuals with Hyperostosis Frontalis Interna in our study sample was 9.45%, which is noteworthy. Although the influence of estrogen has been the predominant factor in the etiopathogenesis of HFI thus far, the observed rate in males will illuminate new research and findings, facilitating a comprehensive exploration of the factors potentially involved in etiopathogenesis.

Given the unclear etiology, pathogenesis, and clinical presentation of HFI, it may be advantageous for clinicians to explore a potential relationship between HFI and the reported high to moderate symptoms. Furthermore, further research is necessary to accurately represent the current population of HFI patients and their associated symptoms. In extensive case groups, both cadaveric and forensic science cases, as well as living HFI instances, will be identified through radiological imaging, particularly in endocrine, neurology, and psychiatry clinics. This will facilitate multidisciplinary research, early diagnosis of HFI, and the development of novel treatment modalities in the relevant clinics.

Description

This article contains a portion of the research that was delivered as an oral presentation at the 2nd International Congress on Sports, Anthropology, Nutrition, Anatomy, and Radiology (SANAR), which took place from the 20th to the 23rd of July, 2020. The summary text was published in the booklet that contained the proceedings of the congress, namely on pages 100-105.

Compliance with Ethical Standards

This project is exempt from ethical approval as it employs course cadavers from the Albert Einstein College of Medicine C&DA Department, consistent with the exemption categories specified in Einstein-IRB-citation104(d).

Conflict of Interest

The author have no conflicts of interest relevant to this article.

Author Contributions

HC: The hypothesis of the study; HC: The Study desing; HC: Project development; HC: Literature search; HC: Analysis; HC: Manuscript writing; HC: Critical review.

Financial Disclosure

The author declare that this study has received no financial support.

References

1. Talarico EFJr, Prather AD, Hardt KD. A case of extensive hyperostosis frontalis interna in an 87-year-old female human cadaver. *Clin Anat*. 2008;21(3);259-268. doi:10.1002/ca.20613
2. Moore S. Hyperostosis Cranii. Springfield, Illinois: Charles C. Thomas. 1955. p 3-195.
3. Hershkovitz I, Greenwald C, Rothschild BM, et al. Hyperostosis frontalis interna: An anthropological perspective. *Am J Phys Anthropol*. 1999;109:303-325.
4. Moore S. Hyperostosis frontalis interna. *Surg Gyn Obst*. 1935;61:345.
5. Moore S. Calvarial hyperostosis and accompanying symptom complex. *Arch Neurol Psychiatr*. 1936;35:975-981.
6. Moore S. The Troell-Junet syndrome. *Acta Radiol*. 1953;39:485-93.
7. Perou ML. Cranial Hyperostosis. Springfield, Illinois: Charles C. Thomas. 1964.
8. She R, Szakacs J. Hyperostosis frontalis interna: Case report and review of Literature. *Annals of Clinical and Laboratory Science*. 2004;34(2):206-208. http://www.annclinlabsci.org/content/34/2/206.short
9. Murphy E, Kortyna R, Flaherty D. Hyperostosis Frontalis. *JBJS Journal of Orthopaedics for Physician Assistants*. 2018;6(2), e17. doi:10.2106/JBJS.JOPA.17.00032
10. Morita K, Nagai A, Naitoh M, Tagami A, Ikeda Y. A rare case of hyperostosis frontalis interna in an 86-year-old Japanese female cadaver. *Anatomical Science International*. 2021;96:315-318.
11. Greenwald C, Rothschild BM, Latimer B, Dutour O, Jellema LM, Wish-Baratz S. Hyperostosis frontalis interna: An anthropological perspective. Hershkovitz I. *American Journal of Physical Anthropology*. 1999;109:303-325.
12. Shahin A, Alhoseiny S, Aldali M. Hyperostosis frontalis interna: An Egyptian case referred to the second dynasty (2890-2650 BC) from Tarkhan-Egypt. *The Egyptian Rheumatologist*. 2014;36:41-45.
13. Bracanovic D, Djonic D, Nikolic S, et al. 3D-Microarchitectural patterns of Hyperostosis frontalis interna: a micro-computed tomography study in aged women. *J Anat*. 2016;229(5):673-680. doi:10.1111/joa.12506
14. Glinskii OV, Abraha TW, Turk JR, et al. Microvascular network remodeling in dura mater of ovariectomized pigs: role for angiopoietin-1 in estrogen-dependent control of vascular stability. *Am J Physiol Heart Circ Physiol*. 2007;293(2):H1131-H1137.
15. Yun SP, Lee MY, Ryu JM, et al. Role of HIF-1alpha and VEGF in human mesenchymal stem cell proliferation by 17- beta-estradiol: involvement of PKC, PI3K/Akt, and MAPKs. *Am J Physiol Cell Physiol.* 2009;296:C317-C326.
16. Liu LH, Lai Y, Linghu LJ, et al. Effect of different concentrations of medroxy-progesterone acetate combined with 17bestradiol on endothelial progenitor cells. *Eur Rev Med Pharmacol Sci*. 2015;19:1790-1795.
17. Peng J, Lai ZG, Fang ZL, et al. Dimethyloxalylglycine prevents bone loss in ovariectomized c57bl/6j mice through enhanced angiogenesis and osteogenesis. *PLoS One*. 2014;9(11):e112744.

doi:10.1371/journal.pone.0112744
18. Brandi ML, Collin-Osdoby P. Vascular biology and the skeleton. *J Bone Miner Res*. 2006;21(2):183-92. doi: 10.1359/JBMR.050917
19. Pufe T, Claassen H, Scholz-Ahrens KE, et al. Influence of estradiol on vascular endothelial growth factor expression in bone: a study in Gottingen miniature pigs and human osteoblasts. *Calcif Tissue Int.* 2007;80:184-191. doi:10.1007/s00223-006-0275-0
20. Griffith JF, Wang YX, Zhou H, et al. Reduced bone perfusion in osteoporosis: likely causes in an ovariectomy rat model. *Radiology*. 2010;254(3):739-46. doi:10.1148/radiol.09090608
21. Armelagos GJ, Chrisman OD. Hyperostosis frontalis interna: A Nubian case. *Am J Phys Anthropol*. 1988;76:25-28.
22. Anderson T. A medieval example of meningiomatous hyperostosis. *Br J Neurosurg*. 1991;5:499-504.
23. Anderson T. An example of meningiomatous hyperostosis from medieval Rochester. *Med Hist*. 1992;36:207-213.
24. Ruhli FJ, Boni T, Henneberg M. Hyperostosis frontalis interna: Archaeological evidence of possible microevolution of human sex steroids? *HOMO*. 2004;55:91-99.
25. Mulhern DM, Wilczak CA, Dudar JC. Brief communication: Unusual finding at Pueblo Bonito: Multiple cases of hyperostosis frontalis interna. *Am J Phys Anthropol.* 2006;130:480-484.
26. Perou ML. Cranial Hyperostosis. Springfield, Illinois: Charles C. Thomas; 1964.
27. Henschen F. Morgagni's Syndrome. Hyperostosis Frontalis Iinterna, Virilismus, Obesitas. Edinburgh, London: Oliver and Boyd; 1949:1-167.
28. Rudali G. Experimental production of hyperostosis frontalis interna in mice. *Isr J Med Sci*. 1968;4:1230-1235.
29. Yamakawa K, Mizutani K, Takahashi M, Matsui M, Mezaki T. Hyperostosis frontalis interna associated with hypogonadism in an elderly man. *Age Aging*. 2006;35:202-203.
30. Pawlikowski M, Komorowski JM. Hyperostosis frontalis and prolactin secretion. *Exp Clin Endocrinol*. 1987;89:109-111.
31. Fulton JD, Shand J, Ritchie D, McGhee J. Hyperostosis frontalis interna, acromegaly and hyperprolactinaemia. *Postgrad Med J*. 1990;66:16-19.
32. Glab H, Szostek K, Kaczanowski K. Hyperostosis frontalis interna, a genetic disease?: Two medieval cases from Southern Poland. *HOMO*. 2006;57:19-27.
33. Ruhli FJ, Henneberg M. Are hyperostosis frontalis interna and leptin linked? A hypothetical approach about hormonal influence on human microevolution. *Med Hypotheses*. 2002;58:378-381.
34. Stiene JM, Frank PW. Hyperostosis Frontalis Interna and a Question on Its Pathology: A Case Report. *Am J Case Rep*. 2022;23:e937450. doi:10.12659/AJCR.937450
35. May H, Peled N, Dar G, et al. Hyperostosis frontalis interna and androgen suppression. *Anat Rec (Hoboken). 2010;293(8):1333-6. doi:10.1002/ar.21175*
36. Belcastro MG, Todero A, Fornaciari G, Mariotti V. Hyperostosis frontalis interna (HFI) and castration: the case of the famous singer Farinelli (1705-1782). *J Anat*. 2011;219(5):632-637. doi:10.1111/j.1469-7580.2011.01413.x
37. Mutlu U, Telci Caklili O, Barburoglu M, Yarman S. Frequency of hyperostosis frontalis interna in patients with active acromegaly: is there a possible role of GH excess or hyperprolactinemia in its etiopathogenesis?. *Hormones (Athens)*. 2023;22(1):25-32. doi:10.1007/s42000-022-00401-x
38. Morgagni GB. Adversaria anatomica VI. Animadversio LXXIV. Vulporius, Padua; 1719.
39. Morel F. L'hyperostose frontale interne. Geneva: Chapalay and Mottier; 1929.
40. Stewart RM. Localized cranial hyperostosis in the insane. *J Neurol Psychopathol*. 1928;8:321.
41. Gilbert T, Ait S, Delphin F, Raharisondraibe E, Bonnefoy M. Frontal cortex dysfunction due to extensive hyperostosis frontalis interna. *BMJ Case Rep*. 2012;2012:bcr0720114439. doi:10.1136/bcr.07.2011.4439
42. Alvarez L, Corrigan W, McGonegal C, et al. The clinical manifestations of hyperostosis frontalis interna: A qualitative systematic review of cases. *Clin Anat*. 2024;37(5):505-521. doi:10.1002/ca.24147
43. Roybal PG, Wu NL, Sun J, Ting MC, Schafer CA, Maxson RE. Inactivation of Msx1 and Msx2 in neural crest reveals an unexpected role in suppressing heterotopic bone formation in the head. *Dev Biol*. 2010;343(1-2):28-39. doi:10.1016/j.ydbio.2010.04.007
44. Kawai M, Herrmann D, Fuchs A, et al. Fgfr1 conditional-knockout in neural crest cells induces heterotopic chondrogenesis and osteogenesis in mouse frontal bones. *Med Mol Morphol*. 2019;52(3):156-163. doi:10.1007/s00795-018-0213-z
45. Torrealba-Acosta G, Mandel J. Hyperostosis frontalis interna diagnosed after a provoked seizure. *BMJ Case Rep*. 2020;13(7):e236520. Published 2020 Jul 1. doi:10.1136/bcr-2020-236520
46. May H, Mali Y, Dar G, Abbas J, Hershkovitz I, Peled N. Intracranial volume, cranial thickness, and hyperostosis frontalis interna in the elderly. *Am J Hum Biol*. 2012;24(6):812-819. doi:10.1002/ajhb.22325
47. De Rose J, Laing B, Ahmad M. Skull Abnormalities in Cadavers in the Gross Anatomy Lab. *Biomed Res Int*. 2020;2020:7837213. doi:10.1155/2020/7837213
48. Loskutova N, Honea RA, Vidoni ED, Brooks WM, Burns JM. Bone density and brain atrophy in early Alzheimer's disease. *J Alzheimers Dis*. 2009;18(4):777-785. doi:10.3233/JAD-2009-1185
49. Bascou A, Savall F, Vergnault M, et al. Finding of Hyperostosis Frontalis Interna During the Autopsy Procedure: Forensic Issues. *J Forensic Sci*. 2019;64(6):1929-1932. doi:10.1111/1556-4029.14100
50. Moreno-Ballesteros A, León-Asuero-Moreno I, Marín-Melero I, García-Gómez AFJ. Hyperostosis frontalis interna by bone scintigraphy. *Jpn J Clin Oncol*. 2021;51(4):664-665. doi:10.1093/jjco/hyaa150

doi: 10.53446/actamednicomedia.1659648

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

MULTI-APPROACH ANALYSIS OF MMP-9 IN PROM AND PPROM: HISTOPATHOLOGICAL AND NETWORK-BASED PERSPECTIVES

PROM VE PPROM'DA MMP-9'UN ÇOK YÖNLÜ ANALİZİ: HİSTOPATOLOJİK VE AĞ TABANLI YAKLAŞIMLAR

Tuğcan Korak[1*], Gurler Akpinar[1], Hayat Ayaz[2], Fırat Aşır[2], Elif Ağaçayak[3], Ayşegül Aşır[4], Merve Gulsen Bal Albayrak[1], Murat Kasap[1]

[1]Kocaeli University, Faculty of Medicine, Department of Medical Biology, Kocaeli, Türkiye. [2]Dicle University, Faculty of Medicine, Department of Histology and Embryology, Diyarbakır, Türkiye. [3]Dicle University, Faculty of Medicine, Department of Gynecology and Obstetrics, Diyarbakır, Türkiye. [4]Gazi Yasargil Training and Research Hospital, Division of Pediatrics, Diyarbakır, Türkiye.

ABSTRACT

Objective: This study investigates the role of matrix metalloproteinase-9 (MMP-9) in prelabor rupture of membranes (PROM) and preterm PROM (PPROM) by integrating histological analysis with bioinformatics-based molecular network assessment.

Methods: Placental tissue samples were collected from 25 control, 25 PROM, and 25 PPROM cases. MMP-9 expression was analyzed via immunohistochemistry, and histological changes were examined. Modular network analysis of MMP-9 was performed using Cytoscape and STRING databases, with clustering conducted using the MCODE plugin and functional enrichment assessed via Enrichr platform. P-values less than 0.05 were considered statistically significant.

Results: Immunohistochemical analysis revealed a progressive increase in MMP-9 expression from control to PROM and PPROM placentas, with the highest expression observed in PPROM cases. Structural disruptions, including fibrin deposition and connective tissue irregularities, were more pronounced in PPROM ($p<0.05$). Network analysis identified four MMP-9-associated molecular clusters, of which two were significantly enriched in key biological pathways ($p<0.05$). Module 1 was associated with interleukin signaling (IL-4, IL-10, and IL-13) and extracellular matrix (ECM) degradation, while Module 2 was linked to neutrophil degranulation and collagen breakdown.

Conclusion: These findings indicate that MMP-9 plays a crucial role in ECM remodeling and inflammatory activation, contributing to membrane weakening and rupture in PROM and PPROM. The identified molecular clusters and signaling pathways provide insights into the underlying mechanisms and highlight MMP-9 as a therapeutic target for preventing premature rupture of membranes.

Keywords: MMP-9, prelabor rupture of membranes, placenta, immunohistochemistry, bioinformatics

ÖZ

Amaç: Bu çalışma, matriks metalloproteinaz-9 (MMP-9) ekspresyonunun preterm doğum öncesi membran rüptürü (PROM) ve preterm PROM (PPROM) patogenezindeki rolünü histolojik ve biyoinformatik yaklaşımlarla araştırmayı amaçlamaktadır.

Yöntem: Çalışmada 25 kontrol, 25 PROM ve 25 PPROM vakasından plasental doku örnekleri toplandı. MMP-9 ekspresyonu immünohistokimya yöntemiyle analiz edildi ve histolojik değişiklikler incelendi. MMP-9 modüler ağ analizi Cytoscape ve STRING veri tabanları kullanılarak gerçekleştirildi; kümelenme MCODE eklentisiyle tespit edildi ve fonksiyonel zenginleştirme analizi Enrichr platformu kullanılarak yapıldı. $P<0,05$ olan değerler istatistiksel olarak anlamlı kabul edildi.

Bulgular: İmmünohistokimyasal analiz sonuçları, kontrol grubundan PROM ve PPROM'a doğru MMP-9 ekspresyonunun kademeli olarak arttığını ve en yüksek seviyeye PPROM vakalarında ulaştığını ortaya koydu ($p<0,05$). Fibrin birikimi ve bağ dokusundaki düzensizlikler, özellikle PPROM grubunda daha belirgindi. Yolak analizinde, MMP-9 ile ilişkili dört moleküler küme tespit edildi; bunlardan ikisi biyolojik açıdan önemli yolaklarla anlamlı düzeyde zenginleşme gösterdi ($p<0,05$). Modül 1, interlökin sinyalleşmesi (IL-4, IL-10 ve IL-13) ve ekstrasellülar matriks (ECM) yıkımı ile ilişkilendirilirken, Modül 2 nötrofil degranülasyonu ve kollajen yıkımı ile bağlantılı olarak bulundu.

Sonuç: Bu bulgular, MMP-9'un ECM yeniden şekillenmesi ve inflamatuar aktivasyonda kritik bir rol oynadığını ve bunun membran zayıflaması ve rüptürüne katkıda bulunduğunu göstermektedir. Belirlenen moleküler kümeler ve sinyal yolları, PROM ve PPROM'un altında yatan mekanizmalara ışık tutmakta ve MMP-9'u potansiyel terapötik hedef olarak öne çıkarmaktadır.

Anahtar Kelimeler: MMP-9, doğum öncesi membran rüptürü, plasenta, immünohistokimya, biyoinformatik

***Corresponding author/İletişim kurulacak yazar:** Tuğcan Korak; Kocaeli University, Faculty of Medicine, Department of Medical Biology, Kocaeli, Türkiye.
Phone/Telefon: +90 (262) 303 75 75, **e-mail/e-posta:** tugcankorak@gmail.com/tugcan.korak@kocaeli.edu.tr
Submitted/Başvuru: 17.03.2025 • **Accepted/Kabul:** 16.05.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

The membranes typically rupture during labor. Prelabor rupture of membranes (PROM) occurs before the membranes begin labor after the 37th week.[1] Preterm PROM (PPROM) is defined as the rupture of membranes before 37 weeks of gestation. It contributes to 40% to 50% of all preterm births. Preterm PROM contributes more to neonatal mortality and morbidity than any other disease group.[2-4] The interaction of physiological weakening of the membranes with the pressure caused by uterine contractions and intraamniotic infection are the major risk factors for PROM. Risk factors for PPROM include a history of PROM, short cervix length, second or third trimester vaginal bleeding, excessive uterine distension, low body mass index (BMI), and smoking. Timely and accurate diagnosis of PROM is essential for the continuation of pregnancy and prevention of neonatal mortality and morbidity.[5,6]

PPROM can be triggered by bacterial infection or inflammation of the membranes, known as chorioamnionitis. Amniochorion membranes derive their tensile strength from a collagen-rich extracellular matrix (ECM); therefore, it is of great importance to understand the enzymes and processes that can degrade the membrane ECM. Matrix metalloproteinases (MMPs) are a class of enzymes that can degrade collagen and other components of the ECM and can be induced by inflammation.[7] Matrix metalloproteinase-9 (MMP-9) is the primary MMP involved in normal labor and also plays an important role in pathological labor. MMP-9 is the most active MMP in the amnion and has been found to be significantly increased in amniotic membranes after the onset of contractions. Matrix metalloproteinase, also known as matrixin, plays an important role in the breakdown and remodeling of the ECM, ultimately causing both preterm labor and PPROM. PPROM increases MMP-9 concentrations in amniotic fluid, causing amniotic fluid infection, threatened preterm labor, and poor birth outcome. Manipulation of MMP may play a role in preventing spontaneous preterm labor.[8]

Given its critical role in ECM remodeling and inflammation-induced membrane rupture, this study aims to elucidate the contribution of MMP-9 to PROM and PPROM pathophysiology through histopathological and in silico approaches by analyzing its interactions with key protein networks involved in these processes.

Methods

Placenta Collection

This study was conducted with the approval of the Non-Interventional Clinical Ethics Committee, Dicle University Faculty of Medicine (date 20.12.2023 and number 2023/6). In the study, pregnant women aged between 18-49 years and without any systemic disease or secondary disease were examined at the Gynecology and Obstetrics Clinic of Dicle University Faculty of Medicine. The patient groups consisted of 25 term pregnant women who gave birth in hospital with the diagnosis of PROM and 25 PROM pregnant women. The control group consisted of 25 pregnant women who gave birth in hospital with the diagnosis of healthy pregnancy. Placental tissue samples were taken from all three groups. The patients whose placental tissues would be collected after delivery were informed about the study and their informed consent was obtained.

Placenta Tissue Preparation

Placenta tissues taken from the maternity clinic after delivery were reduced in size in a manner suitable for histological follow-up. Placenta tissues were first kept in formalin solution for one day. Then, they were kept in running water overnight. Placenta tissues were passed through an ascending ethanol series (50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol) to remove water from the tissues. Tissues were kept in xylene solution 3 times for 30 minutes to remove alcohol. Then, tissues were taken in molten paraffin liquid at 58°C. In the final stage, tissues were embedded in paraffin blocks and 4-6 µm thick sections were taken with a microtome (catalog no: Leica RM2265, Wetzlar, Germany).[9]

Hematoxylin-Eosin Staining

Sections obtained from paraffin blocks of placental tissues were placed in a bain-marie set at 50°C. Sections were left in a 60°C oven overnight to allow the tissues on the sections to stick to the slide and to melt excess paraffin. Sections were removed from the oven, left at room temperature and allowed to cool. To remove paraffin residues from the sections, sections were kept in renewed xylene solutions for 15 minutes three times. After excess paraffin melted, sections were kept in a decreasing ethanol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes and excess alcohol was cleaned in distilled water. Harris hematoxylin stain was first applied to the sections for 8 minutes. Sections were kept under tap water for 5 minutes to clean excess staining. Then, the cleaned sections were kept in alcoholic (5%) eosin for 6 minutes. After the staining stage was completed, the sections were quickly dipped in a rapidly rising ethanol series. In order for the tissue in the sections to appear clear and clean, the sections were kept in xylene solutions for 3x15 minutes. Covering medium was added to the sections, they were covered with a slide and stored for examination.[10]

Immunohistochemical Staining

Sections were washed in phosphate buffer solution (PBS) for 3x5 minutes. After epitope retrieval in ethyl diamine tetra-acetic acid (EDTA) solution (pH: 8.0, catalogue no: ab93680, Abcam, Cambridge, USA), sections were treated with hydrogen peroxide solution (catalogue no: TA-015-HP, ThermoFischer, Fremont, CA, USA) for 20 minutes. Nonspecific staining was blocked with blocking solution (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, USA) Primary antibody MMP-9 (catalog no: sc-393859, Santa Cruz Biotechnology, Heidelberg,

Germany, dilution ratio: 1/100) was dipped onto the tissues and left overnight at +4°C. following biotinylated secondary antibody (catalog no: TP-015-BN, ThermoFischer, Fremont, CA, USA), biotin-streptavidin complex was formed (catalog no: TS-015-HR, ThermoFischer, Fremont, CA, USA). Diaminobenzidine (DAB) (catalog no: TA-001-HCX, ThermoFischer, Fremont, CA, USA) was used as a chromogen. Gill III hematoxylin staining was used as a counter stain. Sections were quickly passed through an increasing ethanol series, cleared in xylene and mounted analyze and visualize with a Zeiss Imager A2 light microscope.[11]

Semi-quantitative Histological Scoring

For the semiquantitative assessment of MMP-9 expression, staining intensity was measured using ImageJ software (version 1.53, http://imagej.nih.gov/ij). Ten microscopic fields per sample were analyzed in each group, and the quantification results were documented. Brown coloration indicated positive antibody staining, whereas blue represented negative staining. Signal intensity (expression) within each area was obtained by dividing the stained antibody area by the total examined area. For each sample, the ratio of positively stained area relative to the total area was calculated across ten fields, and subsequently, a mean value for each group was determined. These mean values were then used for semiquantitative immunohistochemical scoring.

Statistical Analysis

The statistical analysis of our study was performed using IBM SPSS 29.0 software (IBM, Armonk, New York, USA). Shapiro-Wilk's test was used to assess the normality assumption. Continuous variables were presented as mean ± standard deviation (SD). Comparisons of more than two groups were carried out with the one-way analysis of variance (ANOVA), and pairwise group comparisons were made with the Tukey and Dunnett tests. Significance was considered for p values <0.05.

Modular Network and Enrichment Analysis of MMP-9 Protein Targets

To investigate the potential mechanisms associated with MMP-9 in PROM and PPROM, a modular network and pathway enrichment analysis was conducted. Initially, a protein-protein interaction (PPI) network was generated in Cytoscape (v.10.3.3) using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, incorporating an additional 100 interacting proteins. The confidence score threshold was set at 0.400. Subsequently, module identification was performed using the MCODE plugin in Cytoscape to detect functionally relevant clusters within the PPI network. The MCODE parameters were defined as follows: maximum depth=100, node score cutoff=0.2, degree cutoff=2, and K-core=2. The identified modules were further analyzed for functional and pathway enrichment using the Reactome database via the Enrichr platform. Pathways with a p-value less than 0.05 were considered statistically significant, and the top 10 enriched pathways were ranked based on ascending p-values for further interpretation.

Results

Demographic properties of patients

The clinical and demographic characteristics of the study groups are summarized in Table 1. Maternal age and BMI did not differ significantly among the groups (p> 0.05). However, gravida and parity were significantly higher in PROM and PPROM cases (p<0.05). Gestational age at delivery and birth weight were significantly lower in these groups, with the most pronounced decrease in PPROM (p<0.001).

Table 1. Clinical and demographic of patients

Parameters (n=25)	Control (mean ± SD)	PROM (mean ± SD)	PPROM (mean ± SD)	Significance
Maternal age, year	28.35 ± 3.57	32.34 ± 2.68	34.72 ± 2.28	p>0.05
Gravida, n	1.73 ± 0.58	3.47 ± 1.28	4.72 ± 2.28	p<0.05
Parity, n	1.10 ± 0.45	2.25 ± 1.12	2.87 ± 1.66	p<0.05
Gestational age at delivery, week	38.61 ± 1.15	35.24 ± 2.26	32.15 ± 2.43	p<0.001
Birth weight, gr	3815.27 ± 357.49	2504.29 ± 415.82	2147.72 ± 358.46	p<0.001
BMI, kg/m²	28.25 ± 4.58	30.57 ± 4.75	31.24 ± 3.86	p>0.05

SD: Standard deviation

PPROM and PPROM Showed Significant Increase in MMP-9 Expression

Images labeled A, B, and C represent placentas from control, PROM, and PPROM groups, respectively, immunostained for MMP-9 (Figure 1). In the control group (Figure 1A), there was mild expression of MMP-9 in chorionic villi, which exhibited preserved structural integrity, regular connective tissue distribution, and low-level staining in the trophoblast layer. The PROM group (Figure 1B) showed increased MMP-9 expression compared to controls, with noticeable fibrin deposition, mild villous irregularities, and increased staining intensity in the trophoblast layer. Furthermore, moderate congestion was observed in the intervillous spaces. The PPROM group (Figure 1C) demonstrated significantly elevated and widespread MMP-9 expression along with pronounced fibrin accumulation, notable disruption of villous structural integrity, marked irregularities in

connective tissue, and intense staining within the trophoblast layers. Semi-quantitative measurement of MMP-9 expression in groups were presented in Figure 1D. Statistically, MMP-9 expression was increased in PROM and PPROM groups compared to control group, however, no statistical difference was observed between PROM and PPROM groups. These findings suggest that MMP-9 expression is progressively enhanced from control to PROM and most markedly in PPROM, correlating with structural disruptions and increased fibrin deposition.

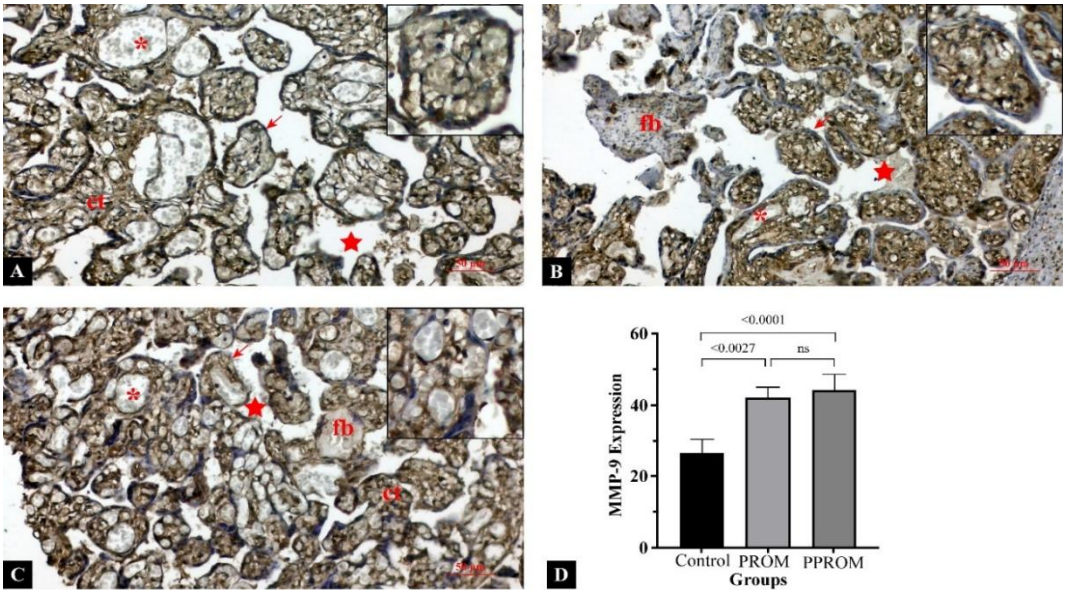


Figure 1. Cross sections of placental tissue in A: Control, B: PROM, C: PPROM groups.: A close-up of MMP-9 expression in placental tissues. D: Semi-quantitative MMP-9 expression in groups. inset Arrow: chorionic villus, ct: connective tissue, fb: fibrin deposition, *: capillary, star: intervillous area. MMP-9 immune staining, inset: Scale Bar: 20 µm (magnification: 40X).

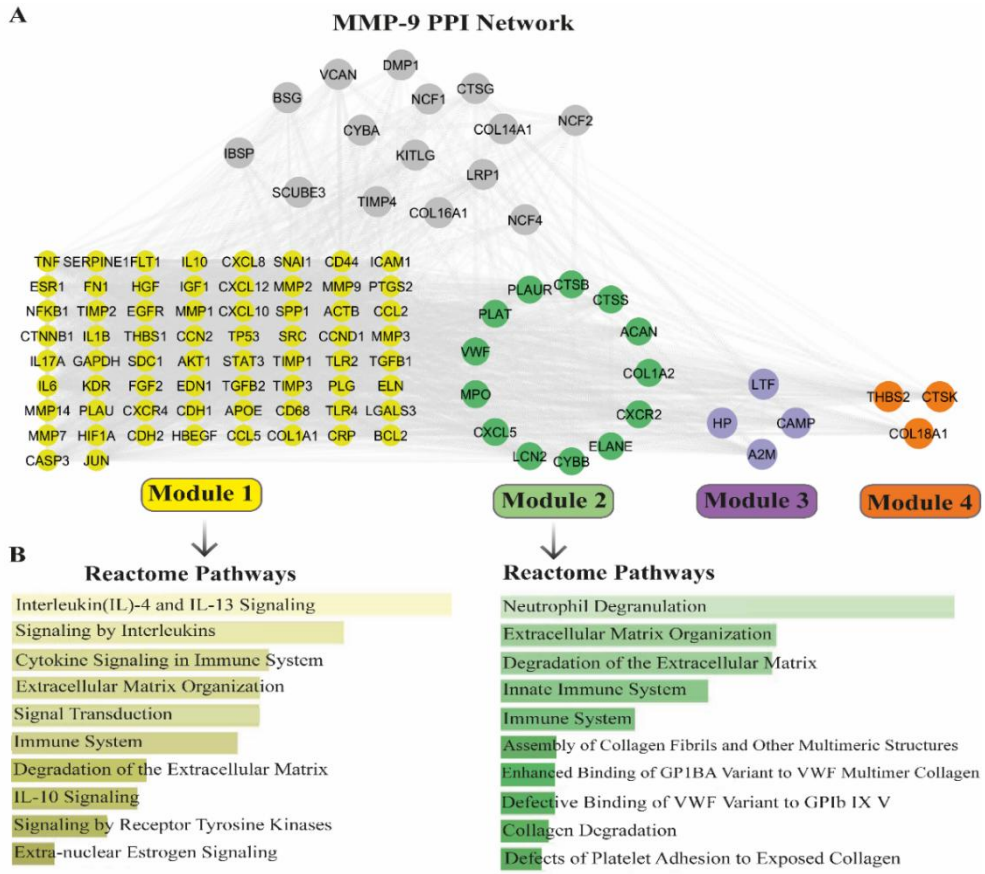


Figure 2. Modular network analysis of the MMP-9 PPI network. A. The MMP-9-centered PPI network was generated using STRING and clustered in Cytoscape with MCODE, identifying four modules: Module 1 (66 proteins), Module 2 (13 proteins), Module 3 (4 proteins), and Module 4 (3 proteins). B. Bar charts represent the significant functional annotation of Module 1 and Module 2, showing enriched pathways ranked in ascending order of p-values.

Modular Network Identification and Functional Enrichment Analysis of MMP-9 PPI Network

Modular network analysis of the MMP-9 PPI network identified four distinct clusters (Figure 2A). However, Module 3 (4 proteins) and Module 4 (3 proteins) were excluded from further analysis due to their small size and lack of biologically meaningful interaction networks. Functional annotation was performed on Module 1 (66 proteins) and Module 2 (13 proteins), which exhibited significant enrichment in pathways related to immune signaling, ECM remodeling, and inflammatory processes. Module 1 was primarily associated with Interleukin signaling (IL-4, IL-10, and IL-13), cytokine regulation, and ECM degradation, while Module 2 showed enrichment in neutrophil degranulation, innate immune response, and collagen degradation (Figure 2B).

Discussion

PROM refers to the spontaneous rupture of fetal membranes before the onset of labor at term (≥37 weeks of gestation). PPROM occurs before 37 weeks of gestation and is associated with significant maternal and neonatal complications, including preterm birth, neonatal sepsis, and increased perinatal morbidity and mortality.[2-4] MMP-9 is a member of the MMP family, which plays a critical role in ECM remodeling.[12] MMP-9 is primarily involved in degrading type IV collagen, a major component of the fetal membranes. Increased MMP-9 activity has been implicated in the weakening of the amniotic sac, contributing to membrane rupture. Its dysregulation has been associated with pathological conditions such as premature rupture of membranes, chorioamnionitis, and preterm labor.[13]

The pathophysiology of PROM and PPROM involves complex biochemical and mechanical processes. Inflammatory cytokines such as IL-6, IL-1β, and TNF-α upregulate MMP-9 expression, leading to ECM degradation and membrane weakening.[14] Increased reactive oxygen species (ROS) contribute to ECM degradation by stimulating MMP-9 expression. Microbial invasion of the amniotic cavity triggers an immune response, enhancing MMP-9 production and leading to premature membrane rupture.[15] Excessive mechanical forces on the fetal membranes may activate MMP-9, promoting ECM breakdown and membrane rupture.[16]

Our study demonstrated that MMP-9 expression was significantly elevated in PROM and PPROM placental tissues compared to the control group, with the highest expression observed in PPROM cases. These findings align with previous studies suggesting that excessive MMP-9 activity contributes to preterm membrane rupture and premature delivery. Studies have reported increased MMP-9 expression in preterm labor, particularly in cases associated with infection and inflammation. MMP-9 levels in amniotic fluid were significantly higher in patients with PPROM, correlating with microbial invasion and inflammatory responses. Furthermore, higher MMP-9 concentrations have been observed in fetal membranes of PPROM cases compared to term pregnancies, supporting the role of ECM degradation in preterm rupture.[17,18] In addition, our findings are consistent with the hypothesis that MMP-9-mediated degradation of type IV collagen weakens the fetal membranes, making them susceptible to rupture. This mechanism is exacerbated in PPROM due to persistent inflammatory stimulation and infection-associated upregulation of MMP-9.

The clinical significance of our findings highlights the potential role of MMP-9 as a biomarker for predicting membrane integrity and the risk of preterm birth. Elevated MMP-9 levels in placental tissues and amniotic fluid may serve as an early indicator of membrane weakening, guiding clinical decisions regarding patient management. Targeted Therapies: Inhibitors of MMP-9 (e.g., tissue inhibitors of metalloproteinases) may be explored as therapeutic agents to prevent premature membrane rupture in high-risk pregnancies. MMP-9 expression profiling in amniotic fluid or maternal blood could help identify women at risk for PROM and PPROM, allowing for early intervention strategies. Patients with elevated MMP-9 levels could benefit from close monitoring, prophylactic antibiotics (in cases of suspected infection), and corticosteroid administration to improve neonatal outcomes.[19,20]

Our bioinformatics analysis identified two key MMP-9-associated molecular clusters in PROM and PPROM, revealing distinct yet interconnected mechanisms. Module 1 was enriched in immune signaling pathways, notably interleukins (ILs) IL-4, IL-10, and IL-13, while Module 2 was primarily associated with neutrophil degranulation, innate immune activation, and collagen degradation. These findings align with our histological results, which showed progressively increased MMP-9 expression from control to PROM and PPROM, suggesting its role in extracellular matrix (ECM) remodeling and immune-mediated membrane weakening. The enrichment of cytokine signaling pathways in Module 1 implies that dysregulated immune responses may contribute to PROM and PPROM progression by promoting ECM degradation. Similarly, the pathways identified in Module 2 highlight the role of innate immune activation and proteolytic enzyme release in weakening fetal membranes. These observations are consistent with previous studies demonstrating MMP-9 upregulation in preterm labor, infection-induced inflammation, and preterm membrane rupture.[21,22] For instance, elevated MMP-9 levels have been detected in fetal plasma of cases with preterm PROM, implicating its role in membrane rupture mechanisms. Additionally, activated neutrophils have been shown to upregulate MMP-9 and prostaglandin E2 release in fetal membranes, contributing to their weakening.[18,22] Moreover, studies have shown that increased MMP-9 expression is associated with the degradation of type IV collagen in fetal membranes, leading to their weakening and rupture.[23] This suggests that MMP-9-mediated ECM remodeling plays a crucial role in the pathogenesis of PROM and PPROM. On the

other hand, polymorphisms in the MMP-9 promoter region have been linked to altered enzyme expression, potentially influencing individual susceptibility to PROM and PPROM.[24] Together, the integration of our modular network analysis with histological and literature-based evidence suggests that MMP-9 contributes to membrane weakening via IL-driven immune responses and neutrophil-mediated proteolytic activity. Moreover, its association with type IV collagen degradation and genetic polymorphisms further supports its involvement in membrane instability. Collectively, these insights highlight MMP-9 as a potential biomarker and therapeutic target, highlighting the importance of continued investigation into its regulatory pathways and potential therapeutic approaches.

This study provides valuable insights into the role of MMP-9 in PROM and PPROM through an integrated histopathological and bioinformatics approach. However, several limitations should be acknowledged. First, the sample size, while balanced across groups, remains relatively small, potentially limiting the statistical power and generalizability of the findings. Second, while the study utilized immunohistochemical methods to assess MMP-9 expression, quantitative assays such as ELISA or Western blotting could provide a more precise evaluation of protein levels. Third, the bioinformatics network analysis was based on publicly available databases and predicted interactions, which may not fully capture tissue-specific or gestational age-specific variations in protein-protein interactions. Additionally, functional validation of the identified molecular clusters and pathways was not performed, leaving open questions regarding the causal role of these pathways in membrane rupture. Future studies should aim to validate these findings in larger, multicentric cohorts and include in vitro or ex vivo experimental models to elucidate the functional consequences of MMP-9 modulation. Investigating upstream regulatory mechanisms and potential MMP-9 inhibitors may offer novel therapeutic avenues for preventing PROM and PPROM, especially in high-risk pregnancies. Furthermore, longitudinal studies examining the temporal dynamics of MMP-9 expression could improve understanding of its role in the progression from membrane weakening to rupture.

In conclusion, this study highlights MMP-9's pivotal role in PROM and PPROM pathogenesis, demonstrating its increased expression in placental tissues, particularly in PPROM cases, and its contribution to ECM remodeling and inflammatory activation leading to membrane rupture. A key novelty of this study is the integration of bioinformatics-driven modular network analysis, identifying the specific protein clusters through which MMP-9 exerts its effects. These clusters are primarily involved in immune signaling, neutrophil degranulation, and ECM degradation, shedding light on the molecular interactions underlying membrane weakening and preterm birth risk. Additionally, the findings support the hypothesis that excessive MMP-9 activity disrupts type IV collagen, making fetal membranes more vulnerable, especially in the presence of chronic inflammation and infection. By combining histological analysis with network-based profiling, this study offers a systems biology perspective, providing potential biomarkers and therapeutic targets for preventing preterm birth in high-risk pregnancies. These insights highlight MMP-9 as a crucial mediator in PROM and PPROM, emphasizing the need for further research into its regulatory mechanisms and therapeutic targeting to improve maternal-fetal health outcomes.

Ethical Approval

This study was conducted with the approval of the Non-Interventional Clinical Ethics Committee, Dicle University Faculty of Medicine (date 20.12.2023 and number 2023/6).

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

TK: Study design, hypothesis, in silico analysis, manuscript preparation; GA, MGBA, MK: Evaluation of molecular aspects, critical review of manuscript; HA, FA: Material preparation, experimental data analysis; EA and AA: Evaluation of clinical aspects, review.

Financial Support

None

References

1. Zaman F, Aşır F, Ermiş IS, Tuncer MC, Deveci E, Oğlak SC. Immunohistochemical analysis of vimentin and zonula occludens-1 in placentas of patients with PPROM. *Clin Exp Obstet Gynecol*. 2024;51:18. doi:10.31083/j.ceog5101018
2. Menon R, Richardson LS. Preterm prelabor rupture of the membranes: A disease of the fetal membranes. *Semin Perinatol*. 2017;41:409-419. doi:10.1053/j.semperi.2017.07.012
3. Mercer BM, Crouse DT, Goldenberg RL, et al. The antibiotic treatment of PPROM study: systemic maternal and fetal markers and perinatal outcomes. *Am J Obstet Gynecol* 2012;206:145.e1-145.e9. doi:10.1016/j.ajog.2011.08.028
4. Delorme P, Lorthe E, Sibiude J, Kayem G. Preterm and term prelabour rupture of membranes: A review of timing and methods of labour induction. *Best Pr Res Clin Obstet Gynaecol*. 2021;77:27-41. doi:10.1016/j.bpobgyn.2021.08.009
5. Lin D, Hu B, Xiu Y, et al. Risk factors for premature rupture of membranes in pregnant women: a systematic review and meta-analysis. *BMJ Open*. 2024;14:e077727. doi:10.1136/bmjopen-2023-077727
6. Medina TM, Hill DA. Preterm premature rupture of membranes: diagnosis and management. *Am Fam Physician*. 2006;73:659-664.
7. Nguyen LM, Aronoff DM, Eastman AJ. Matrix metalloproteinases in preterm prelabor rupture of membranes in the setting of chorioamnionitis: A scoping review. *Am J Reprod Immunol*. 2023;89:e13642. doi:10.1111/aji.13642
8. Sulistyowati S, Zakia Y, Khasan S. High MMP-9 and TNF-α expression increase in preterm premature rupture of

membranes. *Universa Med*. 2016;35:33-39. doi:10.18051/univmed.2016.v35.33-39
9. Aşır F, Oğlak SC, Ağaçayak E, Alabalık U. Homeobox A Cluster 7 (HOXA7) protein expression increased in the placentas of patients with preterm delivery. *Perinatal Journal*. 2023;31:213-218. doi:10.59215/prn.23.0313006
10. Asir F, Oglak SC, Korak T, et al. Placental vimentin expression in preeclampsia and gestational diabetes mellitus. *Gynecol Obstet Reprod Med*. 2024:1-9. doi:10.21613/gorm.2023.1469
11. Öcal E, Akalın SA, Aşır F. Role of cited-1 and caspase-6 expression in HELLP syndrome. *Eur Rev Méd Pharmacol Sci*. 2023;27:3082-3087. doi:10.26355/eurrev_202304_31942
12. Nikolov A, Popovski N. Role of gelatinases MMP-2 and MMP-9 in healthy and complicated pregnancy and their future potential as preeclampsia biomarkers. *Diagnostics*. 2021;11:480. doi:10.3390/diagnostics11030480
13. Cabral-Pacheco GA, Garza-Veloz I, Rosa CC-D la, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020;21:9739. doi:10.3390/ijms21249739
14. Yabluchanskiy A, Ma Y, Iyer RP, Hall ME, Lindsey ML. Matrix Metalloproteinase-9: Many shades of function in cardiovascular disease. *Physiology*. 2013;28:391-403. doi:10.1152/physiol.00029.2013
15. Chen J, Khalil RA. Chapter four matrix metalloproteinases in normal pregnancy and preeclampsia. *Prog Mol Biol Transl Sci*. 2017;148:87-165. doi:10.1016/bs.pmbts.2017. 04.001
16. Barrett DW, John RK, Thrasivoulou C, et al. Targeting mechanotransduction mechanisms and tissue weakening signals in the human amniotic membrane. *Sci Rep*. 2019;9:6718. doi:10.1038/s41598-019-42379-4
17. Bhunia S, O'Brien S, Ling Y, Huang Z, Wu P, Yang Y. New approaches suggest term and preterm human fetal membranes may have distinct biomechanical properties. *Sci Rep*. 2022;12:5109. doi:10.1038/s41598-022-09005-2
18. Romero R, Chaiworapongsa T, Espinoza J, et al. Fetal plasma MMP-9 concentrations are elevated in preterm premature rupture of the membranes. *Am J Obstet Gynecol*. 2002;187:1125-1130. doi:10.1067/mob.2002.127312
19. Athayde N, Edwin SS, Romero R, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. *Am J Obstet Gynecol*. 1998;179:1248-1253. doi:10.1016/s0002-9378(98)70141-3
20. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases. *Circ Res*. 2003;92:827-839. doi:10.1161/01.res.0000070112.80711.3d
21. Lal CV, Xu X, Jackson P, et al. Ureaplasma infection-mediated release of matrix metalloproteinase-9 and PGP: a novel mechanism of preterm rupture of membranes and chorioamnionitis. *Pediatr Res*. 2017;81:75-79. doi:10.1038/pr.2016.176
22. Tong M, Smith AH, Abrahams VM. Activated neutrophils propagate fetal membrane inflammation and weakening through ERK and neutrophil extracellular trap-induced TLR-9 signaling. *J Immunol*. 2021;206:1039-1045. doi:10.4049/jimmunol.2001268
23. Zuo G, Dong J-X, Zhao F-F, Chen Y. Expression of matrix metalloproteinase-9 and its substrate level in patients with premature rupture of membranes. *J Obstet Gynaecol*. 2017;37:441-445. doi:10.1080/01443615.2016.1250734
24. Ferrand PE, Parry S, Sammel M, et al. A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. *Mol Hum Reprod*. 2002;8:494-501. doi:10.1093/molehr/8.5.494

doi: 10.53446/actamednicomedia.1677998

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

INCIDENCE AND CLINICAL OUTCOMES OF CONGENITAL HYPOTHYROIDISM: A RETROSPECTIVE STUDY BASED ON NEWBORN SCREENING DATA FROM MUĞLA PROVINCE, TÜRKİYE

KONJENİTAL HİPOTİROİDİ SIKLIĞI VE KLİNİK SONUÇLARI: MUĞLA İLİNDE YENİDOĞAN TARAMA VERİLERİNE DAYALI RETROSPEKTİF BİR ÇALIŞMA

Gülay Can Yılmaz[1*], Gizem Ger[2], Elif Söbü[3]

[1]Muğla Sıtkı Koçman University, Faculty of Medicine, Department of Pediatric Endocrinology, Muğla, Türkiye. [2]Muğla Sıtkı Koçman University, Faculty of Medicine, Department of Pediatrics, Muğla, Türkiye. [3]Uskudar University, Faculty of Medicine, Department of Pediatric Endocrinology, İstanbul, Türkiye.

ABSTRACT

Objective: To evaluate the incidence, clinical characteristics, and treatment outcomes of congenital hypothyroidism (CH) identified through neonatal screening in Muğla province, with a particular focus on differentiating between transient and permanent CH.

Methods: This retrospective study included 95 infants referred between 2020 and 2022 due to elevated thyroid-stimulating hormone (TSH) levels detected through heel-prick screening. Demographic, clinical, and biochemical data were collected. Infants were classified either as diagnosed and treated CH cases or as having transient TSH elevation without requiring treatment. Among the treated cases, CH was further categorized as transient or permanent based on follow-up findings. The predictive value of the levothyroxine (LT4) dose at six months of age was also analyzed.

Results: The average annual incidence of CH was 114.4 per 100,000 live births. Of the 95 infants, 33 (34.7%) received treatment for CH, while 62 (65.3%) had transient TSH elevation and did not require treatment. Among the treated cases, 19 (57.6%) were diagnosed with permanent CH and 14 (42.4%) with transient CH. The mean LT4 dose at six months was significantly lower in transient cases compared to permanent ones. LT4 dose cut-off value of 3.35 µg/kg/day at six months demonstrated high sensitivity and moderate specificity in predicting transient CH.

Conclusion: The incidence of CH in Muğla province exceeds global averages, with a notable proportion of transient cases. The LT4 dose at six months may serve as a useful marker for differentiating between transient and permanent CH, enabling more individualized follow-up and management strategies.

Keywords: Congenital hypothyroidism, neonatal screening, levothyroxine, transient hypothyroidism, permanent hypothyroidism

ÖZ

Amaç: Bu çalışmada, Muğla ilinde yenidoğan taraması ile saptanan konjenital hipotiroidi (KH) olgularının insidansı, klinik özellikleri ve tedavi sonuçları değerlendirilmiş; geçici ve kalıcı KH ayrımı üzerine odaklanılmıştır.

Yöntem: Bu retrospektif çalışmaya, 2020-2022 yılları arasında topuk kanı taramasında tiroid stimülan hormon (TSH) düzeyi yüksekliği nedeniyle yönlendirilen 95 bebek dahil edilmiştir. Demografik, klinik ve biyokimyasal veriler kaydedilmiştir. Bebekler, KH tanısı alıp tedavi başlananlar ve tedavi gerektirmeyen geçici TSH yüksekliği olanlar şeklinde iki gruba ayrılmıştır. Tedavi başlananlar ise takip sonuçlarına göre geçici veya kalıcı KH olarak sınıflandırılmıştır. Altıncı ayda uygulanan levotiroksin (LT4) dozunun ayırt edici değeri ROC analizi ile değerlendirilmiştir.

Bulgular: Yıllık ortalama KH insidansı 100.000 canlı doğumda 114,4 olarak saptanmıştır. 95 bebeğin 33'üne (%34,7) tedavi başlanmış, 62'si (%65,3) tedavi gerekmemiştir. Tedavi alanlar arasında 19'u (%57,6) kalıcı, 14'ü (%42,4) geçici KH olarak sınıflandırılmıştır. Altıncı ayda LT4 dozu geçici KH grubunda anlamlı olarak daha düşüktü. 3,35 µg/kg/gün kesim değeri, geçici KH'yi %100 duyarlılık ve %63,2 özgüllükle öngörmüştür (AUC: 0,925).

Sonuç: Muğla ilinde KH insidansı küresel ortalamanın üzerindedir ve önemli bir kısmı geçicidir. Altıncı aydaki LT4 dozu, geçici ve kalıcı KH ayrımında faydalı bir belirteç olabilir ve bireyselleştirilmiş takip stratejilerine katkı sağlayabilir.

Anahtar Kelimeler: Konjenital hipotiroidi, yenidoğan taraması, levotiroksin, geçici hipotiroidi, kalıcı hipotiroidi

***Corresponding author/İletişim kurulacak yazar:** Gülay Can Yılmaz; Muğla Sıtkı Koçman University, Faculty of Medicine, Department of Pediatric Endocrinology, Muğla, Türkiye.
Phone/Telefon: +90 (533) 495 46 43, **e-mail/e-posta:** gulaycan@mu.edu.tr
Submitted/Başvuru: 21.04.2025 • **Accepted/Kabul:** 22.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Congenital hypothyroidism (CH) is a leading cause of preventable intellectual disability when not diagnosed and treated early. Since affected neonates are typically asymptomatic, clinical recognition without systematic screening is challenging.[1] Newborn screening programs enable early biochemical detection and timely treatment. When levothyroxine (LT4) therapy is initiated within the first two weeks of life, affected children can achieve neurodevelopmental outcomes comparable to those of their healthy peers.[2]

In Türkiye, a nationwide newborn screening program for CH has been in place since 2006 under the coordination of the Ministry of Health. Thyroid-stimulating hormone (TSH) levels are measured from heel-prick blood samples collected between the third and fifth days of life. According to national guidelines, infants with a TSH value >20 µIU/mL in the first sample or >5.5 µIU/mL in a second sample are referred to pediatric endocrinology centers for confirmatory evaluation.[3,4] This structured approach has enabled earlier diagnosis and improved access to treatment.

Before nationwide screening, incidence estimates in Türkiye were based on regional studies, ranging from 1 in 2736 to 1 in 2326 live births.[5,6] Following the program's launch, reported CH incidence rose sharply—reaching 1 in 888 in 2008 and 1 in 469 in 2010.[7] Similar increases occurred in other countries such as the United States and Canada, likely due to enhanced screening protocols and lower TSH thresholds.[8,9] In Türkiye, the screening TSH cut-off was initially set at 20 µIU/mL and later reduced to 10 µIU/mL, allowing earlier detection of borderline and subclinical cases.[10]

As sensitivity has improved, an increasing proportion of infants diagnosed with CH are now identified as having transient rather than permanent disease. This shift is particularly evident in regions where iodine deficiency or perinatal factors contribute to temporary disruptions in thyroid function.[1] Turkish studies report wide variation in the proportion of transient CH among screen-positive infants. For instance, Kara et al. found that 52% of treated infants were ultimately classified as transient, while Asena et al. reported a rate of 24% in a different regional cohort.[10,11] Such variability highlights the importance of early distinction to avoid unnecessary prolonged treatment.

Current guidelines recommend re-evaluation after age three for children without definitive diagnosis, usually via monitored LT4 withdrawal.[1,2] However, in clinical practice, early differentiation remains challenging, as both transient and permanent CH can initially present with similar biochemical profiles. While imaging findings such as thyroid agenesis or ectopy can suggest a permanent etiology, most screen-positive infants have eutopic glands, making diagnosis less straightforward.[10,12,13]

In recent years, treatment response—particularly levothyroxine dosage during follow-up—has been explored as a potential surrogate marker for predicting disease course. Several studies have demonstrated that lower levothyroxine doses at specific intervals are associated with transient CH, though the proposed cut-off values and predictive accuracies vary.[11,14]

This study aimed to evaluate the incidence, clinical characteristics, and outcomes of infants referred with abnormal TSH through newborn screening in the Muğla province of Türkiye over three years. Particular attention is given to the distribution of transient and permanent CH and to the evaluation of early treatment parameters—specifically levothyroxine dose at six months—as potential predictors of disease permanence.

Methods

This retrospective cohort study was conducted to evaluate the incidence and characteristics of CH diagnosed through the neonatal screening program in Muğla province between January 1, 2020, and December 31, 2022. Data were obtained from hospital records of infants who were referred due to elevated TSH levels (>10 µIU/mL) on neonatal screening and were subsequently evaluated clinically and biochemically by a pediatric endocrinologist.

Neonatal screening in Türkiye is a nationwide, government-funded program initiated by the Turkish Ministry of Health, which screens all newborns for congenital hypothyroidism using TSH measurement from heel-prick blood samples collected within the first 48-72 hours of life. The TSH threshold was progressively lowered over time, and a cut-off of 10 µIU/mL is currently used to prompt referral for further evaluation.[7,10]

Live birth data for the corresponding years were obtained from the Turkish Statistical Institute and used to calculate annual incidence rates. [15-17]

For each infant, demographic, clinical, and biochemical parameters were recorded, including sex, birth weight, age at diagnosis, TSH and free T4 levels at presentation, maternal and family history of hypothyroidism, and thyroid ultrasonography findings. Levothyroxine treatment was initiated in infants with persistent TSH elevation (>10 µIU/mL) and/or decreased free T4 levels, in accordance with guidelines.[1,2] The initial LT4 dose ranged between 10-15 µg/kg/day depending on the severity of hypothyroidism.

Patients were classified into two main categories based on their clinical evaluation and treatment course. The first group consisted of infants who were diagnosed with congenital hypothyroidism and initiated on levothyroxine therapy due to persistently elevated TSH levels and/or low free T4 concentrations. The second group included those who exhibited transient neonatal TSH elevation but did not require any treatment following confirmatory testing, as their thyroid function normalized spontaneously without pharmacologic intervention.

Among treated patients, a further distinction was made between permanent and transient CH based on follow-up findings. Children were followed with regular clinical and biochemical assessments. At approximately 3 years of

age, a trial off therapy was performed by discontinuing LT4 for 4–6 weeks, followed by evaluation of serum TSH and free T4 levels. CH was considered permanent if abnormal thyroid function persisted after treatment cessation or if structural thyroid abnormalities (agenesis, ectopy, hypoplasia) were evident on imaging.[1,2] Conversely, CH was defined as transient in children who demonstrated normal thyroid function and did not require LT4 re-initiation during follow-up.

This study was approved by the Medical Sciences Ethics Committee of Muğla Sıtkı Koçman University (Decision date: December 5, 2024; Protocol No: 240222; Decision No: 157).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using both visual methods (histograms, probability plots) and analytical tests (Kolmogorov-Smirnov and Shapiro-Wilk). Variables with normal distribution were presented as mean ± standard deviation, while non-normally distributed variables were presented as median and interquartile range (IQR). Categorical variables were expressed as percentages. Group comparisons were conducted using the independent samples t-test or Mann–Whitney U test for continuous variables and the Chi-square or Fisher's exact test for categorical variables. A p-value of <0.05 was considered statistically significant. The Youden index was used to determine the optimal cut-off value for levothyroxine (LT4) dose in the ROC curve analysis.

Results

Live birth data for the corresponding years were retrieved from the Turkish Statistical Institute (TÜİK): 9,952 in 2020, 9,643 in 2021, and 9,256 in 2022. The annual incidence of CH was calculated by dividing the number of confirmed cases by the total number of live births per year and multiplying by 100,000.

A total of 95 infants were included in the study. Of these, 33 (34.7%) were diagnosed with congenital hypothyroidism and started on levothyroxine therapy, while 62 (65.3%) were followed without treatment due to transient neonatal TSH elevation. Among the 33 treated cases, 19 (57.6%) were classified as permanent CH and 14 (42.4%) as transient CH after follow-up (Table 1).

The mean birth weight of the cohort was 3,169 ± 459.7 grams, and all infants were born at term. The median age at first evaluation was 16 days (IQR: 12). The yearly incidence of CH was 160.7 per 100,000 live births in 2020, 72.6 in 2021, and 108.0 in 2022. The average annual CH incidence over the three years was 114.4 per 100,000 live births, equivalent to 1 in 874. The mean annual incidence of permanent CH was 65.9 per 100,000 (1/1,517), and for transient CH, it was 48.5 per 100,000 (1/2,062).

Thyroid ultrasonography was available for 35 treated infants; 32 had normal thyroid anatomy, 2 had thyroid agenesis, and 1 had thyroid hypoplasia. The median TSH and free T4 values at diagnosis were 81.9 mIU/L (IQR: 48) and 8.6 ng/dL (IQR: 5.4) in treated cases, compared to 13.2 mIU/L (IQR: 8.1) and 18 ng/dL (IQR: 3.4) in untreated cases (p<0.001 for both) (Table 1).

The median treatment initiation age was 20 days (IQR: 16), and the mean starting dose of levothyroxine was 10.9 ± 4.1 µg/kg/day. At 6 months, the mean dose was 2.3 ± 0.5 µg/kg/day in the transient group and 3.6 ± 0.7 µg/kg/day in the permanent group (p<0.001) (Table 2). The ROC curve analysis revealed that a levothyroxine dose ≤3.35 µg/kg/day at 6 months predicted transient CH with 100% sensitivity and 63.2% specificity (AUC: 0.925; 95% CI: 0.84–1.00; p<0.001) (Figure 1).

Table 1. Comparison of demographic and clinical characteristics between infants diagnosed with Congenital Hypothyroidism and those with transient neonatal TSH elevation

Parameter	CH Group (n = 33)	Transient TSH Elevation (n = 62)	p-value
Sex (Female/Male)	18 / 15	22 / 40	0.07
Family history of hypothyroidism (%)	9 (27.3%)	11 (17.7%)	0.27
Maternal hypothyroidism history (%)	11 (33.3%)	32 (51.6%)	0.08
Birth weight (g), mean ± SD	3145 ± 487.5	3182 ± 447.7	0.61
Age at initial evaluation (days), median (IQR)	20 (14.5)	14 (9.2)	**<0.001**
TSH (mIU/L), median (IQR)	81.9 (48.0)	13.2 (8.1)	**<0.001**
Free T4 (ng/dL), median (IQR)	8.6 (5.4)	18.0 (3.4)	**<0.001**
Thyroid ultrasound findings (n)	28	7	**<0.001**
Normal	25	7	
Agenesis	2	0	
Hypoplasia	1	0	

P-values were calculated using the Mann–Whitney U test for non-normally distributed variables (presented as median and IQR) and the independent samples t-test for normally distributed variables (presented as mean ± SD).

IQR: interquartile range, SD: Standard deviation, CH: Congenital Hypothyroidism, TSH: thyroid-stimulating hormone

Table 2. Comparison between permanent and transient congenital hypothyroidism within the treated group

Parameter	Transient CH (n = 14)	Permanent CH (n = 19)	p-value
Sex (Female/Male)	9 / 5	9 / 10	0.33
Family history of hypothyroidism (%)	4 (28.6%)	5 (26.3%)	0.88
Maternal hypothyroidism history (%)	5 (37.4%)	6 (31.6%)	0.80
Birth weight (g), mean ± SD	3174 ± 464	3124 ± 515	0.07
Age at initial evaluation (days), median (IQR)	17 (13.7)	26 (14.0)	0.08
TSH (mIU/L), median (IQR)	72.5 (71.0)	95.0 (41.0)	0.21
Free T4 (ng/dL), median (IQR)	8.1 (5.6)	8.7 (6.2)	0.82
Thyroid ultrasound findings (n)	14	14	0.05
Normal	14	11	
Agenesis	0	2	
Hypoplasia	0	1	
Initial levothyroxine dose (µg/kg/day), mean ± SD	11.5 ± 4.6	10.5 ± 3.6	0.49
LT4 dose at 6 months (µg/kg/day), mean ± SD	2.3 ± 0.5	3.6 ± 0.7	**<0.001**

P-values were calculated using the Mann–Whitney U test for non-normally distributed variables (presented as median and IQR) and the independent samples t-test for normally distributed variables (presented as mean ± SD).
IQR: interquartile range, SD: Standard deviation, CH: Congenital Hypothyroidism, TSH: thyroid-stimulating hormone, LT4: levothyroxine

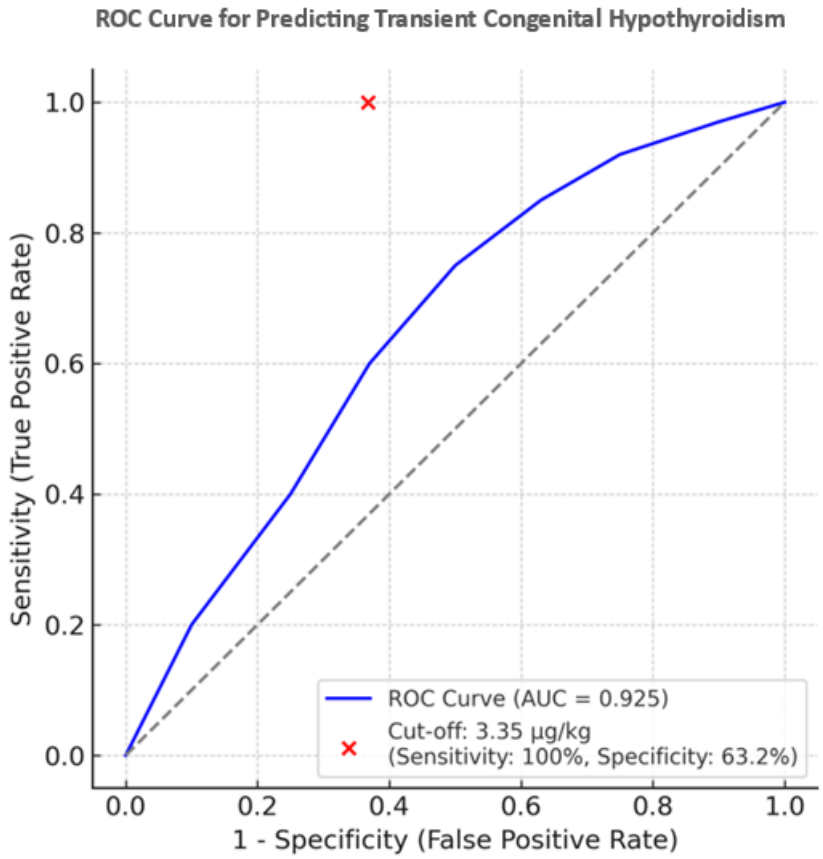


Figure 1. Receiver Operating Characteristic (ROC) Curve for Predicting Transient Congenital Hypothyroidism Based on the Levothyroxine Dose at 6 Months. The optimal cut-off value of the levothyroxine dose at 6 months for predicting transient congenital hypothyroidism was determined to be 3.35 µg/kg/day, with a sensitivity of 100%, specificity of 63.2%, and an area under the curve (AUC) of 0.925 (95% confidence interval: 0.84-1.00; p<0.001).

Discussion

The incidence of CH observed in this province during the years 2020-2022 was approximately 114 per 100,000 live births (1 in 874), which is notably higher than the classically reported global range of 1:2000 to 1:3000 births. This elevated incidence is consistent with previously published national screening reports from Türkiye and with findings from other iodine-deficient regions, such as parts of Iran, where incidence rates have been reported as high as 1:500 to 1:800.[7,18] In Türkiye, national newborn screening data showed a marked increase in CH detection after the implementation of universal screening in 2006, with incidence rates rising from 1:888 in 2008 to 1:469 in 2010.[7] Our findings align with this trend and may reflect both methodological changes and region-specific risk factors.

A key factor contributing to the rising CH incidence is the progressive lowering of TSH cut-off values used in neonatal screening. In Türkiye, the threshold was reduced from 20 to 10 µIU/mL, increasing the likelihood of detecting milder or transient forms of CH.[10] Similar effects have been observed internationally in countries that lowered screening thresholds.[9] Additionally, factors such as prematurity and low birth weight-frequently observed in regions with limited prenatal care-have been associated with increased rates of false-positive results in newborn screening programs, likely due to immature hypothalamic-pituitary-thyroid axis function.[18] Genetic and familial factors also play a role, especially in populations with high rates of consanguinity, where dyshormonogenesis and other hereditary defects are more prevalent.[19]

In our cohort, 57.6% of infants diagnosed with CH were classified as having permanent hypothyroidism, while 42.4% had transient CH. This distribution closely mirrors the findings of other Turkish studies, which report transient CH rates between 40% and 60% among screen-detected cases.[10,11] Notably, a recent multicenter Turkish study also reported a 53.6% rate of transient CH, highlighting the consistency of these rates across different regions and emphasizing the national relevance of our findings.[20]

A major challenge in clinical practice is distinguishing transient from permanent CH early in the course of treatment, ideally before age three. Identifying infants who do not require lifelong LT4 therapy can prevent overtreatment and reduce caregiver burden. In our study, we evaluated the predictive value of LT4 dose at 6 months of age. ROC curve analysis identified a cut-off value of 3.35 µg/kg/day, with 100% sensitivity and 63.2% specificity for predicting transient CH (AUC: 0.925). This result is consistent with existing literature showing that lower LT4 requirements in early infancy are associated with transient disease. For example, Oron et al. reported that a 6-month LT4 dose of ≤2.2 µg/kg/day predicted transient CH with moderate sensitivity and specificity.[21] Similarly, Özer et al. found that an LT4 dose of <2.0 µg/kg/day at the time of treatment discontinuation predicted transient CH with high specificity (94.5%), further validating the role of LT4 dosing in guiding clinical decisions.[20] Chen et al., in a large cohort study, proposed a 24-month LT4 dose threshold of <2.9 µg/kg/day for predicting transient CH.[22]

While our cut-off value of 3.35 µg/kg/day demonstrated excellent sensitivity, its specificity was moderate, indicating that some infants with permanent CH also maintained euthyroidism on low LT4 doses by 6 months. In our cohort, approximately one-third of permanent CH cases had LT4 needs below the identified threshold. This limitation has been similarly reported in other studies, where infants with mild permanent CH-especially those with eutopic thyroid glands-may initially appear transient.[20,21] Moreover, up to 62-86% of infants with mildly elevated TSH and normal ultrasound findings have been shown to have permanent hypothyroidism upon long-term follow-up.[13] These findings highlight that no early marker is perfectly reliable when used in isolation, and that clinical, biochemical, and imaging data must be integrated cautiously. Current international guidelines endorse a cautious approach. The American Academy of Pediatrics (AAP) recommends that in all cases where a definitive diagnosis of permanent hypothyroidism has not been established, LT4 treatment should be continued until 3 years of age, followed by a 4- to 6-week discontinuation to reassess thyroid function.[23] Similarly, the European Society for Pediatric Endocrinology (ESPE) suggests that a child with a eutopic gland and a 6-month LT4 dose below 3.0 µg/kg/day may be considered for earlier re-evaluation, though only under close clinical supervision.[2] In our study, most clinicians adhered to a conservative approach; the median age for treatment discontinuation among transient CH cases was approximately 2.4 years. This practice balances patient safety with the desire to avoid unnecessary prolonged therapy and is in line with international recommendations. Nevertheless, monitoring LT4 requirements throughout infancy remains a valuable tool to support individualized care planning.

In summary, our findings from Muğla province confirm a relatively high incidence of congenital hypothyroidism, consistent with national data from Turkey and reports from other iodine-deficient populations.[9,10,18] A substantial proportion of cases (42.4%) were transient, emphasizing the importance of distinguishing between transient and permanent forms to optimize treatment duration. Our analysis supports the use of LT4 dose at six months of age as a practical and sensitive early predictor of transient CH, in agreement with both national and international studies.[11,20,21] While early dose thresholds should not replace formal re-evaluation at 2–3 years of age, they can provide valuable guidance for individualized follow-up.

To our knowledge, this study is among the few that have comprehensively evaluated CH incidence and treatment outcomes in the southwestern Aegean region of Türkiye, providing valuable regional data to complement national reports. These findings reinforce the clinical relevance of early dose monitoring and may support the development of individualized follow-up protocols. A key limitation of

our study is its retrospective, single-center design and relatively small sample size, which may affect generalizability. In addition, iodine status and genetic testing data were not available, limiting the ability to determine underlying etiologies. Long-term neurodevelopmental outcomes were also not assessed, which may have provided a more comprehensive evaluation of treatment efficacy. Further multicenter studies with long-term follow-up are warranted to validate early predictive markers and improve CH management strategies across different populations.

Ethical Approval

This study was approved by the Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (Date: 05.12.2024, Protocol No: 240222, Decision No: 157). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Conflict of Interest

There is no conflict of interest to declare.

Author Contributions

GCY: Study conception and design, data analysis, manuscript drafting, final approval; GG: Data collection, literature review, manuscript revision; ES: Methodological supervision, interpretation of results, critical revision of the manuscript.

Financial Support

None

References

1. Van Trotsenburg P, Stoupa A, Léger J, et al. Congenital Hypothyroidism: A 2020-2021 Consensus Guidelines Update-An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid*. 2021;31(3):387-419. doi:10.1089/thy.2020.0333
2. Léger J, Olivieri A, Donaldson M, et al. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. *J Clin Endocrinol Metab*. 2014;99(2):363-384. doi:10.1210/jc.2013-1891
3. T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü. Ulusal Yenidoğan Tarama Programı [İnternet]. Erişim: https://hsgm.saglik.gov.tr/tr/tarama-programlari/ntp.html. Erişim tarihi: 15 Nisan 2025.
4. T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü. TSH tarama ve yönlendirme akış şeması [İnternet]. Erişim: https://hsgm.saglik.gov.tr/depo/birimler/cocuk-ergen-sagligi-db/Programlar/TSH_Akis_Semasi.pdf. Erişim tarihi: 15 Nisan 2025.
5. Yordam N, Calikoğlu AS, Hatun S, et al. Screening for congenital hypothyroidism in Turkey. *Eur J Pediatr*. 1995;154(8):614-616. doi:10.1007/BF02079061
6. Simşek E, Karabay M, Safak A, Kocabay K. Congenital hypothyroidism and iodine status in Turkey: a comparison between the data obtained from an epidemiological study in school-aged children and neonatal screening for congenital hypothyroidism in Turkey. *Pediatr Endocrinol Rev*. 2003;1 Suppl 2:155-161.
7. Dilli D, Özbaş S, Acıcan D, Yamak N, Ertek M, Dilmen U. Establishment and development of a national newborn screening programme for congenital hypothyroidism in Turkey. *J Clin Res Pediatr Endocrinol*. 2013;5(2):73-79. doi:10.4274/Jcrpe.929
8. Harris KB, Pass KA. Increase in congenital hypothyroidism in New York State and in the United States [published correction appears in *Mol Genet Metab*. 2008 May;94(1):140]. *Mol Genet Metab*. 2007;91(3):268-277. doi:10.1016/j.ymgme.2007.03.012
9. Saleh DS, Lawrence S, Geraghty MT, et al. Prediction of congenital hypothyroidism based on initial screening thyroid-stimulating-hormone. *BMC P*ediatr. 2016;16:24. doi:10.1186/s12887-016-0559-0
10. Kara C, Günindi F, Can Yılmaz G, Aydın M. Transient congenital hypothyroidism in Turkey: An analysis on frequency and natural course. *J Clin Res Pediatr Endocrinol*. 2016;8(2):170-179. doi:10.4274/jcrpe.2345
11. Asena M, Demiral M, Unal E, Öcal M, Demirbilek H, Özbek MN. Validity of six month L-Thyroxine dose for differentiation of transient or permanent congenital hypothyroidism. *J Clin Res Pediatr Endocrinol*. 2020;12(3):275-280. doi:10.4274/jcrpe.galenos.2020.2019.0170
12. Oron T, Lazar L, Ben-Yishai S, et al. Permanent vs transient congenital hypothyroidism: Assessment of predictive variables. *J Clin Endocrinol Metab*. 2018;103(12):4428-4436. doi:10.1210/jc.2018-00362
13. Rabbiosi S, Vigone MC, Cortinovis F, et al. Congenital hypothyroidism with eutopic thyroid gland: analysis of clinical and biochemical features at diagnosis and after re-evaluation. *J Clin Endocrinol Metab*. 2013;98(4):1395-1402. doi:10.1210/jc.2012-3174
14. Kang MJ, Chung HR, Oh YJ, Shim YS, Yang S, Hwang IT. Three-year follow-up of children with abnormal newborn screening results for congenital hypothyroidism. *Pediatr Neonatol.* 2017;58(5):442-448. doi:10.1016/j.pedneo.2017.01.002
15. Türkiye İstatistik Kurumu (TÜİK). (2021, Mayıs 18). Birth Statistics, 2020 [Bülten No: 37229]. https://data.tuik.gov.tr/Bulten/Index?p=Birth-Statistics-2020-37229
16. Türkiye İstatistik Kurumu (TÜİK). (2022, Mayıs 24). Birth Statistics, 2021 [Bülten No: 45514]. https://data.tuik.gov.tr/Bulten/Index?p=Birth-Statistics-2021-45514
17. Türkiye İstatistik Kurumu (TÜİK). (2023, Mayıs 25). Birth Statistics, 2022 [Bülten No: 53720]. https://data.tuik.gov.tr/Bulten/Index?p=Birth-Statistics-2022-53720
18. Mehran L, Khalili D, Yarahmadi S, et al. Evaluation of the congenital hypothyroidism screening programme in Iran: a 3-year retrospective cohort study. *Arch Dis Child Fetal Neonatal Ed.* 2019;104(2):F176-F181. doi:10.1136/archdischild-2017-313720
19. Tanyeri D, Anık A, Cengiz A, Polat YD, Ünüvar T, Anık A. Etiological evaluation of congenital hypothyroidism in cases referred from the national screening program. *The Journal of Pediatric Research*. 2021;8(1):1-6.
20. Özer Y, Anık A, Sayılı U, et al. High frequency of transient congenital hypothyroidism among infants referred for suspected congenital hypothyroidism from the Turkish National screening program: thyroxine dose may guide the

prediction of transients. *J Endocrinol Invest*. 2024;47(9):2213-2224. doi:10.1007/s40618-024-02348-9

21. Oron T, Lazar L, Ben-Yishai S, et al. Permanent vs transient congenital hypothyroidism: assessment of predictive variables. *J Clin Endocrinol Metab*. 2018;103(12):4428-4436. doi:10.1210/jc.2018-00362
22. Chen SH, Yang BC, Li JY, Xu P, Wang F. Diagnostic re-evaluation and predictors of congenital hypothyroidism with eutopic thyroid gland in Jiangxi, China. *J Pediatr Endocrinol Metab*. 2021;34(9):1139-1146. doi:10.1515/jpem-2020-0733
23. Rose SR, Wassner AJ, Wintergerst KA, et al. Congenital hypothyroidism: screening and management. *Pediatrics*. 2023;151(1):e2022060420. doi:10.1542/peds.2022-060420

doi: 10.53446/actamednicomedia.1693078

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Araştırma Makalesi | Research Article

METABOLİK SENDROMUN ÖTESİNDE: METS-IR, TYG İNDEKSİ VE EPİKARDİYAL YAĞ DOKUSUNUN FARKLI VASKÜLER YATAKLARDAKİ SUBKLİNİK ATEROSKLEROZLA İLİŞKİSİ

BEYOND METABOLIC SYNDROME: THE RELATIONSHIP OF METS-IR, TYG INDEX, AND EPICARDIAL ADIPOSE TISSUE WITH SUBCLINICAL ATHEROSCLEROSIS IN DIFFERENT VASCULAR BEDS

Şenol Coşkun[1,2*]

[1]Kocaeli Sağlık ve Teknoloji Üniversitesi, Kocaeli. [2]Özel Bursa Acıbadem Hastanesi, Bursa.

ÖZ

Amaç: Bu çalışmada, metabolik insülin direnci göstergeleri (METS-IR ve TyG indeksi) ile epikardiyal yağ dokusu (EYD) miktarının, subklinik ateroskleroz göstergeleri olan koroner arter kalsiyumu (CAC) ve karotis intima-media kalınlığı (IMT) ile ilişkisi değerlendirildi.

Yöntem: Koroner arter hastalığı şüphesiyle değerlendirilen 108 hastanın retrospektif verileri analiz edildi. METS-IR ve TyG skorları laboratuvar ve antropometrik verilerden hesaplandı; EYD kalınlığı ve hacmi bilgisayarlı tomografi ile ölçüldü. CAC skoru ve karotis IMT standart yöntemlerle değerlendirildi. İlişkiler korelasyon ve çok değişkenli regresyon analizleriyle incelendi, ROC analizi ile tanısal performans değerlendirildi.

Bulgular: Epikardiyal yağ kalınlığı (AUC=0,802) ve hacmi (AUC=0,781), CAC varlığını öngörmede yüksek ayırt edicilik gösterdi. EYD kalınlığı için belirlenen 5,9 mm eşik değeri %75 duyarlılık ve %71 özgüllük sağladı. METS-IR skoru hem CAC (AUC=0,670) hem de karotis IMT ile bağımsız ilişkili bulunurken, TyG indeksi sadece CAC ile zayıf bir ilişki gösterdi (AUC=0,661). Çok değişkenli analizlerde, EYD ölçümleri ve METS-IR skoru, konvansiyonel risk faktörlerinden bağımsız olarak subklinik ateroskleroz belirteçleriyle anlamlı ilişkiler gösterdi.

Sonuç: METS-IR, TyG ve özellikle epikardiyal yağ ölçümleri, subklinik aterosklerozun erken tanısında potansiyel biyobelirteçlerdir. Bu parametrelerin bireyselleştirilmiş kardiyovasküler risk değerlendirmelerine entegrasyonu, ileri çalışmalarla desteklenmelidir

Anahtar kelimeler: METS-IR, TyG İndeksi, Epikardiyal Yağ Dokusu, Subklinik Ateroskleroz, Koroner Arter Kalsiyumu

ABSTRACT

Objective: This study aimed to evaluate the relationship between metabolic insulin resistance indicators (METS-IR and TyG index) and the amount of epicardial adipose tissue (EAT), with subclinical atherosclerosis markers such as coronary artery calcium (CAC) and carotid intima-media thickness (IMT).

Methods: Retrospective data from 108 patients evaluated for suspected coronary artery disease were analyzed. METS-IR and TyG scores were calculated using laboratory and anthropometric data. EAT thickness and volume were measured by computed tomography. CAC score and carotid IMT were assessed using standard methods. Correlations and multivariate regression analyses were used to examine associations, and ROC analysis was performed to evaluate diagnostic performance.

Results: Epicardial fat thickness (AUC=0.802) and volume (AUC=0.781) showed high discriminative power in predicting the presence of CAC. A threshold value of 5.9 mm for EAT thickness provided 75% sensitivity and 71% specificity. The METS-IR score was independently associated with both CAC (AUC=0.670) and carotid IMT, while the TyG index showed only a weak association with CAC (AUC=0.661). In multivariate analyses, EAT measurements and METS-IR scores were significantly associated with subclinical atherosclerosis markers, independent of conventional risk factors.

Conclusion: METS-IR, TyG, and particularly epicardial fat measurements appear to be potential biomarkers for the early detection of subclinical atherosclerosis. Integration of these parameters into individualized cardiovascular risk assessments should be supported by further studies.

Keywords: METS-IR, TyG Index, Epicardial Adipose Tissue, Subclinical Atherosclerosis, Coronary Artery Calcium

***Corresponding author/İletişim kurulacak yazar:** Şenol Coşkun; Kocaeli Sağlık ve Teknoloji Üniversitesi, Kocaeli. Özel Bursa Acıbadem Hastanesi, Bursa.
Phone/Telefon: +90 (533) 238 76 04, **e-mail/e-posta:**scoskun2@yahoo.com
Submitted/Başvuru: 07.05.2025 • **Accepted/Kabul:** 14.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Giriş

Aterosklerotik süreç, uzun yıllar belirti vermeden ilerleyerek ciddi kardiyovasküler olaylara zemin hazırlar. Subklinik aterosklerozun erken tespiti, risk altındaki bireylerin belirlenmesi ve koruyucu stratejilerin uygulanabilmesi için kritik öneme sahiptir. Bu amaçla, koroner arter kalsiyumu (CAC) skoru ve karotis intima-media kalınlığı (IMT) gibi non-invaziv görüntüleme yöntemleri, farklı vasküler yataklardaki subklinik aterosklerotik yükü değerlendirmede yaygın olarak kullanılmaktadır.[1,2]

İnsülin direnci (İD) ve visseral adipozite, ateroskleroz gelişiminde temel belirleyicilerdir.[3,4] İD, metabolik sendromun temel bileşenlerini tetikleyerek vasküler inflamasyonu artırır; visseral yağ dokusu ise aktif bir endokrin organ gibi davranarak inflamatuar ve pro-aterojenik mediatörler üretir.[4] Bu süreçler hem sistemik hem de lokal düzeyde damar sağlığını olumsuz etkiler.

İnsülin direncini pratik şekilde yansıtmak için geliştirilen Trigliserid-Glukoz İndeksi (TyG) ve daha yeni bir parametre olan Metabolik İnsülin Direnci Skoru (METS-IR), kardiyometabolik riskin belirlenmesinde dikkat çekmektedir.[5–10] METS-IR, vücut kitle indeksi ve HDL-kolesterolü de hesaba katarak insülin direncini daha kapsamlı yansıtmayı hedefler.

Öte yandan, epikardiyal yağ dokusu (EYD) da kalp çevresinde bulunan ve koroner arterlerle doğrudan ilişkili olan, metabolik olarak aktif bir visseral yağ depolamasıdır.[11] Artmış EYD kalınlık ve hacmi, koroner arter hastalığı, koroner kalsifikasyon ve majör kardiyak olaylarla ilişkilendirilmiştir.[12–16]

Her bir parametrenin (METS-IR, TyG, EYD) subklinik aterosklerozla ilişkisi farklı çalışmalarda gösterilmiş olmakla birlikte, bu belirteçlerin birlikte değerlendirilerek hangi vasküler yatakta daha güçlü öngörü sağladığı ve geleneksel risk faktörlerinden bağımsız katkıları tam olarak aydınlatılmamıştır.

Bu nedenle, bu çalışmanın amacı, koroner arter hastalığı şüphesiyle BT koroner anjiyografi yapılan bireylerde, METS-IR skoru, TyG indeksi ve epikardiyal yağ ölçümlerinin, CAC skoru ve karotis IMT ile bağımsız ilişkilerini araştırmaktır.

Yöntem

Çalışma Popülasyonu: Tek merkezli retrospektif kohort çalışmamıza, Ocak 2015 – Ocak 2024 tarihleri arasında Özel Bursa Acıbadem Hastanesi'ne KAH şüphesi (örn. tipik/atipik göğüs ağrısı, efor dispnesi, pozitif stres testi veya çoklu risk faktörü varlığı) ile başvurup BTKA ve karotis Doppler ultrasonografisi yapılan 108 ardışık hasta dahil edildi. Dahil edilme kriterleri: ≥18 yaş olmak, klinik endikasyonla BTKA yapılmış olması, BTKA ile eş zamanlı veya 3 ay içinde karotis ultrasonu bulunması, METS-IR ve TyG hesaplamaları için gerekli açlık kan şekeri, trigliserid, HDL-K ve VKİ verilerinin mevcut olması, EYD kalınlık/hacim ve CAC ölçümü için uygun kalitede BT görüntülerinin olmasıydı. Dışlama kriterleri: Bilinen KAH öyküsü (miyokard infarktüsü veya revaskülarizasyon geçirmiş olmak), akut koroner sendrom bulguları, ciddi kalp kapak hastalığı, belirgin kardiyomiyopati veya konjenital kalp hastalığı, aktif enfeksiyon ya da inflamatuar hastalık, malignite öyküsü, ileri böbrek yetmezliği (eGFR < 30 mL/dk/1.73 m²), BT görüntülerinde belirgin artefakt varlığı veya eksik veri bulunması olarak belirlendi. Bu kriterlere uyan tüm hastalar çalışma kapsamına alındı. Çalışma, Helsinki Deklarasyonu'na uygun yürütüldü.

Görüntüleme ve Ölçümler: Tüm hastalarda kontrastsız kardiyak BT ile koroner arter kalsiyum skoru (CAC) elde edildi. CAC skoru, Agatston yöntemiyle 3 mm kesit kalınlığında aksiyel görüntülerde hesaplandı.[1] CAC skorunun >0 olması koroner kalsifikasyon varlığı olarak tanımlandı. Kontrastlı BTKA ile koroner arter darlık dereceleri değerlendirildi. Her hasta için en ciddi darlık derecesine göre KAH ciddiyet skoru şu şekilde sınıflandırıldı: 0 = anlamlı plak yok; 1 = <%50 luminal darlık (non-obstrüktif plak); 2 = %50–69 darlık; 3 = ≥%70 darlık (obstrüktif).[14,17] Bu sınıflama CAD-RADS raporlama sistematiği temel alınarak yapıldı.[17] Epikardiyal yağ dokusu, BTKA görüntülerinin aksiyel kesitlerinde perikardın visseral tabakası ile miyokard arasındaki düşük atenüasyonlu yağ dokusu olarak tanımlandı. EYD kalınlığı, sağ ventrikül serbest duvarı üzerinde perikardiyal sınırdan miyokarda dik olarak ölçülen en büyük kalınlık olarak (mm cinsinden) belirlendi. EYD hacmi, kontrastlı BTKA aksiyel kesitlerinde perikard içi tüm yağ dokusunun planimetrik yöntemle konturlanması ve üç boyutlu rekonstrüksiyonuyla (mL cinsinden) hesaplandı. Karotis IMT, yüksek çözünürlüklü B-mod ultrason ile, sağ ve sol ortak karotis arter distal duvarında, karotis bifürkasyonuna yakın 1 cm'lik segmentin ortalama intima-media kalınlığı olarak ölçüldü.

Laboratuvar ve İndeks Hesaplamaları: Hastaların antropometrik ölçümleri (boy, kilo, VKİ) ve açlık laboratuvar değerleri (glukoz, lipid paneli) hastane kayıtlarından temin edildi. METS-IR ve TyG indeksleri aşağıdaki formüller kullanılarak hesaplandı:

METS-IR = [Ln ((2 * AKŞ) + TG) * VKİ] / Ln(HDL-K)
TyG indeksi = Ln ((TG * AKŞ) / 2)

İstatistiksel Analiz

Çalışmada verileri özetlemek amacıyla temel tanımlayıcı istatistikler kullanıldı. Verilerin normal dağılıp dağılmadığı Kolmogorov-Smirnov testi ile kontrol edildi ve normal dağılmayan değişkenler (örn. CAC skoru) için logaritmik transformasyon gibi uygun yöntemler uygulandı. Değişkenler arasındaki ilişkileri değerlendirmek için Spearman korelasyon analizi yapıldı. Koroner kalsiyum varlığı (CAC>0), herhangi bir koroner arter hastalığı varlığı (KAH≥1) ve karotis intima-media kalınlığı (IMT) ile ilişkili faktörleri belirlemek amacıyla lojistik ve lineer regresyon analizleri kullanıldı. Bu analizlerde özellikle METS-IR, TyG indeksi ve epikardiyal yağ dokusu (EYD) ölçümlerinin bağımsız etkileri, yaş, cinsiyet, diyabet ve hipertansiyon gibi potansiyel karıştırıcı faktörler kontrol edilerek değerlendirildi. Ek olarak, METS-IR, TyG ve EYD

ölçümlerinin koroner kalsiyum varlığını (CAC>0) ayırt etme performansını karşılaştırmak için ROC (Receiver Operating Characteristic) eğrisi analizi yapıldı ve eğri altında kalan alanlar (AUC) hesaplandı. İstatistiksel anlamlılık düzeyi p<0,05 olarak kabul edildi.

Bulgular

1. Katılımcıların Temel Özellikleri (n = 108)

Çalışmaya dahil edilen 108 katılımcının temel demografik ve klinik özellikleri Tablo 1'de sunulmuştur.

Tablo 1. Katılımcıların Temel Özellikleri

Kategori	Değer
Cinsiyet	Erkek %61 (66) Kadın %39 (42)
Yaş (yıl)	51,8 ± 9,5
Tip 2 Diyabet (Var)	%19 (21)
Hipertansiyon (Var)	%62 (67)
Sigara (Var)	%38 (41)
Vücut Kitle İndeksi (VKİ kg/m ²)	30,7 ± 5,8
Açlık Glukoz (mg/dL)	108 ± 30
Trigliserid (mg/dL)	169 ± 94
HDL Kolesterol (mg/dL)	42 ± 11
TyG İndeksi	8,8 ± 0,6
METS-IR Skoru	2,39 ± 0,21
Epikardiyal Yağ Kalınlığı (mm)	6,5 ± 2,0
Epikardiyal Yağ Hacmi (mL)	110 ± 41
Koroner Arter Kalsiyum (CAC > 0)	%49 (53)
CAC Skoru (Pozitif Olanlarda)	Medyan 57 (10–232)
Koroner Plak Varlığı (≥1)	%44 (47)
Plak Darlık Derecesi	28 < %50, 11 %50–69 8 ≥ %70
Karotis IMT (mm)	0,90 ± 0,21*
IMT ≥ 1 mm Olanlar	%31

*Not: Değerler ortalama ± standart sapma, medyan (çeyrekler arası aralık) veya yüzde (sayı) olarak verilmiştir. VKİ: Vücut Kitle İndeksi; TyG: Trigliserid-Glukoz; METS-IR: Metabolik İnsülin Direnci Skoru; EYD: Epikardiyal Yağ Dokusu; CAC: Koroner Arter Kalsiyum; KAH: Koroner Arter Hastalığı; IMT: İntima-Media Kalınlığı. IMT ölçümü 90 hastada mevcuttu.

Katılımcıların temel demografik, klinik ve laboratuvar özellikleri Tablo 1'de özetlenmiştir. Kohortun çoğunluğunu (%61) erkekler oluşturmakta olup, ortalama yaş 51,8 (±9,5) yıldır. Tip 2 diyabet, hipertansiyon öyküsü ve sigara kullanımı oranları sırasıyla %19, %62 ve %38'dir. Obezite prevalansı %40 olup, ortalama VKİ 30,7 (±5,8) kg/m²'dir. İnsülin direnci belirteçleri olarak ortalama TyG indeksi 8,8 (±0,6) ve METS-IR skoru 2,39 (±0,21) bulunmuştur.

Epikardiyal yağ kalınlığı ve hacmi sırasıyla 6,5 (±2,0) mm ve 110 (±41) mL olarak ölçüldü. Katılımcıların %49'unda CAC skoru >0 bulunurken, medyan kalsiyum skoru 57 (IQR 10–232) idi. BT anjiyografide %44 oranında koroner plak saptandı; bunların çoğunluğu non-obstrüktif plaklardı. IMT ölçülebilen hastalarda ortalama IMT 0,90 (±0,21) mm olup, %31'inde değer 1,0 mm'nin üzerindeydi (Tablo 1).

2. Değişkenler Arasındaki Önemli İlişkiler (Korelasyonlar)

Ölçümler arasındaki ilişkiler Spearman korelasyon analizi ile incelendi; anlamlı ilişkiler (p<0,05) Şekil 1'de gösterildi. İstatiksel analizler, epikardiyal yağ ölçümleri ile ateroskleroz göstergeleri arasında anlamlı ilişkiler olduğunu gösterdi (Şekil 1). Epikardiyal yağ kalınlığı ve hacmi arttıkça, hem log-transforme koroner kalsiyum skoru (CAC) hem de koroner arter hastalığı (KAH) skoru ile orta düzeyde pozitif korelasyonlar saptandı (tüm p<0,001). Ayrıca, epikardiyal yağ hacmi karotis intima-media kalınlığı (IMT) ile orta düzeyde (r_s=0,335, p=0,001), yağ kalınlığı ise IMT ile daha zayıf ancak anlamlı bir ilişki gösterdi (r_s=0,238, p=0,024).

Metabolik risk göstergeleri incelendiğinde, METS-IR skoru koroner kalsiyum ile zayıf-orta düzeyde (r_s=0,261, p=0,006) ve IMT ile zayıf düzeyde bir ilişki gösterdi (r_s=0,200, p=0,059). TyG indeksi ise yalnızca koroner kalsiyum skoru ile zayıf ama anlamlı bir korelasyon sergiledi (r_s=0,203, p=0,035); karotis IMT ile anlamlı bir ilişki bulunmadı (p=0,892).

Epikardiyal yağ dokusu hem koroner hem de karotis aterosklerozu ile ilişkilendirildi; METS-IR skoru koroner ve karotis ateroskleroz göstergeleriyle sınırlı bir ilişki gösterirken, TyG indeksi yalnızca koroner kalsifikasyonla zayıf bir bağlantı sergiledi.

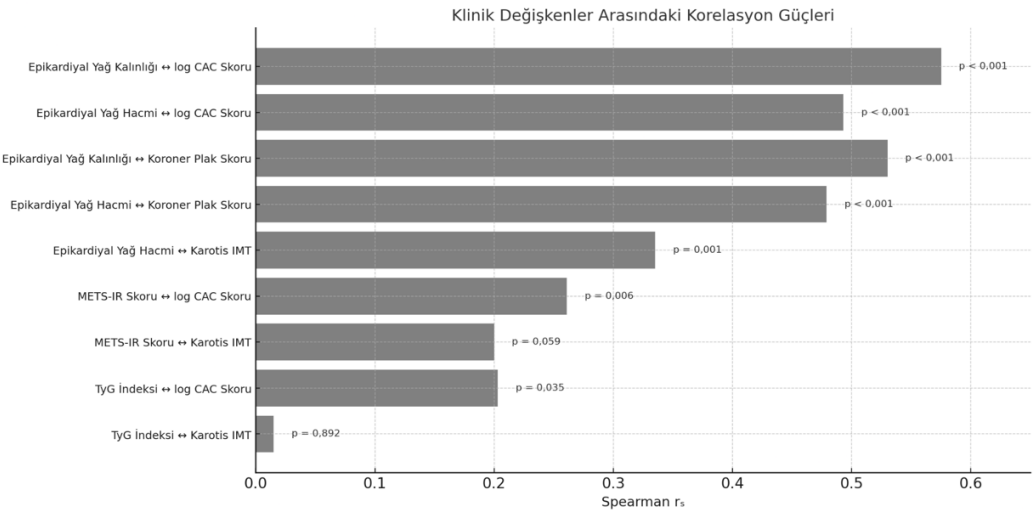
3. Koroner Arter Kalsiyum Varlığını (CAC > 0) Öngören Faktörler (Lojistik Regresyon)

Konvansiyonel risk faktörleri (yaş, cinsiyet, diyabet ve hipertansiyon) kontrol edilerek yapılan çok değişkenli lojistik regresyon analizinde, koroner arterlerde kalsiyum birikimi (CAC > 0) ile ilişkili bağımsız değişkenler belirlendi. Sonuçlar Şekil 2'de sunulmuştur.

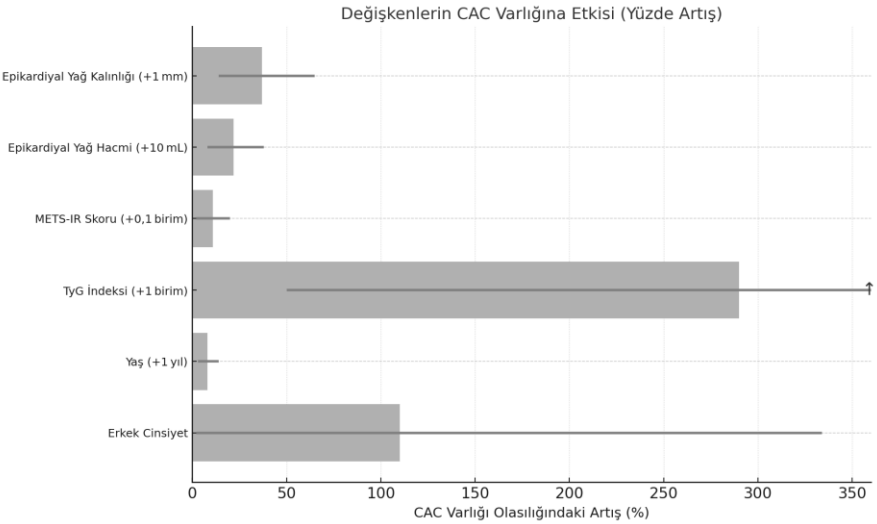
Konvansiyonel risk faktörleri (yaş, cinsiyet, diyabet, hipertansiyon) kontrol edildikten sonra, epikardiyal yağ kalınlığı ve hacminin koroner kalsiyum varlığı (CAC > 0) ile bağımsız ve güçlü bir şekilde ilişkili olduğu bulundu. Epikardiyal yağ kalınlığındaki her 1 mm'lik artış kalsiyum olasılığını 1,37 kat, hacimdeki her 10 mL'lik artış ise 1,22 kat artırmaktaydı (her ikisi için p<0,001).

Metabolik göstergelerden METS-IR skoru ve TyG indeksi de CAC varlığı ile bağımsız ilişkiliydi. METS-IR'daki her 0,1 birimlik artış, CAC pozitifliğini %11 artırırken (p=0,015), TyG indeksindeki her 1 birimlik artış yaklaşık 3,9 katlık bir risk artışıyla ilişkiliydi (p=0,005).

Yaş ve erkek cinsiyet CAC pozitifliğiyle anlamlı şekilde ilişkiliyken, diyabet ve hipertansiyonun bağımsız etkisi istatistiksel anlamlılık göstermedi (p>0,05).



Şekil 1. Önemli korelasyon katsayıları (Spearman r_s)



Şekil 2. Koroner arter kalsiyum varlığı (CAC > 0) için bağımsız öngörücüler (Multivariable Lojistik Regresyon Modeli)

Ayrıca, Karotis IMT'yi öngören çok değişkenli lineer regresyon analizinde, yalnızca METS-IR skoru bağımsız bir belirleyici olarak saptandı (β=0,11; p=0,042). Epikardiyal yağ kalınlığı ve TyG indeksi IMT ile anlamlı ilişki göstermedi.

METS-IR ve TyG indeksinin her ikisinin de insülin direnciyle ilişkili olması nedeniyle, birlikte kullanıldıkları modellerde kolinerite potansiyeline dikkat edilmesi gerektiği gözlendi.

4. Koroner Kalsiyum Pozitifliğini Saptamada Belirteçlerin ROC Analizi

Belirteçlerin CAC > 0 varlığını öngörme performansları ROC analizi ile değerlendirildi; AUC değerleri ve optimum kesim noktalarındaki duyarlılık ve özgüllük oranları Tablo 2'de sunulmuştur.

CAC > 0 için Belirteçlerin AUC, Duyarlılık ve Özgüllük Değerleri: ROC analizi sonuçlarına göre, CAC varlığını öngörmede en iyi tanısal performans epikardiyal yağ ölçümleriyle elde edildi. Epikardiyal yağ kalınlığı için AUC 0,802, hacmi için ise 0,781 olarak bulundu; her iki ölçüm "iyi" düzeyde ayırt edicilik sergiledi. Kalınlık için belirlenen 5,9 mm kesim noktası %75 duyarlılık ve %71 özgüllük, hacim için belirlenen 95 mL kesim noktası ise %66 duyarlılık ve %79 özgüllük sağladı.

METS-IR ve TyG indeksleri de CAC varlığını ayırt etmede istatistiksel olarak anlamlı performans gösterdi (AUC sırasıyla 0,670 ve 0,661), ancak epikardiyal yağ ölçümlerine göre daha düşük bir tanısal güce sahipti. İstatistiksel karşılaştırmalarda, epikardiyal yağ kalınlığının AUC değeri hem METS-IR (p=0,018) hem de TyG indeksinden (p=0,011) anlamlı olarak yüksekti. Epikardiyal yağ hacmi, TyG indeksinden daha üstün performans gösterdi (p=0,035), ancak METS-IR ile fark anlamlı düzeye ulaşmadı (p=0,06).

Bu bulgular, epikardiyal yağ dokusunun kalınlık ve hacim ölçümlerinin, metabolik skorlarla karşılaştırıldığında CAC varlığını öngörmede daha güvenilir biyobelirteçler olabileceğini göstermektedir.

Tablo 2. CAC > 0 için belirteçlerin AUC, duyarlılık ve özgüllük değerleri

Belirteç	AUC (95 % GA)	Optimum Kesim Noktası	Duyarlılık (%)	Özgüllük (%)	Yorum
Epikardiyal Yağ Kalınlığı	0,802 (0,718–0,886)	5,9 mm	75	71	CAC varlığını saptamada **iyi** bir ayırt edicilik gösteriyor (AUC ≈ 0,8).
Epikardiyal Yağ Hacmi	0,781 (0,693–0,869)	95 mL	66	79	CAC varlığını saptamada **iyi** bir ayırt edicilik gösteriyor (AUC ≈ 0,78).
METS-IR Skoru	0,670 (0,569–0,771)	2,35	70	56	CAC varlığını saptamada **orta düzeyde** ayırt edici (AUC ≈ 0,67).
TyG İndeksi	0,661 (0,557–0,765)	8,8	61	65	CAC varlığını saptamada **orta düzeyde** ayırt edici (AUC ≈ 0,66).

Tartışma

Bu çalışmada, orta yaşlı erişkinlerde METS-IR skoru, TyG indeksi ve epikardiyal yağ dokusu (EYD) kalınlık/hacminin koroner kalsifikasyon (CAC) ve karotis intima-media kalınlığı (IMT) gibi subklinik ateroskleroz göstergeleri ile anlamlı ve bağımsız ilişkiler gösterdiği bulundu. İlişkilerin yaş, cinsiyet ve komorbiditelere rağmen korunması, insülin direnci ve visseral adipozitenin ateroskleroz gelişiminde merkezi bir rol oynadığını desteklemektedir. Bulgular ayrıca, METS-IR, TyG ve EYD ölçümlerinin klinik pratikte subklinik ateroskleroz riskinin değerlendirilmesinde potansiyel yardımcı belirteçler olabileceğini göstermektedir.

Bulgularımız, epikardiyal yağ kalınlığı ve hacminin subklinik koroner aterosklerozun güçlü belirteçleri olduğunu ve bu ilişkinin koroner arterlere anatomik yakınlık ve pro-aterojenik faktör salınımı ile açıklanabileceğini gösteren önceki çalışmalarla uyumludur.[11,14]

Framingham Kalp Çalışması'nda EYD miktarı ile koroner arter kalsifiye plak yükü arasında belirgin bir ilişki saptanmış ve EYD'nin, koroner arter hastalığı risk skorları kontrol edildiğinde dahi bağımsız bir risk faktörü olabileceği öne sürülmüştür.[12] Genel popülasyonda yürütülen Heinz Nixdorf Recall çalışması, EYD kalınlığının ileriye dönük miyokard infarktüsü gelişimiyle ilişkili olduğunu bildirmiştir.[13] Bizim çalışmamızda da EYD kalınlığı, multivariable analizde koroner arterlerde subklinik lezyon varlığını (CAC>0) yaklaşık 1,4 kat artıran bağımsız bir belirleyici olarak bulunmuştur.

Çalışmamızda EYD hacmi için de benzer şekilde, CAC varlığıyla yaklaşık 1,22 kat artmış bir risk saptadık. Diyabetik hastalarda yapılan bir çalışmada, yüksek EYD hacminin koroner kalsifikasyon varlığı ile bağımsız olarak ilişkili olduğu bildirilmiştir.[16]

Epikardiyal yağ hacminin yalnızca koroner plak varlığıyla değil, aynı zamanda plakların riskli morfolojik özellikleriyle de ilişkili olabileceği gösterilmiştir. Dey ve arkadaşlarının ileri görüntüleme çalışmasında, EYD hacmi ve düşük atenüasyonlu "aktif" EYD'nin, yüksek riskli plak karakteristikleri ve majör kardiyak olaylarla anlamlı şekilde ilişkili olduğu bildirilmiştir.[15] Bu bulgular, epikardiyal yağ miktarının ve kompozisyonunun yalnızca aterosklerozun varlığını değil, aynı zamanda klinik önemini de etkileyebileceğini düşündürmektedir.

Çalışmamızda, epikardiyal yağ ölçümleri sadece koroner kalsifikasyonla değil, sistemik aterosklerozun invaziv olmayan bir göstergesi olan karotis IMT ile de anlamlı ilişkiler gösterdi. Özellikle EYD hacmindeki artış, karotis IMT kalınlaşması ile orta düzeyde pozitif bir ilişki sergiledi. Benzer şekilde, önceki çalışmalarda da tip 2 diyabetli hastalarda ve obez bireylerde EYD artışının karotis IMT ve arteriyel sertlikle ilişkili olduğu bildirilmiştir.[18,19] Yılmaz ve arkadaşlarının çalışmasında da, diyabetik bireylerde EYD kalınlığının artmış karotis IMT ve azalmış arteriyel elastikiyet ile bağımsız bir ilişki gösterdiği saptanmıştır.[18] Cabrera-Rego ve ark.[19], obez çocuklarda epikardiyal yağ kalınlığındaki artışın karotis arter duvar kalınlığı ve sertliği ile anlamlı şekilde ilişkili olduğunu ve her 1 mm'lik artışın subklinik ateroskleroz riskini yaklaşık üç kat artırdığını göstermiştir. Çalışmamızda, epikardiyal yağ kalınlığının karotis IMT ile doğrudan ilişkisi tam anlamıyla istatistiksel bağımsızlık göstermese de, ham korelasyon analizleri ve literatürdeki veriler birlikte değerlendirildiğinde, epikardiyal yağ fazlalığının sistemik ateroskleroz sürecinin bir yansıması veya katkı sağlayan bir faktör olabileceği düşünülmektedir.

METS-IR ve TyG gibi metabolik insülin direnci indeksleri çalışmamızda subklinik ateroskleroz göstergeleriyle anlamlı ilişkiler sergiledi. METS-IR skoru hem CAC skoru hem de karotis IMT ile pozitif korelasyon gösterdi ve çok değişkenli analizlerde her iki belirteç için de bağımsız bir öngörücü olarak saptandı. Bu bulgular, METS-IR'ın klasik risk faktörlerinin ötesinde ek prognostik bilgi sağlayabileceğini düşündürmektedir.

Wang ve ark.'nın asemptomatik bireylerde yaptığı çalışmada, METS-IR skorunun yüksek olduğu grupta CAC pozitifliğinin anlamlı şekilde arttığı ve METS-IR'ın koroner kalsifikasyonun bağımsız bir belirleyicisi olduğu bildirilmiştir.[8] Benzer şekilde, Wu ve ark.'nın çalışması da METS-IR'ın orta-ileri yaş popülasyonda kardiyovasküler olay riskini öngördüğünü göstermiştir.[9] Çalışmamızda ise METS-IR skoru, karotis IMK artışıyla bağımsız şekilde ilişkili bulunmuştur.

Literatürde, hipertansif hastalarda yüksek METS-IR değerlerinin artmış IMT ve mikroalbuminüri gibi hedef organ hasarı göstergeleriyle ilişkili olduğu bildirilmiştir.[10] Ayrıca, NHANES verilerinin analizinde yüksek METS-IR düzeylerinin yaklaşık 14 yıllık takipte hem tüm nedenli hem de kardiyovasküler ölümlerle ilişkili olduğu gösterilmiştir.[20] Bu bulgular, METS-IR'ın güçlü bir

kardiyometabolik risk belirteci olduğunu desteklemektedir.

Öte yandan, TyG indeksi bu çalışmada subklinik aterosklerozla METS-IR kadar tutarlı bir ilişki göstermemiştir. TyG, CAC>0 öngörüsünde bağımsız bir belirteç olarak anlamlı bulunmasına rağmen, METS-IR ile birlikte modele dahil edildiğinde bağımsızlığı zayıflamış; karotis IMT ile ise anlamlı bir ilişki saptanmamıştır. Bu durum, TyG indeksinin yalnızca açlık trigliserid ve glukozu içermesi nedeniyle insülin direncinin tüm metabolik ve vasküler etkilerini yeterince yansıtamayabileceğini düşündürmektedir.[8,9]

Çalışmamızda METS-IR ve TyG indeksleri arasında güçlü bir ilişki olmasına rağmen, METS-IR'ın VKİ ve HDL gibi ek parametreleri içermesi, özellikle karotis IMT gibi sistemik ateroskleroz göstergeleriyle ilişkide avantaj sağlamış görünmektedir. TyG indeksinin literatürdeki performansı değişken olup, genel popülasyonda kardiyovasküler olay riskini öngördüğü bildirilse de[7] bazı çalışmalarda IMT ve arteriyel sertlik ile anlamlı ilişki göstermediği bildirilmiştir.[21] Bulgularımız, TyG indeksinin tek başına değil, METS-IR gibi daha kapsamlı metabolik risk skorlarıyla birlikte değerlendirilmesinin daha doğru bir yaklaşım olabileceğini düşündürmektedir.[22]

Çalışmamız, KAH riski değerlendirmesinde METS-IR skoru ve epikardiyal yağ ölçümlerinin potansiyel kullanımını desteklemektedir. Özellikle, koroner arterlerde sessiz aterosklerozun saptanmasında epikardiyal yağ kalınlığı ve hacmi güçlü göstergeler olarak öne çıkmıştır. EYD ölçümünün koroner BT anjiyografiye ek maliyet veya radyasyon yükü getirmeksizin kolaylıkla yapılabilmesi, klinik pratiğe entegrasyonunu desteklemektedir. METS-IR skoru ise basit klinik ve laboratuvar verileri ile hesaplanabilir olması sayesinde non-invaziv risk değerlendirmesinde pratik olarak kullanılabilir. Bu bulgular, bireyselleştirilmiş primer koruma stratejilerinin planlanmasında bu parametrelerin yardımcı olabileceğini düşündürmekte olup, prospektif çalışmalarla desteklenmelidir.

Çalışmamızın bazı sınırlamaları bulunmaktadır. Retrospektif tasarımı nedeniyle nedensellik ilişkisi kurulamamakta ve belirteçlerin prognostik değerleri doğrudan değerlendirilememektedir. Örneklem büyüklüğünün nispeten küçük ve tek merkezden seçilmiş olması, sonuçların genellenebilirliğini sınırlayabilir. Ayrıca, metabolik indeksler açlık ölçümleri temel alınarak hesaplanmış; insülin düzeyleri veya altın standart yöntemlerle (örneğin klemp tekniği) karşılaştırma yapılmamıştır. Karotis IMT ölçümleri tüm hastalarda mevcut olmayıp, detaylı segmental plak analizi gerçekleştirilmemiştir. Son olarak, epikardiyal yağ dokusunun yalnızca kalınlık ve hacmi değerlendirilmiş, dokunun inflamatuar özellikleri veya kompozisyonu analiz edilmemiştir. Gelecekteki çalışmalarda EYD'nin hem niceliksel hem de niteliksel özelliklerinin birlikte değerlendirilmesi faydalı olacaktır.

Sonuç olarak, METS-IR ve TyG indeksleri ile epikardiyal yağ ölçümleri, subklinik aterosklerozun erken saptanmasında geleneksel risk faktörlerinden bağımsız katkı sağlayabilecek potansiyel belirteçlerdir. Bu bulguların klinik uygulamaya entegrasyonu için daha geniş örneklemli ve prospektif çalışmalara ihtiyaç vardır.

Etik Standartlara Uygunluk

Bu çalışmanın etik kurul onayı Acıbadem Üniversitesi Etik Kurulu'ndan alınmıştır (Sayı:ATADEK-2025/09, Karar numarası: 2025-09/76).

Kaynaklar

1. Greenland P, Blaha MJ, Budoff MJ, Erbel R, Watson KE. Coronary Calcium Score and Cardiovascular Risk. *J Am Coll Cardiol.* 2018;72(4):434-447. doi:10.1016/j.jacc.2018.05.027
2. O'Leary DH, Polak JF, Kronmal RA, et al. Carotid artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med.* 1999;340(1):14-22. doi:10.1056/NEJM199901073400103
3. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an AHA/NHLBI Scientific Statement. Circulation. 2005;112(17):2735-2752. doi:10.1161/CIRCULATIONAHA.105.169404
4. Iacobellis G, Corradi D, Sharma AM. Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. Nat Clin Pract Cardiovasc Med. 2005;2(10):536-543. doi:10.1038/ncpcardio0319
5. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The Triglyceride and Glucose Index is associated with cardiovascular risk factors in normal-weight children and adolescents. *Pediatr Res.* 2017;82(6):920-924. doi:10.1038/pr.2017.177
6. Dong Z, Wang Z, Chen R, et al. Triglyceride-glucose index is associated with arterial stiffness and coronary artery calcification: a systematic review and meta-analysis. *Cardiovasc Diabetol.* 2023;22(1):18. doi:10.1186/s12933-023-01750-y
7. Sánchez-Íñigo L, Navarro-González D, Fernández-Montero A, Pastrana-Delgado J, Martínez JA. The TyG index is associated with fatal and non-fatal cardiovascular events: a meta-analysis. *Diabetes Metab Syndr.* 2020;14(5):1279-1287. doi:10.1016/j.dsx.2020.07.010
8. Wang S, Wang J, Li J, et al. Metabolic score for insulin resistance as a novel predictor of coronary artery calcification in asymptomatic adults: a cross-sectional study. *BMC Endocr Disord.* 2022;22(1):29. doi:10.1186/s12902-022-00940-x
9. Wu Z, Cui H, Zhang Y, et al. The relationship between metabolic score for insulin resistance and cardiovascular disease. *Front Cardiovasc Med.* 2023;10:1139128. doi:10.3389/fcvm.2023.1139128
10. Tanindi A, Erkan AF, Ekici B, et al. Metabolic score for insulin resistance (METS-IR) is associated with target organ damage in patients with essential hypertension. *Clin Exp Hypertens*. 2022;44(2):141-147. doi:10.1080/10641955.2021.1991777

11. Iacobellis G. Epicardial adipose tissue in contemporary cardiology. *Nat Rev Cardiol.* 2022;19(9):593-606. doi:10.1038/s41569-022-00688-z
12. Mahabadi AA, Massaro JM, Rosito GA, et al. Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. *Eur Heart J.* 2009;30(7):850-856. doi:10.1093/eurheartj/ehp023
13. Mahabadi AA, Berg MH, Lehmann N, et al. Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: the Heinz Nixdorf Recall Study. *J Am Coll Cardiol.* 2013;61(13):1388-1395. doi:10.1016/j.jacc.2012.11.062
14. Bettencourt N, Toschke AM, Leite D, et al. Epicardial adipose tissue is an independent predictor of coronary atherosclerotic burden. *Int J Cardiol.* 2012;158(1):26-32. doi:10.1016/j.ijcard.2010.12.089
15. Dey D, Nakazato R, Pimentel R, et al. Epicardial fat volume and attenuation: association with high-risk plaque features, coronary stenosis, and major adverse cardiovascular events. *Radiology.* 2021;301(1):E372-E381.doi:10.1148/radiol.2021204390
16. Cosson E, Nguyen MT, Rezgani I, et al. Epicardial adipose tissue volume and coronary calcification among people living with diabetes: a cross-sectional study. *Cardiovasc Diabetol.* 2021;20(1):35. doi:10.1186/s12933-021-01229-0
17. Cury RC, Abbara S, Achenbach S, et al. CAD-RADS™ Coronary Artery Disease – Reporting and Data System: an expert consensus document of the Society of Cardiovascular Computed Tomography, American College of Radiology and North American Society for Cardiovascular Imaging. *J Cardiovasc Comput Tomogr.* 2016;10(4):269-281. doi:10.1016/j.jcct.2016.04.005
18. Yılmaz Y, Kurt Omurlu İ, Sadıç M, et al. Epicardial fat thickness is associated with arterial stiffness and carotid intima-media thickness in patients with type 2 diabetes mellitus. *Angiology.* 2016;67(3):267-273. doi:10.1177/0003319715588600
19. Cabrera-Rego JO, Iacobellis G, Castillo-Herrera JA, et al. Epicardial fat thickness correlates with carotid intima-media thickness, arterial stiffness, and cardiac geometry in children and adolescents. *Pediatr Cardiol.* 2014;35(3):450-456. doi:10.1007/s00246-013-0799-9
20. Duan M, Zhao X, Li S, et al. Metabolic score for insulin resistance (METS-IR) predicts all-cause and cardiovascular mortality in the general population: evidence from NHANES 2001–2018. *Cardiovasc Diabetol.* 2024;23(1):243. doi:10.1186/s12933-024-02334-8
21. Raimi TH, Saheed O, Saidu H, et al. Predictive performance of triglyceride-glucose index for subclinical atherosclerosis among adults in a Nigerian community. *PLoS One.* 2023;18(3):e0283049. doi:10.1371/journal.pone.0283049

doi: 10.53446/actamednicomedia.1698649

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

ANTERIOR ILIAC BLOCK FOR BONE GRAFT HARVESTING: A CADAVERIC STUDY

KEMİK GREFTİNDE ANTERİOR İLİAK BLOK: KADAVRA ÇALIŞMASI

Hadi Ufuk Yörükoğlu[1*], Abdullah Örs[2], Serdar Demiröz[3], Volkan Alparslan[1], Sevim Cesur[1], Özgür Çakır[4], Can Aksu[1]

[1]Kocaeli University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kocaeli, Türkiye. [2]Kocaeli University, Faculty of Medicine, Department of Anatomy, Kocaeli, Türkiye. [3]Kocaeli University, Faculty of Medicine, Department of Orthopedics and Traumatology, Kocaeli, Türkiye. [4]Kocaeli University, Faculty of Medicine, Department of Radiology, Kocaeli, Türkiye.

ABSTRACT

Objective: Anterior iliac crest (AIC) is frequently used as a donor site for various constructive surgeries. However, it is associated with severe postoperative pain that increases morbidity. To provide analgesia in the donor site with a low volume, a novel technique was described named anterior iliac block (AIB). It provides effective analgesia, but the mechanism of action is not clear. We designed this study to demonstrate the mechanism of AIB.

Methods: In this cadaveric study, AIB was performed with 10 mL (right side) and 20 mL (left side) of drug containing methylene blue and radiopaque dye on opposite sides. Following the block application, the spread on both sides was evaluated with 3D computed tomography. Afterwards, systematic layer-by-layer cadaver dissection was carried out by an orthopedic surgeon and anatomist experienced in pelvic anatomy.

Results: The spread was more extensive on the left side, it predominantly extended in a cranial direction. In contrast, on the right side, where a lower volume was administered, the injectate remained localized within the fascial plane surrounding the iliac crest. On the left side, spread was observed in the fascial plane above the iliacus muscle, and the cutaneous branches of spinal nerves were stained. Additionally, dye was observed in the ilioinguinal nerve. On the right side, dye staining in the cutaneous branches above the iliac crest was observed.

Conclusion: This cadaveric study demonstrates that AIB effectively targets the cutaneous branches of the subcostal, iliohypogastric, and ilioinguinal nerves with low volume.

Keywords: Regional anesthesia, anterior iliac block, cadaveric study, postoperative analgesia

ÖZ

Amaç: Anterior iliak krest (AİK), rekonstrüktif cerrahilerde sıklıkla greft donör sahası olarak tercih edilmektedir. Ancak bu bölgeye yönelik girişimler, morbiditeyi artıran ciddi postoperatif ağrılarla ilişkilidir. Donör bölgesine düşük hacimde lokal anesik ile analjezi sağlamak amacıyla "anterior iliak blok (AİB)" adı verilen yeni bir teknik tanımlanmıştır. AİB etkili bir analjezi sağlasa da etki mekanizması açık değildir. Bu çalışmayı, AIB'nin etki mekanizmasını araştırmayı amaçladık.

Yöntem: Bu kadavra çalışmasında, AİB sol tarafa 20 mL ve sağ tarafa 10 mL olacak şekilde metilen mavisi ve radyoopak boya içeren ilaçla uygulandı. Blok uygulamasının ardından, her iki taraftaki yayılım üç boyutlu bilgisayarlı tomografi ile değerlendirildi. Daha sonra, pelvik anatomi konusunda deneyimli bir ortopedi cerrahı ve bir anatomist tarafından sistematik olarak katman katman kadavra diseksiyonu gerçekleştirildi.

Bulgular: Sol tarafta yayılım daha genişti ve özellikle kraniyal yönde ilerleme gözlendi. Buna karşılık, daha düşük hacmin uygulandığı sağ tarafta, enjeksiyon sıvısı iliak krest çevresindeki fasyal plan içinde sınırlı kaldı. Sol tarafta, iliakus kası üzerindeki fasyal planda yayılım gözlendi ve spinal sinirlerin kutanöz dalları boyandı. Ayrıca, ilioinguinal sinirde de boya izlendi. Sağ tarafta ise iliak krest üzerindeki kutanöz dallarda boya tutulumu saptandı.

Sonuç: Bu kadavra çalışması, AİB'nin düşük hacimle uygulandığında subkostal, iliohipogastrik ve ilioinguinal sinirlerin kutanöz dallarını etkili bir şekilde hedef aldığını göstermektedir.

Anahtar Kelimeler: Rejyonal anestezi, anterior iliak blok, kadavra çalışması, postoperatif analjezi

***Corresponding author/İletişim kurulacak yazar:** Hadi Ufuk Yörükoğlu; Kocaeli University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kocaeli, Türkiye.
Phone/Telefon: +90 (262) 303 75 75, **e-mail/e-posta:** ufukyorukoglu@gmail.com
Submitted/Başvuru: 13.05.2025 • **Accepted/Kabul:** 18.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Anterior iliac crest (AIC) is frequently used as a donor site for various constructive surgeries.[1] It is preferred because it can be accessed in the supine position. However, it is associated with severe postoperative pain that increases morbidity.[2]

To provide analgesia in the donor site, local anesthesia infiltration can be used.[3] Nevertheless, it is performed after the harvesting and has a short duration of action. There are several regional anesthesia techniques that can be performed to provide analgesia in the donor site such as transversus abdominis plane (TAP) block, erector spinae plane (ESP) block, transmuscular quadratus lumborum block (QLB) and transversalis fascia plane (TFP) block in order to block the T12-L1 spinal nerves which innervates AIC.[4-7] However, these techniques are fascial plane blocks and high volumes of local anesthetic is needed. Considering the primary surgical procedure and the need for high-dose regional blocks (e.g., brachial plexus block, sciatic nerve block) to achieve adequate analgesia, the additional use of a high-volume fascial plane block for the donor site may substantially increase the risk of local anesthetic systemic toxicity.

Therefore, we described a new technique called anterior iliac block (AIB) which requires low volumes, such as 10 mL.[8] The injection point is in the vicinity of the donor site, medial to anterior iliac crest, between the iliacus muscle and aponeurosis of transversus abdominis muscle, which lies below internal oblique muscle. The injection point is also the junction where the thoracolumbar fascia merges with the iliac fascia, where small cutaneous branches that innervates anterior iliac crest may exist, this may also explain why a small volume is effective. However, the mechanism of action is not clear and cadaveric studies demonstrating the spread of the injectate are needed.

We designed this study to demonstrate the mechanism of the anterior iliac block. In this cadaveric study, we aimed to compare the spread of 10 mL and 20 mL of drug injected on opposite sides, in order to assess whether a higher volume is essential. Our primary objective was to assess the spread of the injectate using computer tomography (CT) imaging, and the secondary objective was to identify the cutaneous nerve branches blocked.

Methods

Cadaver selection and preparation

Dissections were performed on a cadaver, from woman aged 65. The human tissue, acquired via an institutional body donation program with agreement for exclusive use in teaching and research, were embalmed using Thiel's method. The cadaver had no history of surgical interventions or significant operations in the pelvic or abdominal area. The study was conducted following the procedures approved by the Non-Interventional Ethical Committee of Kocaeli University School of Medicine (GOKAEK-2024/13.31).

Solution

For the anterior iliac block, a total of 30 mL mixture containing 1% methylene blue was obtained using 15 ml of 2% methylene blue, 13 mL of 0.9% saline solution, and 2 mL of radiopaque dye (Iohexol, Kopaq 350 mg/1 ml, Koçsel).[9]

Ultrasound guided anterior iliac block

The probe was placed on the anterior aspect of the anterior iliac crest (Figure 1A). Afterward, the probe was advanced medially and tilted cranially to visualize the iliacus muscle where it attaches to AIC. In the ultrasound image, the iliacus muscle, internal oblique muscle above, aponeurosis of transversus abdominis muscle between these muscles, and ilium were seen (Figure 1B). With an in-plane approach, an 80-mm block needle was advanced from lateral to medial, and 10 mL of the solution on the right side and 20 mL of solution on the left side was injected in the plane between the iliacus and aponeurosis of transversus abdominis muscle.

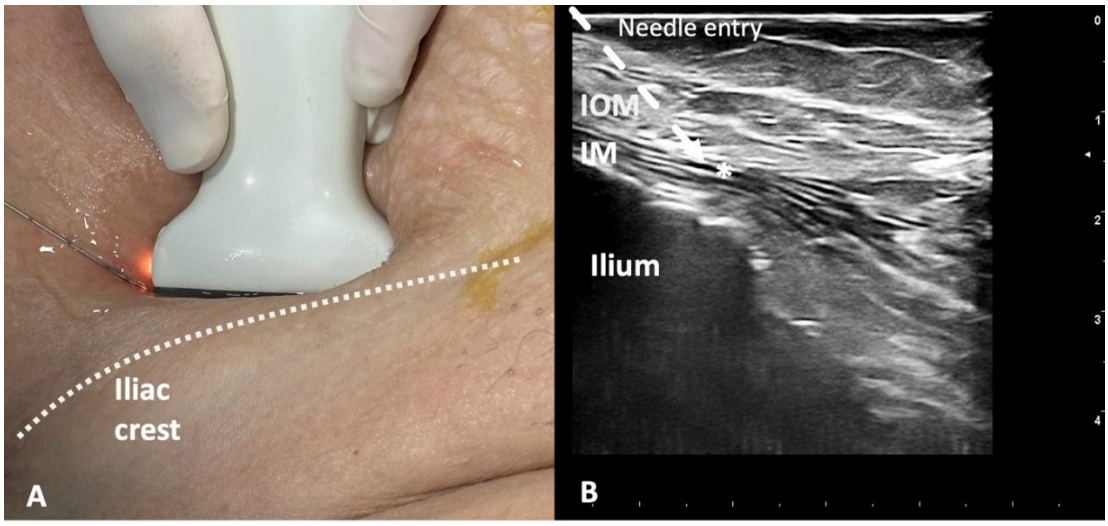


Figure 1. Ultrasound guided anterior iliac block. A: Probe position, B: Ultrasound image, IOM: Internal oblique muscle, IM: Iliacus muscle, *Injection point

Computed Tomographic Scanning

One hour after the block application, the imaging procedures described below were applied to both sides in order to valuate post-injection spread:

Scanning was conducted with the cadaver in the supine position using a 640-slice CT apparatus (Aquilion One, Canon). Images scanned in helical mode at 120 kV with a rotation duration of 0.5 seconds were examined in the soft tissue window (level: 40, width: 400) using Vital Vitrea software (Canon Group, Minnetonka, MN, USA). The radiologist assessed the images in the axial, coronal, and sagittal planes.

Anatomical Dissection and Stain Spread Analysis

Following the imaging procedures, systematic layer-by-layer cadaver dissection was carried out by an orthopedic surgeon and anatomist experienced in pelvic anatomy (2 hours after the block application).

Results

The three-dimensional distribution of the injectate, as visualized on computed tomography, is presented in Figure 2 (Figure 2A: right side, Figure 2B: left side). Although the spread was more extensive on the left side, it predominantly extended in a cranial direction. In contrast, on the right side, where a lower volume was administered, the injectate remained localized within the fascial plane surrounding the iliac crest.

On the right side, methylene blue spread was observed in the fascial plane above the iliacus muscle. In addition, dye staining in the cutaneous branches of spinal nerves above the iliac crest was seen (Figure 3). On the left side, dye staining in the cutaneous branches above the iliac crest was observed (Figure 4A). Additionally, dye was observed in the ilioinguinal nerve (Figure 4B).

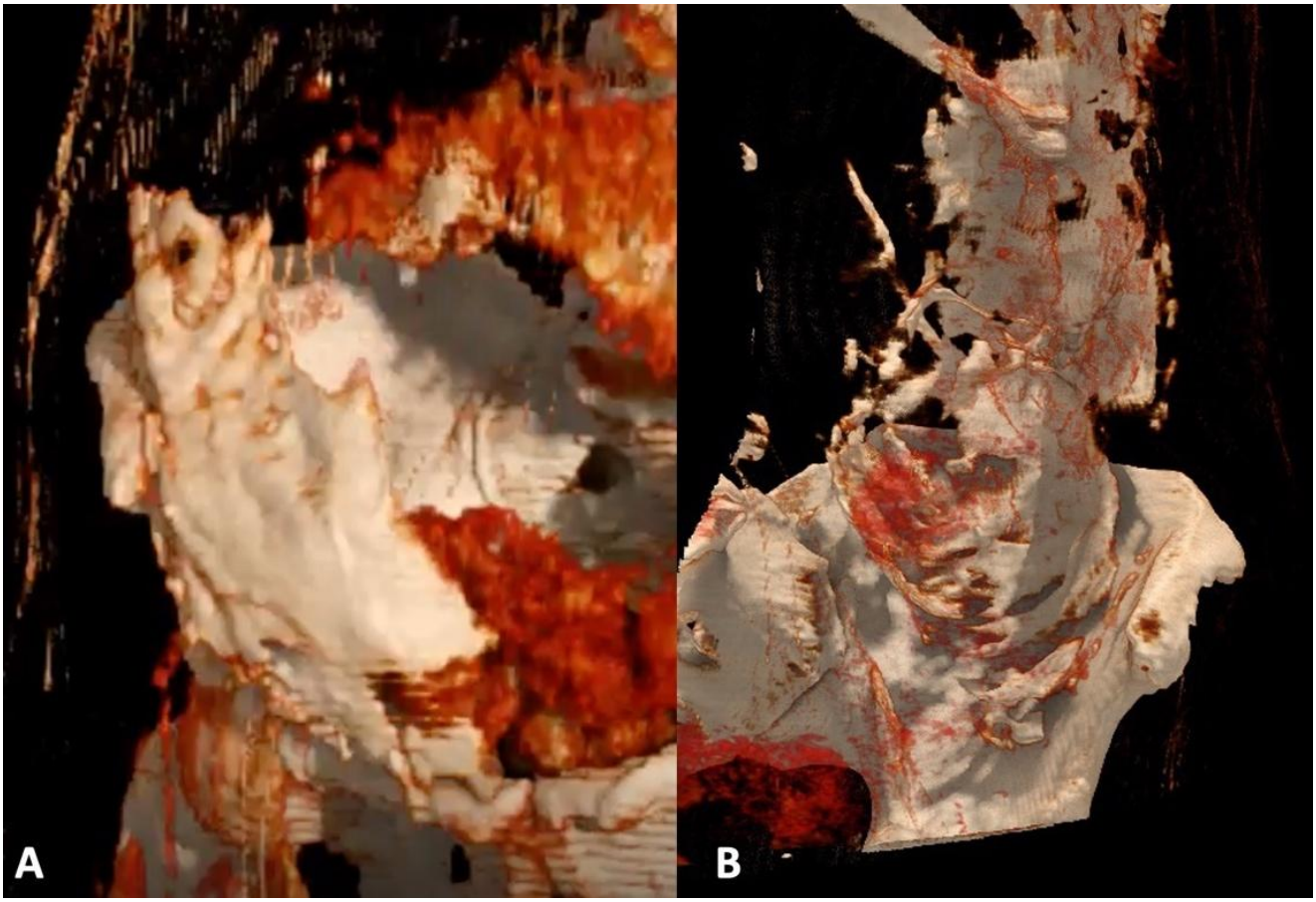


Figure 2. 3D CT images of the drug spread. A: Spread on the right side (10 mL), B: Spread on the left side (20 mL)

Figure 3. Dissection of the left side (20 mL). White arrows show the cutaneous branches of spinal nerves.

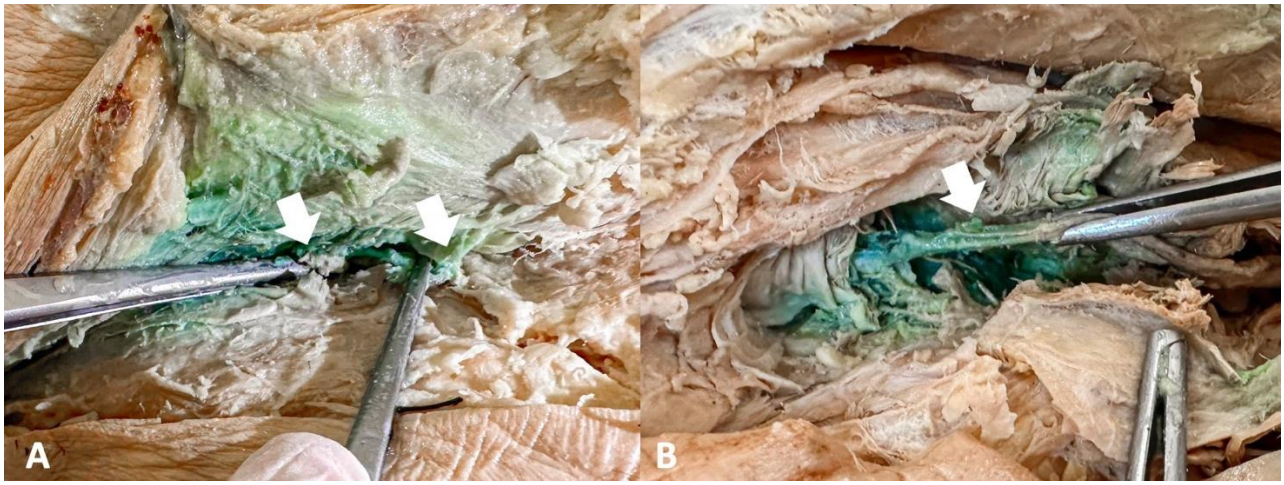


Figure 4. Dissection of the right side (10 mL). A: White arrows show the cutaneous branches of spinal nerves, B: White arrow shows the ilioinguinal nerve

Discussion

In this cadaveric study investigating the mechanism of action of the anterior iliac block and comparing the effects of low and high volumes, we observed that the cutaneous branches of spinal nerves innervating the anterior iliac crest as well as the ilioinguinal nerve were consistently involved. Our findings suggest that a high injectate volume is not necessary for the effectiveness of this technique.

Innervation of the ilium is complex, however anterior iliac crest is mainly innervated by subcostal, ilioinguinal, and iliohypogastric nerves.[10, 11] Cutaneous branches of these nerves are responsible with this innervation. These nerves also innervate the skin and the subcutaneous tissue above the iliac crest. Cutaneous branches of these nerves and the ilioinguinal nerve are located above the iliacus muscle in the vicinity of anterior iliac crest.[12-14] Therefore, we thought that low-volume local anesthetic injection at this site can provide analgesia at both the anterior iliac crest—the graft harvest site—and the surgical incision area and defined the anterior iliac block (AIB).[8] Indeed, we found that low-volume injection was effective in providing postoperative analgesia. With this cadaveric study, we demonstrated the mechanism of action of the AIB and confirmed that low volumes are sufficient to block the nerves involved in the innervation of the anterior iliac crest. While high-volume injection resulted in more cranial spread and potentially involved additional dermatomes, this extended distribution is

unlikely to contribute to postoperative analgesia for bone graft harvesting from the iliac crest.

There are a number of limitations to this study. The first involves a cadaver. Drug spread might usefully be investigated with more cadavers. In addition, the contrast material added to visualize the spread with CT may also have affected the drug diffusion. Finally, this was a cadaver study, and the tissues being non-living may also have impacted the drug spread. Although cadavers embalmed using the Thiel method are regarded as providing the best physical and functional characteristics in investigating the mechanisms of regional techniques, the present research now needs to be supported by in vivo clinical studies.

In conclusion, this cadaveric study demonstrates that AIB effectively targets the cutaneous branches of the subcostal, iliohypogastric, and ilioinguinal nerves, which are responsible for the sensory innervation of the anterior iliac crest. Our findings indicate that low-volume local anesthetic injection at the defined site may be sufficient to achieve postoperative analgesia in the graft harvest region, without the need for higher volumes. However, further studies are needed to validate the mechanism of action.

Ethical Approval

The study was conducted following the procedures approved by the Non-Interventional Ethical Committee of Kocaeli University School of Medicine (GOKAEK-2024/13.31).

Conflict of Interest

There is no conflict of interest to declare.

Author Contributions

HUY: Study conception and design, data collection and analysis, manuscript drafting, final approval; AÖ: Study conception and design, data collection, manuscript drafting; SD: Data collection, manuscript revision; VA: Data collection, literature review, manuscript drafting; SC: Literature review, manuscript revision; ÖÇ: Study conception and design, data collection and analysis, methodological supervision; CA: Study conception and design, data collection, methodological supervision.

Financial Support

None

References

1. Dissaux C, Ruffenach L, Bruant-Rodier C, George D, Bodin F, Rémond Y. Cleft Alveolar bone graft materials: literature review. *Cleft Palate Craniofac J.* 2022;59(3):336-346. doi:10.1177/10556656211007692.
2. Morgan SJ, Jeray KJ, Saliman LH, Miller HJ, Williams AE, Tanner SL, et al. Continuous infusion of local anesthetic at iliac crest bone-graft sites for postoperative pain relief. A randomized, double-blind study. *J Bone Joint Surg Am.* 2006;88(12):2606-2612. doi:10.2106/JBJS.E.00984.
3. Cowan N, Young J, Murphy D, Bladen C. Double-blind, randomized, controlled trial of local anesthetic use for iliac crest donor site pain. *J Neurosci Nurs*. 2002;34(4):205-210. doi:10.1097/01376517-200208000-00006.
4. Black ND, Malhas L, Jin R, Bhatia A, Chan VWS, Chin KJ. The analgesic efficacy of the transversalis fascia plane block in iliac crest bone graft harvesting: a randomized controlled trial. *Korean J Anesthesiol*. 2019;72(4):336-343. doi:10.4097/kja.d.18.00352.
5. Sondekoppam RV, Ip V, Johnston DF, Uppal V, Johnson M, Ganapathy S, Tsui BCH. Ultrasound-guided lateral-medial transmuscular quadratus lumborum block for analgesia following anterior iliac crest bone graft harvesting: a clinical and anatomical study. *Can J Anaesth*. 2018;65(2):178-187. doi:10.1007/s12630-017-1021-y.
6. Sowapark J, Sumphaongern T. Does Ultrasound-guided transversus abdominis plane block reduce donor site pain after harvesting anterior iliac crest bone grafts. *J Oral Maxillofac Surg*. 2021;79(2):333-342. doi:10.1016/j.joms.2020.09.017.
7. Gürkan Y, Aksu C. Iliac crest bone graft donor site analgesia: a new indication for erector spinae plane block. *Can J Anaesth*. 2019;66(3):338-339. doi:10.1007/s12630-018-01276-6.
8. Yörükoğlu HU, Cesur S, İzgin Avci İ, et al. Retrospective evaluation of postoperative analgesia efficacy of a new technique in anterior iliac crest bone graft harvesting: anterior iliac block. *BMC Anesthesiol*. 2024;24(1):443. doi:10.1186/s12871-024-02829-7.
9. Wong S, Hon S, Parry S, Boesch JM, Pearson E, de Miguel Garcia C. Image analysis comparison of nerve staining with food dye, methylene blue or tissue marker. *Vet Anaesth Analg*. 2024;51(1):36-43. doi:10.1016/j.vaa.2023.09.073.
10. Howard MA, Dickie SR. Chapter 9: comprehensive trunk anatomy. İçinde: Neligan PC, ed. Plastic surgery. 3. Baskı, New York, NY: Elsevier Health; 2013:220–238.
11. Birch R. Chapter 80: pelvic girdle, gluteal region and thigh. İçinde: Standring S, ed. Gray's anatomy: the anatomical basis of clinical practice. 41. Baskı, Londra: Elsevier; 2016:1337–1375.
12. Hebbard PD, Barrington MJ, Vasey C. Ultrasound-guided continuous oblique subcostal transversus abdominis plane blockade: description of anatomy and clinical technique. *Reg Anesth Pain Med*. 2010;35(5):436-441. doi:10.1097/aap.0b013e3181e66702.
13. van Ramshorst GH, Kleinrensink GJ, Hermans JJ, Terkivatan T, Lange JF. Abdominal wall paresis as a complication of laparoscopic surgery. *Hernia*. 2009;13(5):539-543. doi:10.1007/s10029-009-0473-6.
14. Whiteside JL, Barber MD, Walters MD, Falcone T. Anatomy of ilioinguinal and iliohypogastric nerves in relation to trocar placement and low transverse incisions. *Am J Obstet Gynecol*. 2003;189(6):1574-1578. doi:10.1016/s0002-9378(03)00934-7.

doi: 10.53446/actamednicomedia.1703990

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

COMBINATION THERAPY WITH SELENOUREA AND ETHACRYNIC ACID TARGETING GST INHIBITION AND REVEALS SOME APOPTOSIS-CLEAVED PROTEINS IN BREAST CANCER

SELENOUREA VE ETAKRINIK ASIT ILE KOMBINASYON TEDAVISI GST INHIBISYONUNU HEDEFLIYOR VE MEME KANSERINDE APOPTOZLA AYRILAN BAZI PROTEINLERI ORTAYA ÇIKARIYOR

Berna Ozdem[1*], Isil Yildirim[2],

[1]Inonu University, Health Science Institute, Department of Medical Biology and Genetics, Malatya, Turkiye. [2]Biruni University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Turkiye.

ABSTRACT

Objective: Glutathione S-transferase (GST) participates in the maintenance of cellular redox homeostasis through several mechanisms. Therefore, compounds or drug-like drugs that GSTs target are important for preclinical and clinical studies. We hypothesized that inhibition of GST by selenourea and Etacrynic acid combination sensitizes breast cancer cells to apoptotic signaling by altering redox homeostasis, leading to the identification of specific apoptosis-cleaved proteins that drive cell death pathways. **Methods:** This study was carried out to demonstrate the binding target molecular docking, cell proliferation inhibition with MTS method, and protein expression with western blot analysis of the combination therapy with selenourea and Etacrynic acid to target GST inhibition. **Results:** In the results, it was found that selenourea acts through targeting by indirect S-glutathionylation modification of cysteine residues in target proteins, although its polar covalent bond, hydrogen bond, and ionic interaction bind to other amino acids in all sub-types of GST. The combination of selenourea and etacrynic acid dose-dependently inhibited cell proliferation at $p<0.0001$ levels. Selenourea only exhibited 52% and 50% inhibition on MDA-MB-231 and MCF7 cells, respectively. In contrast, the combination of Selenourea and Etacrynic acid showed 41% and 38% inhibition on MDA-MB-231 and MCF7 cells, respectively. This combination also revealed apoptosis-cleaved proteins in estrogen-positive MCF7 cells and non-estrogenic MDA-MB-231 breast cancer cells. **Conclusions:** This work may provide a practical guide and useful insights into new therapeutics. This could be considered a very promising strategy for the development of new antineoplastic drugs. Targeting identified proteins, in combination with GST inhibition, enhances therapeutic efficacy.

Keywords: Selenourea, GST, ADME analysis, protein-ligand interaction, signal pathway analysis.

ÖZ

Amaç: Glutatyon S-transferaz (GST), çeşitli mekanizmalar aracılığıyla hücresel redoks homeostazının korunmasına katılır. Bu nedenle, GST'lerin hedef aldığı bileşikler veya ilaç benzeri ilaçlar preklinik ve klinik çalışmalar için önemlidir. GST'nin selenoüre ve Etakrinik asit kombinasyonu ile inhibisyonunun, redoks homeostazını değiştirerek meme kanseri hücrelerini apoptotik sinyale duyarlı hale getirdiğini ve hücre ölüm yollarını yönlendiren spesifik apoptozla ayrılan proteinlerin tanımlanmasına yol açtığını varsaydık. **Yöntem:** Bu çalışma, GST inhibisyonunu hedeflemek için selenoüre ve Etakrinik asit ile kombinasyon tedavisinin bağlanma hedefi moleküler yerleştirme, MTS yöntemi ile hücre proliferasyonu inhibisyonu ve western blot analizi ile protein ekspresyonunu göstermek için gerçekleştirilmiştir **Bulgular:** Sonuçlarda, selenoürenin hedef proteinlerdeki sistein kalıntılarının dolaylı S-glutatyonilasyon modifikasyonu ile hedefleme yoluyla etki ettiği, ancak polar kovalent bağı, hidrojen bağı ve iyonik etkileşiminin GST'nin tüm alt tiplerindeki diğer amino asitlere bağlandığı bulunmuştur. Selenoüre ve etakrinik asit kombinasyonu hücre proliferasyonunu $p<0.0001$ düzeyinde doza bağlı olarak inhibe etmiştir. Selenoüre, MDA-MB-231 ve MCF7 hücreleri üzerinde sırasıyla yalnızca %52 ve %50 inhibisyon sergilemiştir. Buna karşılık, Selenourea ve Etacrynic asit kombinasyonu MDA-MB-231 ve MCF7 hücreleri üzerinde sırasıyla %41 ve %38 inhibisyon göstermiştir. Bu kombinasyon aynı zamanda östrojen pozitif MCF7 hücrelerinde apoptozla parçalanmış proteinler ortaya çıkarmıştır. **Sonuç:** Bu çalışma, yeni terapötikler için pratik bir rehber ve faydalı bilgiler sağlayabilir. Bu, yeni antineoplastik ilaçların geliştirilmesi için çok umut verici bir strateji olarak düşünülebilir. GST inhibisyonu ile birlikte tanımlanmış proteinlerin hedeflenmesi, terapötik etkinliği artırır.

Anahtar Kelimeler: Selenourea, GST, ADME analizi, protein-ligand etkileşimi, sinyal yolu analizi.

***Corresponding author/İletişim kurulacak yazar:** Işil Yildirim; Biruni University, Faculty of Pharmacy, Department of Biochemistry, 34015, Istanbul, Turkiye
Phone/Telefon: +90 (537) 569 86 99, **e-mail/e-posta:**Dr.IsilYildirim@hotmail.com
Submitted/Başvuru: 22.05.2025 • **Accepted/Kabul:** 23.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Selenourea is an organic compound with the formula CH_4N_2Se (Figure 1) and solid white or crystalline solid molecular weight 122.02 g/mol, selenium inorganic or organic forms is rapidly absorbed from the human gut, with total bioavailability of 84% to 97%, respectively.[1,2] Since selenium-containing compounds are less toxic than other inorganic compounds, synthesis and biological evaluation of these compounds. Drug design is the inventive process of finding new medications depending on the knowledge of a biological target such as receptor agonists, antagonists, inverse agonists, or modulators; ion channel openers or blockers; enzyme activators or inhibitors. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the molecular target with which they interact and bind. Therefore, this study was carried out to demonstrate the binding of selenourea compound to glutathione s-transferase enzymes (GSTs) which are a superfamily of proteins found in all cellular organisms, which usually exist as multiple isoforms have many different exogenous and multifunctional enzymes that detoxify endogenous compounds.[3,4] This extensive family has been categorized into at least 13 classes. The 3 big families of proteins cytosolic based upon alpha, beta, delta, epsilon, zeta, theta, mu, nu, pi, sigma, tau, phi, and omega, mitochondrial based Kappa, and microsomal GSTs like Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) superfamily. One general function of these superfamilies is the involvement in detoxication reactions for both endogenous and xenobiotic compounds.[5] Besides their molecular and catalytic role (Figure 2), GSTs may bind to a range of exogenous and endogenous compounds in a non-catalytic manner. These affect hormones, fatty acids, bilirubin, and xenobiotics.[6,7] GSTs are phase II metabolic enzymes that play a key role in drug metabolism.[8] GSTs act as the thiol group of glutathione (GSH) in the active center to the electrophilic site of a second substrate to catalyze conjugation reactions. The glutathione conjugate formed is less toxic and excreted in soluble form GSTs recognize a large number of substrates, although the common features of the substrates are that most of them are hydrophilic, and carry an electrophilic center.[9] The supramolecular structure of cytoskeletal proteins determines exposed thiol groups.[10] This effect has been associated with glutathionylation of these sites affecting protein function by protecting them from irreversible oxidation under stress conditions or inhibiting polymerization.[11,12] In this manner, maintaining cellular homeostasis and participating in various pathological processes may be associated with cancer cell survival, particularly in telomere-targeted compounds.[13] GSTs protect cancer cells from oxidative stress and may contribute to tumor progression by maintaining cellular homeostasis under oxidative conditions.[14] So, inhibition is needed there. Inhibiting GST activity disrupts the cellular defense mechanisms against oxidative stress, which results in an accumulation of reactive oxygen species (ROS). This accumulation can overwhelm cellular repair mechanisms, ultimately triggering apoptotic pathways in cancer cells.[15,16] By targeting GSTs, researchers aim to enhance the efficacy of chemotherapy and reduce cancer cell survival, particularly in drug-resistant tumors.[17,18] Therefore, it is important to determine the inhibition effect of drugs or combinations on GST enzymes. GST inhibitors, such as etacrynic acid disrupt the GSH-GST system. This leads to increased ROS accumulation, making cancer cells more prone to oxidative stress and triggering apoptosis. This study aims to investigate the therapeutic potential of combining selenourea and Etacrynic acid in targeting GST inhibition, while also identifying apoptosis-associated cleaved proteins in breast cancer cells, supported by molecular docking to predict key binding interactions.

Methods

Material

Cells and Reagents

In our study, the anticancer effects of the combination of selenourea and etacrynic acid were investigated on breast cancer cells such as estrogen-positive MCF7 (HTB-22; ATCC, USA), estrogen-negative MDA-MB-231(HTB-26; ATCC, USA), and non-cancer cells. RPMI has been supplemented with 10% fetal bovine serum (FBS), penicillin (50 IU/mL Capricorn Scientific, Germany), streptomycin (50 µg/mL, Capricorn Scientific, Germany), and 2 mM L-glutamine. The cells were incubated in a humidified incubator at 5% CO_2 at 37℃. PBS, FBS, antibiotic (Penicillin-Streptomycin), and Trypsin/EDTA solution were purchased from Sigma-Aldrich (Germany). MTS was purchased from Promega (Germany). Selenourea was purchased from Sigma-Aldrich (Germany), etacrynic acid was purchased from Sigma-Aldrich(Germany).

Used devices

In this study, Nuve marked a laminar flow cabinet, a Thermo-Scientific mark a CO_2 Incubator, and an Emax-Plus Microplate reader were used.

Method

Cell proliferation assay with MTS

The MTS assay is a colorimetric assay for assessing a cell's metabolic activity. The MTS tetrazolium compound is bio-reduced by cells into a colored formazan product that is soluble in the culture medium. This conversion is presumably accomplished by NADPH or NADH produced by dehydrogenase enzymes in metabolically active cells.[19] Measurement of the anti-proliferative effect using the MTS cell proliferation kit was performed in a 96-well plate using human breast cell lines. Cells were grown in the appropriate medium and then seeded in 96 well plates in a 100 µL medium. The presence of 1500 cells in each well were verified and then incubated at 37°C in a 5% CO_2 atmosphere for 24 h. At the end of the time cycle,

different concentrations from 10 to 140 µM of the test sample were added. The cells were then incubated under conditions appropriate for the cell lines ranging from 24 to 72 h. Then, 25 µL of MTS reagent was added to each well and incubated for 1 h. At the end of the incubation time, the optical density (OD) of the color was measured using a microplate reader. The absorbance was measured at a primary wavelength of 490 nm. The mean absorbance values were calculated, and the cell viability percentages were documented using the Excel Office program. Then the IC_{50} values were calculated. The best cell proliferation reducing were found 72 hours and this time, western blot analysis. The results are shown in Figure 3. Apoptosis-cleaved protein expression studies were performed according to the determined IC_{50} values. Results are shown Figure 3.

Western blotting

Western blotting was carried out as described elsewhere.[18] Western blotting was performed to evaluate the protein expression levels of E-cadherin, β-catenin, and β-actin in MCF7 and MDA-MB-231 cells. Cells were lysed using RIPA buffer (Thermo Scientific, Cat# 89901) supplemented with protease inhibitor cocktail (Abcam, Cat# ab271306), phosphatase inhibitor cocktail (Sigma-Aldrich, Cat# P0044), and 1 mM each of NaF and Na_3VO_4. After incubation on ice for 30 minutes, lysates were centrifuged at 14,000 rpm for 15 minutes at 4°C. Protein concentrations were measured by Bradford assay (Bio-Rad, Cat#5000006), and 40 µg of total protein was loaded per lane onto 8–12% SDS-polyacrylamide gels for electrophoresis at 90V, followed by transfer onto PVDF membranes (Millipore, Cat# IPVH00010). The choice of 40 µg total protein was based on prior optimization trials to achieve optimal band intensity and clarity, especially for moderately expressed proteins such as E-cadherin and β-catenin, while maintaining consistent detection of β-actin as a loading control. Membranes were blocked in 5% non-fat dry milk in TBS-T for 1 hour at room temperature and incubated overnight at 4°C with the following mouse monoclonal primary antibodies diluted 1:1000 in 2.5% milk-TBS/T: anti-E-cadherin (Cell Signaling Technology, Cat# 3195, 135 kDa), anti-β-catenin (Cell Signaling Technology, Cat# 8480, 92 kDa), and anti-β-actin (Sigma-Aldrich, Cat# A5441, 43 kDa, internal control). After washing three times with TBS-T, membranes were incubated for 1 hour at room temperature with HRP-conjugated anti-mouse IgG secondary antibodies (Cell Signaling Technology, Cat# 7076, Anti-rabbit IgG, HRP-linked Antibody #7074, dilution 1:2000 in 2.5 % milk-TBS/T). Protein bands were visualized using enhanced chemiluminescence (Bio-Rad Clarity ECL, Cat# 1705061) and imaged with the UVP ChemiDoc-It² system. Results are shown Figure 4.

Protein preparation

The methods presented in this paper are specifically based on machine learning (ML) techniques. The Protein Data Bank was used for protein preparation. Molecular docking analyses were made by using network analysis in systems to predict their binding mode and binding energy. Afterward, in silico analysis to evaluate the pharmacokinetic properties was predicted by using the ADME software program. The Protein Data Bank20 was used for molecular docking analysis. The PDB archive contains information on experimentally determined structures of proteins, nucleic acids, and complex assemblies. As a member of RCSB, PDB compiles and annotates PDB data according to agreed standards. RCS and PDB also provide a variety of tools and resources. Users can perform simple and advanced searches based on annotations related to ranking. PDB IDs and X-RAY Diffraction Resolutions 3EIN (1.13 Å), 1GSD (2.50Å), 2WJU (2.30Å), 1TDI (2.40Å), 1R4W (2.50Å), 1GTU (2.68Å), 1AQW (1.80 Å), 11GS (2.30 Å) A chain structures due to no mutation and homogenization and then all the water and buffer molecules, as well as the ions, were deleted, and all the protein subsequently, hydrogen atoms were added to the system (according to pH 7.4) using the Protein Preparation tool in the playmolecule.com data analysis program and, Auto Dock Vina (4.0).[21,22]

Ligand preparation and optimization

The molecular structures of all of the molecules were sketched using the ChemBioDraw-Ultra-v12.0 (2010) software. The chemical structures of known drugs were retrieved from the PubChem compound database, which is available at NCBI.[23]

ADME, Molecular docking and gene ontology analysis

ADME analysis was performed using the SwissADME software (version 1.0, 2023) program. The results of this analysis are shown in Figure 3. Molecular docking analyses were performed using the Kdeep/data analysis system.[24] Perform virtual screening of the compound was made using a neural-network-based predictor of bind scope and aesthetic 2D diagrams of protein-ligand interactions including hydrogen-bonds and pi-pi stacking were made by Plexview programs (version 1.0, 2023).[25] Results were shown in Figure (5-6) and signaling pathways were made using a state-of-the-art neural networks pathway map program and gene ontology analysis were made using by STRING online tool (Figure 6).[26] Results are shown Table1, Figure 5, and Figure 6.

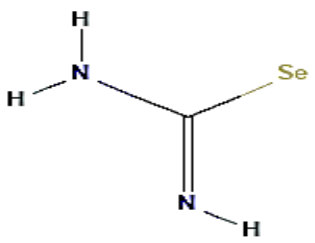


Figure 1. Chemical structure of selenourea ($H_2N–C(=NH)–Se$): A selenium analog of urea. The molecule contains a selenium atom bonded to the central carbon, replacing the oxygen typically found in urea. This compound is of interest due to its applications in coordination chemistry and as a precursor for selenium-containing biomolecules. X-ray crystallographic measurements on crystals at -100°C give average C=Se bond lengths of 1.86 Å and 1.37 Å for C-N. Both

the Se-C-N and N-C-N angles were measured at 120°, as expected for a sp2-hybridized carbon.[27] Both the shortened length of the N-C σ bond and the longer Se=C bond suggest that the lone pair is displaced on amines; the Se=C π bond electrons are attracted to the selenium atom, while the nitrogen lone pair is attracted to the carbonyl carbon.[28] The synthesis of selenium-containing heterocycles and another class of reactions take place by complexation of selenurea with transition metals and metalloids, which is attributed to the electron-donating effect of the amino groups and the consequent stabilization of the selenium-metal π bond.

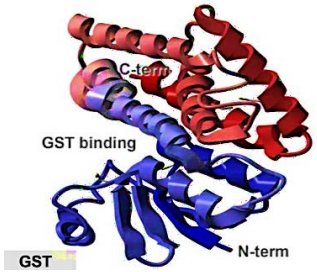


Figure 2. GST terminal structure: Structural representation of the GST (Glutathione S-Transferase) fusion protein. The N-terminal domain (N-term) is shown in blue, and the C-terminal domain (C-term) is highlighted in red. The GST-binding site is labeled and structurally positioned between the two domains, indicating the orientation and domain organization of the protein.

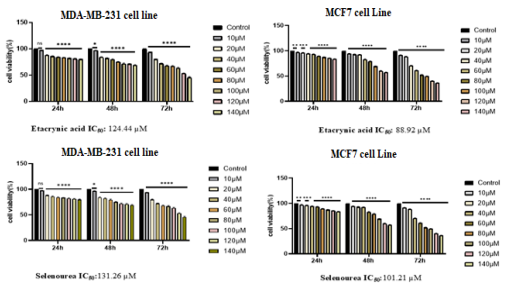
Results

Cells were treated with increasing concentrations (10–140 µM) of each compound for 24, 48, and 72 hours. Cell viability was measured using an MTS assay and is expressed as a percentage of untreated control cells. The best results found at 72 hours in both cells. For 72 hours was the best result, optimization was achieved. We found that selenourea and etacrynic acid combination reduced cell proliferation dose and time. Selenourea only segmented with 50 % inhibition exhibited at 131.26 µM, 101.21µM concentration in MCF7 and MDA-MB231 cell respectively, while it's combination with Etacrynic acid IC_{25} exhibited 41% and 38% at 35-50µM in both cells.

Western blot analyses were performed by combine with selenourea IC_{50} and Ethacrynic acid IC_{25} and IC_{50} doses at 72 hours.

Figure 3A top panels have shown Etacrynic acid treatment resulted in dose- and time-dependent cytotoxicity with IC_{50} values of 124.44 µM (MDA-MB-231) and 88.92 µM (MCF7). Bottom panels: Selenourea also reduced cell viability with IC_{50} values of 131.26 µM (MDA-MB-231) and 101.21 µM (MCF7).

A)



B)

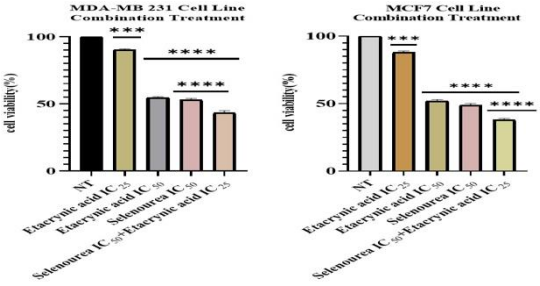


Figure 3. Cell proliferation analysis results of etacrynic acid and selenourea (A) and combination(B) on MDA-MB-231 and MCF7 breast cancer cell lines.

Considering the results of Figure 4, expression slightly increases with EIC_{50} and SIC_{50}, and even more in the combination (E+S) treatment IC_{50} in MDA-MB-231 have shown a partial reversal of Epithelial Mesenchymal Transition (EMT) link to E-cadherin, potentially linked to cell differentiation or apoptosis. Combination treatment, indicating a reduction in β-catenin signaling, which may correlate with decreased Wnt pathway activity, relevant to both proliferation and apoptosis regulation. Generally, the upregulation of E-cadherin and reduction of β-catenin in combination treatment in MDA-MB-231 cell supports a reversal of mesenchymal phenotype, which can be associated with increased susceptibility to apoptosis.

Considering the results of Figure 4, the combined E+S treatment IC_{50} in MCF7 cell have exhibited E-cadherin expression moderate increase compared to non-combination. This upregulation may reflect either enhanced cell-cell adhesion or a response to apoptotic stress (cells often upregulate adhesion molecules in early apoptosis).

Table 1 results suggest that the molecule is hydrophilic. This may limit its ability to passively diffuse through the lipid bilayer of cell membranes. However, it could still be efficiently taken up by active transport mechanisms, which is particularly relevant if the molecule targets intracellular apoptotic pathways. However, compound is well-suited for oral formulations with 84.1mg/ml so, it has excellent solubility enhances bioavailability, allowing the molecule to effectively reach intracellular compartments and exert potential pro-apoptotic effects. Moreover, no CYP enzyme inhibition (CYP1A2, CYP2C9, CYP3A4, etc.) has shown low metabolic interaction and good pharmacokinetic stability. This compound is efficiently absorbed and retained in cells and may be an anticancer agent inducing apoptosis.

Figure 6 top results have shown Reactome pathway enrichment analysis revealed significant upregulation of apoptosis-related signaling, particularly R-HSA-111465 (apoptotic cleavage of cellular proteins), indicating that the treatment triggers both extrinsic caspase activation and executioner-mediated proteolysis. These findings are consistent with western blot data showing caspase-related protein cleavage and align with ADME properties that predict effective intracellular bioavailability.

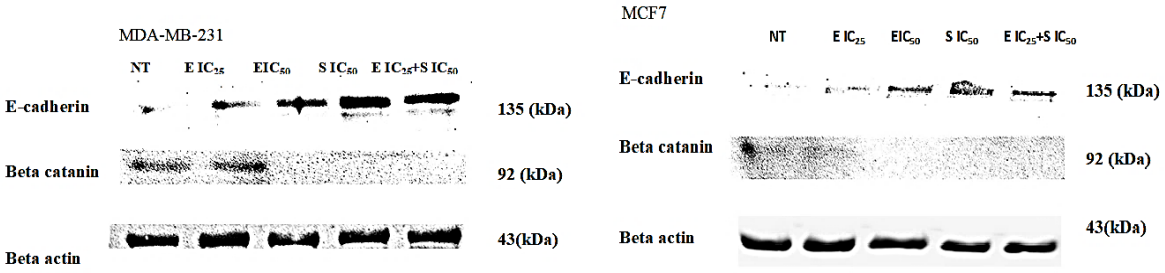


Figure 4. Western blot analysis results.

Table 1. ADME analysis

Category	Property	Abbreviation / Model	Value	Interpretation / Notes
Lipophilicity	Log P (Octanol/Water)	iLOGP	0.00	Neutral lipophilicity
	Log P	XLOGP3	-0.69	Slightly hydrophilic
	Log P	WLOGP	-0.95	Hydrophilic
	Log P	MLOGP	-1.13	Hydrophilic
	Log P	SILICOS-IT	-0.85	Hydrophilic
	Consensus Log P	—	**-0.16**	Overall indicates mild hydrophilicity
Water Solubility	Log S	ESOL	-0.16	Very soluble
	Solubility (mg/mL; mol/L)	ESOL	84.1 mg/mL ; 0.689 mol/L	Very soluble
	Log S	Ali	0.12	Soluble
	Solubility (mg/mL; mol/L)	Ali	160 mg/mL ; 1.31 mol/L	Soluble
	Log S	SILICOS-IT	0.16	Soluble
	Solubility (mg/mL; mol/L)	SILICOS-IT	176 mg/mL ; 1.44 mol/L	Soluble
	Solubility Class	—	Very soluble to soluble	Favorable for absorption
Pharmacokinetics	Gastrointestinal Absorption	GI	High	Good oral bioavailability
	Blood-Brain Barrier Permeant	BBB	No	Not CNS-active
	P-glycoprotein Substrate	P-gp	No	Not effluxed by P-gp transporter
	CYP1A2 Inhibition	CYP1A2	No	Not expected to inhibit
	CYP2C19 Inhibition	CYP2C19	No	—
	CYP2C9 Inhibition	CYP2C9	No	—
	CYP2D6 Inhibition	CYP2D6	No	—
	CYP3A4 Inhibition	CYP3A4	No	—
	Skin Permeability	Log Kp	-7.53 cm/s	Poor skin penetration
Drug-Likeness	Bioavailability Score	—	0.55	Moderate drug-likeness
	Synthetic Accessibility	—	3.15	Moderately easy to synthesize (scale: 1–10)
	Rule of Five Compliance	Lipinski, Veber	Compliant with 3 Lipinski + Veber rules	Drug-like

Log P : Logarithm of partition coefficient (lipophilicity), Log S : Logarithm of solubility, P-gp: P-glycoprotein, CYP:Cytochrome P450 enzymes, GI :Gastrointestinal, BBB:Blood-Brain Barrier, Kp: Skin permeability coefficient, ESOL, Ali, SILICOS-IT:Solubility prediction models, iLOGP, XLOGP3: Lipophilicity prediction models

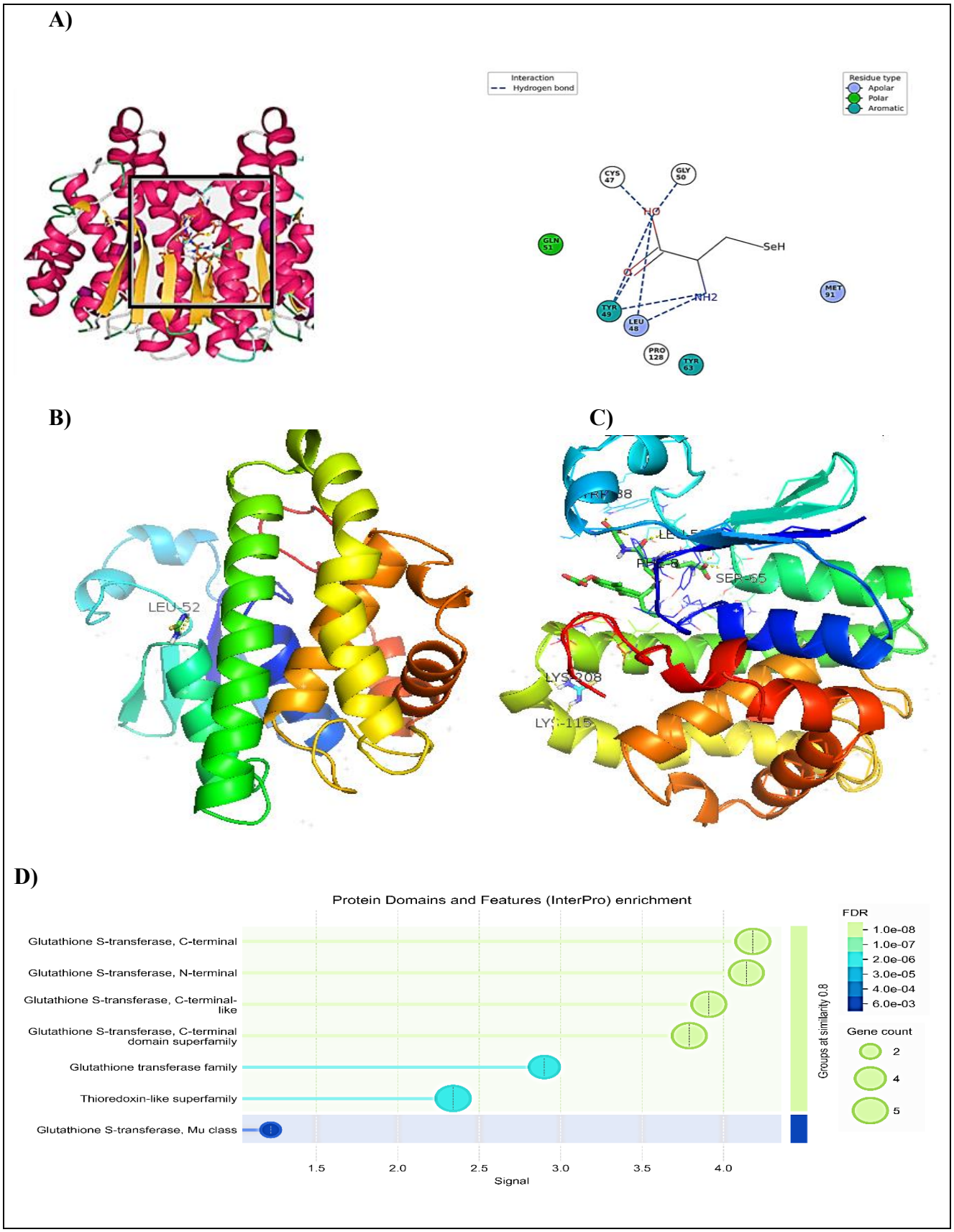


Figure 5. A: Molecular docking analysis for (PDB ID:1AQW; Glutathione S-Transferase in Complex with Glutathione) GST of selenocysteine (A) and selenourea (B) Molecular docking analysis for GST inhibition of selenourea. (C) (PDB ID:11GS; Glutathione S-transferase complexed with etacrynic acid-glutathione conjugate). (D) Protein Domains and features.

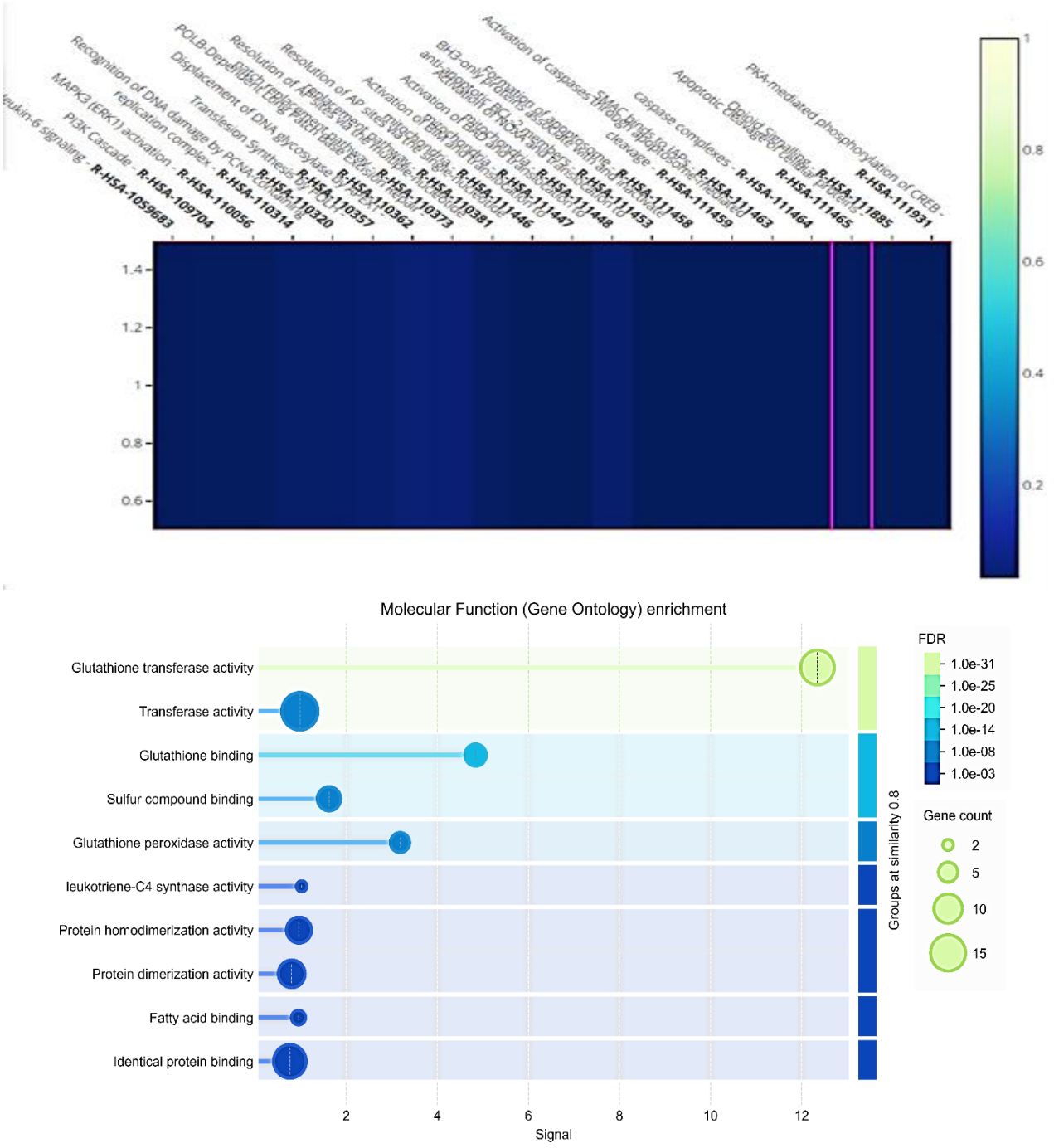


Figure 6. Molecular pathway association with self-normalizing neural networks (top panel) and gene ontology(below pane) of Selenourea

Discussion

Drug designs bind to the biomolecules with which they interact, changing their structure, shape, and charge states. Moreover, they affect many other properties, such as bioavailability, metabolic half-life, side effects, etc.[29] Computer-designed studies have been carried out recently to show the structure-activity relationship of drugs, and improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed. So, this study was conducted using computer-aid protein-ligand analysis of selenourea compound on GST family enzyme (Figure 6) Binding selectivity was defined concerning the binding of Selenourea to a substrate (protein) forming a complex with non-covalent interactions, such as hydrogen bonding or Van der Waals force. Moreover, ΔGs were found to be -3.6, -3.7, -3.1, -3.8, 3.1, and -2.8 kcal/mol for GSTA1-1, GST A2, GSTA3, GST Delta, GST K, and GSTM1, respectively. Selenourea's low energies are associated with small-molecule probability, identifying potential therapeutic compounds capable of modulating the activity of specific biological targets.

This study determined a quantitative structure-activity relationship (QSAR), in which a correlation between calculated biophysical properties of selenourea (Figure 1) and significant interactions were observed between these compounds and with the amino acids of the active site of the protein with amino acid ion charged (Figure 2). For example, the selenourea interacts with GST delta with hydrogen bonds GSH210, GLN124, ASN123, and ARG122 amino acids. (AIt was bound to hydrogen bind of GST alpha (A2) with GSH230, and LEU198 amino acids, but it was not connected to GST A1. GSTA1-1PHE97, GLN199, SER202 hydrogen bond. For GSTM1 ASP8, TYR6 amino acid binds to hydrogen bound. Finally, there could be a target between GST Kappa with SER15 MET102 hydrogen bond ARG204 GLU17 contact amino acid residues. This situation can be associated with GSTK found in mitochondria. Furthermore, selenourea was directly bound by GST with GSH201, LEU52 hydrogen bond ΔG 2.8 kcal/mol, while selenocysteine was bound by CYS47, and GLY50, LEU48, and TYR49 amino acid residue to hydrogen bind with ΔG-3.84 kcal/mol, but selenourea bind to LEU52 amino acid hydrogen bound (Figure 5). GST inhibition of selenourea with complexed with etacrynic acid-glutathione conjugate amino acid residue to hydrogen binds LYS2028, LYS2025 with ΔG-3.0 kcal/mol. A previous study showed that GSTP was subjected to phosphorylation at Thr109, Ser28, Ser154, and Ser184; O-glycosylation at Thr5 amino acids, but unknown placement to N-terminal. When these Figures (5) are compared to Figure 2, selenourea bonds to the GST enzyme N-terminal side. A study reported that the GST N-terminal domain fold is similar to cellular homeostasis, and detoxification proteins such as glutathione peroxidases and glutaredoxin.[30] The accumulation of ROS, particularly following the inhibition of Glutathione S-transferases (GSTs), can trigger apoptosis in cancer cells.[31,32] Identifying cleaved proteins such as PARP and Bid provides valuable insights into the mechanisms by which GST inhibition facilitates apoptosis and may offer potential therapeutic strategies for cancer treatment.[33]

Signaling analysis results (Figure 6) show that selenourea acts to apoptotic cleave cellular proteins (R-HSA-111465) found in the cytosol. These proteins are E-cadherin, Beta-catenin, alpha Fodrin, GAS2, FADK, alpha adducin, HIP-55, and desmoglein and they have critical roles for cell adhesion and maintenance of the cytoskeletal structure, and also these structures target caspase proteins. Moreover, it has been reported that cleavage of proteins such as APC and CIAP1 can forward stimulate apoptosis by producing proapoptotic protein.[34,35] In this study, we conducted E-Cadherin and Beta-catenin protein expression analyses (Figure 4). We found that both the IC_{50} dose of etacrynic acid and the IC_{50} concentration of selenourea, when compared to beta-actin, upregulated E-cadherin expression in the non-estrogenic MDA-MB-231 cell line. Additionally, the combination of selenourea (IC_{50}) and etacrynic acid (IC_{25}) also upregulated E-cadherin expression in these cells. When comparing Beta-catenin expression levels to beta-actin expression, individual treatments resulted in upregulation in the MDA-MB-231 cell line. However, under combination conditions, Beta-catenin expression was downregulated. Furthermore, neither the individual treatments nor the combination had any effect on expression in estrogenic MCF7 cells. In this case, the combination of selenourea (IC_{50}) and etacrynic acid (IC_{25}) also led to downregulation of expression. This effect is associated with the inhibitory action of the selenourea and etacrynic acid combination on GST activity and its selective effect on apoptotic progression.

Apoptosis and deterioration of redox homeostasis are among the main mechanisms that lead to multidrug resistance. Moreover, resistance to cancer chemotherapeutic agents in several oncogenes is mediated by the overexpression of certain GST enzymes associated with their ability to catalyze drug conjugation to GSH. So, in this study, between selenourea and glutathione-S-transferase complexed with Etacrynic acid-glutathione conjugate analysis was made for inhibitory effect. GST inhibitor, Etacrynic acid disrupted the GSH-GST system. This leads to increased ROS accumulation, making cancer cells more prone to oxidative stress and triggering apoptosis. The use of GST inhibitors to manage to prevent resistance among anticancer agents can be promised therapeutic emerging as a pro-agent. Therefore, the effect of medicinally active compounds on metabolic enzyme determination is crucial for drug design studies. Despite the limitations of the study, further *in vitro* and *in vivo* analyses of selenourea on drug metabolism enzymes will increase its biological importance. Proteomic analysis reveals a subset of apoptosis-cleaved proteins, including novel candidates, that regulate cell death in breast cancer.

In conclusion, this study demonstrates that combination therapy involving selenourea and Etacrynic acid, both acting as glutathione S-transferase (GST) inhibitors, holds significant potential in the treatment of breast cancer. By targeting GST, a key enzyme implicated in detoxification and drug resistance, this dual approach disrupts cellular redox balance, leading to the accumulation of reactive oxygen species and the induction of oxidative stress. This oxidative stress, in turn, amplifies apoptotic signaling pathways. Western blot (↑E-cadherin, ↓β-catenin), ADME analysis (good bioavailability, P-gp evasion), and functional enrichment, the data collectively supports that your treatment is effectively inducing apoptosis, likely through mitochondrial and DNA-damage-related pathways. Importantly, our findings reveal that GST inhibition facilitates the identification of apoptosis-associated cleaved proteins, suggesting a direct link between GST suppression and enhanced apoptotic machinery activation. These cleaved proteins may serve as novel biomarkers or therapeutic targets in breast cancer. The observed synergistic effect of selenourea and Etacrynic acid may be attributed to their complementary mechanisms, while both inhibit GST, they may differentially affect metabolic and signaling pathways associated with tumor cell survival.

Overall, this combinatorial strategy not only enhances the efficacy of pro-apoptotic responses in breast cancer cells but also offers insights into the molecular events downstream of GST inhibition. These findings underscore the therapeutic value of simultaneously modulating redox homeostasis and apoptosis in developing more effective breast cancer treatments.

Conflict of interest statement

The author declared no conflict of interest in the manuscript.

Funding

No funding was received for conducting this study.

Acknowledgment

The necessary resources and facilities for the realization of this study were provided by the Tekedereli laboratory of the Department of Medical Biology and Genetics, Faculty of Medicine, Inonu University. For this reason, we would like to thank our advisor Prof Dr. İbrahim Tekedereli.

References

1. Koketsu M, Ishihara H. Thiourea and selenourea and their applications. *Curr Org Synth*. 2006;3:439-455.
2. National Center for Biotechnology Information. PubChem Compound Summary for CID 6327594, Selenourea. https://pubchem.ncbi.nlm.nih.gov/compound/Selenourea. Accessed June 16, 2025.
3. Hayes JD, Lanagan JF, Jowsey IR. Glutathione S-transferases. *Annu Rev Pharmacol Toxicol*. 2005;45:51-88.
4. Ketterer B. A bird's eye view of the glutathione transferase field. *Chem Biol Interact*. 2001;138:27-42.
5. Habig WH, Pabst MJ, Fleischner G, Gatmaitan Z, Arias IM, Jakoby WB. The identity of glutathione S-transferase B with ligandin, a major binding protein of the liver. *Proc Natl Acad Sci U S A*. 1974;71(10):3879-3882.
6. Türkeş C, Demir Y, Beydemir Ş. Infection medications: assessment in vitro glutathione S-transferase inhibition and molecular docking study. *ChemistrySelect*. 2021;6(43):11915-11924.
7. Türkeş C, Kesebir AÖ, Demir Y, Küfrevioğlu Öİ, Beydemir Ş. Calcium channel blockers: the effect of glutathione S-transferase enzyme activity and molecular docking studies. *ChemistrySelect*. 2021;6(40):11137-11143.
8. Erat M, Sakiroglu H. The effect of some antineoplastic agents on glutathione S-transferase from human erythrocytes. *J Enzyme Inhib Med Chem*. 2013;28(4):711.
9. Çağlar MK, Bilgin R. Covalent immobilization and characterization of glutathione-S-transferase enzymes on to magnetic iron nanoparticles via epichlorohydrin intermediate spacer arm. *Ç.Ü Fen ve Mühendislik Bilimleri Dergisi*. 2018;36(5):9-16.
10. Choudhary BS, Chaudhary N, Khan BK. LCN2 promotes focal adhesion formation and invasion by stimulating Src activation. *J Cell Sci*. 2025:jcs263663.
11. Chen W, Seefeldt T, Young A, et al. Microtubule S-glutathionylation as a potential approach for antimitotic agents. *BMC Cancer*. 2012;12:245.
12. Nulton–Persson AC, Starke DW, Mieyal JJ, Szweda LI. Reversible inactivation of alpha-ketoglutarate dehydrogenase in response to alterations in the mitochondrial glutathione status. *Biochemistry*. 2003;42:4235-4242.
13. Ozcan M, Burus A, Mender I, et al. Investigation of the inhibitory effects of the telomere-targeted compounds on glutathione S-transferase P1. *Naunyn Schmiedebergs Arch Pharmacol*. 2025:1-9.
14. Singh SP, Dhanasekara CS, Melkus MW. Relevance of cellular homeostasis-related gene expression signatures in distinct molecular subtypes of breast cancer. *Biomedicines*. 2025;13(5):1058.
15. Burus A, Ozcan M, Canpinar H, et al. The effect of the combination therapy with chlorophyllin, a glutathione transferase P1-1 inhibitor, and docetaxel on triple-negative breast cancer invasion and metastasis in vivo/in vitro. *Naunyn Schmiedebergs Arch Pharmacol*. 2025:1-12.
16. Attique I, Haider Z, Khan M, et al. Reactive oxygen species: from tumorigenesis to therapeutic strategies in cancer. *Cancer Med*. 2025;14(10):e70947.
17. Gu X, Mu C, Zheng R, et al. The cancer antioxidant regulation system in therapeutic resistance. *Antioxidants*. 2024;13(7):778.
18. Arunachalam K, Sreeja PS. MTT assay protocol. In: *Advanced Cell and Molecular Techniques: Protocols for In Vitro and In Vivo Studies*. New York, NY: Springer US; 2025:271-276.
19. Tekedereli I, Akar U, Alpay SN, Lopez-Berestein G, Ozpolat B. Autophagy is required to regulate mitochondria renewal, cell attachment, and all-trans–retinoic acid–induced differentiation in NB4 acute promyelocytic leukemia cells. *J Environ Pathol Toxicol Oncol*. 2019;38(1).
20. RCSB Protein Data Bank. https://www.rcsb.org. Accessed June 29, 2025.
21. Martínez-Rosell G, Giorgino T, De Fabritiis G. PlayMolecule ProteinPrepare: a web application for protein preparation for molecular dynamics simulations. *J Chem Inf Model*. 2017;57(7):1511-1516.
22. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem*. 2010;31:455-461.
23. PubChem Database. https://pubchem.ncbi.nlm.nih.gov. Accessed June 29, 2025.
24. Jiménez J, Škalič M, Martínez-Rosell G, De Fabritiis G. KDEEP: protein–ligand absolute binding affinity prediction via 3D-convolutional neural networks. *J Chem Inf Model*. 2018;58(2):287-296. https://playmolecule.com/Kdeep/data analysis system.
25. PlexView, PlayMolecule. https://www.playmolecule.com/PlexView. Accessed June 29, 2025.
26. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023;51(D1):D638-D646.
27. Jimenez J, Sabbadin D, Cuzzolin A, et al. PathwayMap: molecular pathway association with self-normalizing neural networks. *J Chem Inf Model*. 2018;59(3):1172-1181.
28. Espano JRB. *Understanding the Crystalline Landscape of Metal Chalcogenide Materials* [doctoral dissertation]. Vanderbilt University; 2024.
29. Hughes LP, Szell PM, Blade H, Brown SP. NMR crystallography in pharmaceutical development. 2025.
30. Waring MJ, Arrowsmith J, Leach AR, et al. An analysis of the attrition of drug candidates from four major

pharmaceutical companies. *Nat Rev Drug Discov*. 2015;14(7):475-486.
31. Singh RR, Reindl KM. Glutathione S-transferases in cancer. *Antioxidants*. 2021;10(5):701.
32. Roy N, Paira P. Glutathione depletion and stalwart anticancer activity of metallotherapeutics inducing programmed cell death: opening a new window for cancer therapy. *ACS Omega*. 2024;9(19):20670-20701.
33. Nazar SS, Ayyappan JP. Mechanistic evaluation of myristicin on apoptosis and cell cycle regulation in breast cancer cells. *J Biochem Mol Toxicol*. 2024;38(6):e23740.
34. Storr SJ, Woolston CM, Zhang Y, Martin SG. Redox environment, free radical, and oxidative DNA damage. *Antioxid Redox Signal*. 2013;18:2399-2408.
35. Wee LJ, Tan TW, Ranganathan S. CASVM: web server for SVM-based prediction of caspase substrates cleavage sites. *Bioinformatics*. 2007;23(1):3241-3243.

doi: 10.53446/actamednicomedia.1712079

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

THE CLINICAL CHARACTERISTICS OF INFLUENZA AND OTHER VIRAL RESPIRATORY INFECTIONS IN THE INTENSIVE CARE UNIT: A ONE-YEAR SINGLE-CENTER RETROSPECTIVE STUDY

YOĞUN BAKIM ÜNİTESİNDE İNFLUENZA VE DİĞER SOLUNUM YOLU VİRAL ENFEKSİYONLARININ KLİNİK ÖZELLİKLERİ: BİR YILLIK TEK MERKEZLİ RETROSPEKTİF ÇALIŞMA

Volkan Alparslan[1*], Özlem Güler[2], Samet Kutlu[1], İpek İzgin Avcı[1], Aynur Karadenizli[3], Nur Baykara[1], Alparslan Kuş[1]

[1]Kocaeli University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kocaeli, Türkiye. [2]Kocaeli University, Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Kocaeli, Türkiye. [3]Kocaeli University, Faculty of Medicine, Department of Medical Microbiology, Kocaeli, Türkiye.

ABSTRACT

Objective: The aim of this study was to evaluate the clinical features and prognostic factors associated with mortality in respiratory viral infections in intensive care unit patients.

Methods: This retrospective, single-centre study included adult patients aged ≥18 years who were admitted to the intensive care unit of Kocaeli University Faculty of Medicine between March 2024 and March 2025 and whose respiratory viral agent was detected by BioFire® Respiratory 2.1 Plus Panel test. Demographic data, clinical and pneumonia scoring systems (APACHE II, SOFA, PSI, CURB-65), laboratory parameters and patient prognosis (survived/non survived) were analysed.

Results: A total of 547 out of 2719 intensive care unit patients underwent respiratory panel, and at least one viral agent was detected in 95 (17.4%) of them. The most frequently detected viruses were rhinovirus/enterovirus (n=22), SARS-CoV-2 (n=15) and influenza A-H3 (n=23). The intensive care unit mortality rate was 54.3% in viral positive patients. APACHE II, SOFA, PSI and CURB-65 scores as well as urea and procalcitonin levels were found to be significantly higher in patients with non-survived ($p<0.05$). Although mortality rates due to viral agents differed according to subgroups, this difference was not statistically significant ($p=0.215$).

Conclusion: Respiratory viral infections in intensive care unit patients are associated with mortality, especially in individuals with high clinical severity scores and some laboratory parameters. These findings need to be confirmed with larger sample, multicentre, prospective studies.

Keywords: Respiratory viruses, intensive care unit, influenza, COVID-19, APACHE II, SOFA

ÖZ

Amaç: Bu çalışmanın amacı, yoğun bakım hastalarında saptanan solunum yolu viral enfeksiyonlarının klinik özelliklerini ve mortalite ile ilişkili prognostik faktörleri değerlendirmektir.

Yöntem: Bu retrospektif, tek merkezli çalışmaya, Mart 2024 – Mart 2025 tarihleri arasında Kocaeli Üniversitesi Tıp Fakültesi yoğun bakım ünitesine kabul edilen ve BioFire® Respiratory 2.1 Plus Panel testi ile solunum yolu viral etkeni saptanan ≥18 yaş erişkin hastalar dahil edildi. Demografik veriler, klinik ve pnömoni skorlama sistemleri (APACHE II, SOFA, PSI, CURB-65), laboratuvar parametreleri ve hasta prognozu (yaşam/exitus) analiz edildi.

Bulgular: Toplam 2719 yoğun bakım hastasından 547'sine solunum paneli uygulanmış, bunların 95'inde (%17,4) en az bir viral etken saptanmıştır. En sık tespit edilen virüsler rhinovirüs/enterovirüs (n=22), SARS-CoV-2 (n=15) ve influenza A-H3 (n=23) olmuştur. Viral pozitif hastalarda yoğun bakım mortalite oranı %54,3 bulunmuştur. Ölen hastalarda APACHE II, SOFA, PSI ve CURB-65 skorlarının yanı sıra üre ve prokalsitonin düzeylerinin anlamlı derecede yüksek olduğu görülmüştür ($p<0,05$). Alt gruplara göre viral etkenlere bağlı mortalite oranları farklılık gösterse de bu fark istatistiksel olarak anlamlı bulunmamıştır ($p=0,215$).

Sonuç: Yoğun bakım hastalarında görülen solunum yolu viral enfeksiyonları, özellikle klinik şiddet skorları ve bazı laboratuvar parametreleri yüksek olan bireylerde mortalite ile ilişkilidir. Daha geniş örneklemli, çok merkezli, prospektif çalışmalar ile bu bulguların doğrulanması gerekmektedir.

Anahtar Kelimeler: Solunum yolu virüsleri, yoğun bakım, influenza, COVID-19, APACHE II, SOFA

***Corresponding author/İletişim kurulacak yazar:** Volkan Alparslan; Kocaeli University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kocaeli, Türkiye.
Phone/Telefon: +90 (262) 303 75 75, **e-mail/e-posta:** volkan.alparslan@kocaeli.edu.tr
Submitted/Başvuru: 02.06.2025 • **Accepted/Kabul:** 26.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Bacterial pathogens occur frequently in intensive care, and the diagnosis of viral infections has increased with advances in molecular tests.[1] However, viral infections have threatened global health, as seen with the 2009 H1N1 pandemic and most recently, the 2019 COVID-19 pandemic.[2] Despite advances in the diagnosis of viral infections, antiviral agents used in their treatment are limited compared to antibiotics.[3] The principal defence mechanism against viral infection is vaccination. Viral pathogens can cause life-threatening sepsis, acute respiratory distress syndrome (ARDS), and organ failures.[4] According to studies conducted prior to the COVID-19 pandemic, the incidence of viral respiratory infections can range between 20% and 50%.[5,6] Although the incidence of respiratory viruses has decreased with the use of methods such as masks and disinfection during the COVID-19 pandemic, it has recently begun to rise again as these measures have been lifted.[7] The principal viral respiratory agents most commonly seen in intensive care are influenza, parainfluenza, respiratory syncytial virus, metapneumovirus, coronavirus, adenovirus and rhinovirus.[1]

There is a strong likelihood of a life-threatening pandemic following COVID-19.[8] Therefore, evaluating the clinical effects of viral respiratory infections in intensive care patients is of particular importance. This study aimed to identify the clinical and epidemiological characteristics of patients with viral respiratory infections in the intensive care unit (ICU) of a tertiary hospital between 2024-2025, to determine factors associated with mortality, and to compare these with the current literature. The study hypothesis was that mortality in patients diagnosed with viral respiratory infections would be associated with the causative pathogen, age, comorbidities, severity of infection (Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA), Pneumonia Severity Index (PSI), Confusion, Blood Urea Nitrogen (BUN, optional), respiratory rate, blood pressure (CURB-65)), and various laboratory parameters.

Methods

This retrospective, single-center study included patients who were followed up at the Kocaeli University Faculty of Medicine General and Postoperative Intensive Care Unit, Turkey, between March 2024 and March 2025. Patients aged 18 years or older and with positive BioFire® Respiratory 2.1 Plus Panel (BioFire Diagnostics, Salt Lake City, UT, USA) tests during admission to the ICU or during their ICU stay were included. Approval for the study was granted by the Kocaeli University Non-Interventional Clinical Research Ethics Committee (Approval number: GOKAEK-2025/05/33). All patient data were anonymized. The study data were retrieved through a hospital information management system and retrospective examination of patient files. Data recorded during routine clinical procedures were used.

The variables investigated included demographic data (age, sex, underlying chronic diseases, the presence of immunosuppression, and malignancy), clinical scores (APACHE II and SOFA), type of respiratory viral pathogen, pneumonia scores (PSI and CURB-65), prognosis (survivors or non-survivors), laboratory findings (leukocyte, neutrophil, lymphocyte, hematocrit, C-reactive protein (CRP), procalcitonin(Pct), aspartate transferase (AST), alanine transaminase (ALT), urea, creatinine, pH, pO_2, lactate, international normalized ratio (INR), and activated partial thromboplastin time values (aPTT), and radiological involvement (single lobe, multilobular bilateral infiltration, and pleural effusion).

Inclusion Criteria

1. Age 18 or over
2. Admission to the Kocaeli University Faculty of Medicine Hospital ICU
3. Being followed up between 31 March, 2024, and 31 March, 2025, and
4. Identification of respiratory infection based on pathogen-specific PCR testing and laboratory confirmation

Statistical Analysis

All statistical analyses were performed using IBM SPSS for Windows version 29.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality assumption. Continuous variables were presented with median and interquartile range (IQR) values since the normality assumption did not hold. Categorical variables were presented as the number of observations and percentages. Comparisons between groups were performed using the Mann-Whitney U test. Associations between categorical variables were examined with the Chi-square test. A p-value <0.05 was considered statistically significant.

Results

BioFire® Respiratory 2.1 Plus Panel (BioFire Diagnostics, Salt Lake City, UT, USA) were applied to 547 of the 2,719 patients admitted to our ICU throughout the study period. At least one respiratory tract virus was detected in 95 patients (17.4%).

The distribution of viral agents detected in the positive cases was as follows:

- Rhinovirus/enterovirus: 22 (23.1%)
- SARS-CoV-2: 15 (15.7%)
- Coronavirus OC 43:2 (2.1%)
- Parainfluenza virus-3: 11 (11.5%)
- Parainfluenza virus-4: 6 (6.3%)
- Parainfluenza virus-1: 2 (2.15)
- Influenza A-H3 + Influenza A: 23 (24.2%)
- Influenza A (H1N1) 2009 + Influenza A: 2 (2.1%)
- Influenza A (only positive): 4 (4.2%)
- Influenza B: 1 (1.0%)

- RSV A/B: 2 (2.1%)
- Human bocavirus: 1 (1.0%)
- Metapneumovirus A/B: 1 (1.0%)
- Adenovirus: 1 (1.0%)
- Coronavirus 229E: 1 (1.05)
- Rhinovirus/enterovirus + Coronavirus OC43 coinfection: 1 (1.0%)

Of the 95 virus-positive patients, 53 (55.8%) were women and 42 (44.2%) were men. As one of these patients was transferred to an external center, the individual was excluded from the analysis. Analysis was performed on the remaining 94 patients. Forty-three (45.7%) of these 94 patients were discharged, and 51 (54.3%) died in intensive care.

A comparison of the survivors and non-survivors revealed significantly higher SOFA, APACHE II, PSI, and CURB-65 scores in non-survivor patients (p=0.006, p=0.014, p<0.001, and p<0.001, respectively). These scores, associated with mortality, have emerged as powerful indicators of clinical severity and prognosis in patients with viral infections.

In terms of laboratory parameters, the non-survivor patients exhibited significantly higher urea levels (62.8 mg/dL (39.1-154.4) vs 35.5mg/dL (19.2-59.1); p<0.001), and procalcitonin levels (0.13 ng/mL (0.09-0.93) vs. 0.775 ng/mL (0.23-2.54); p=0.002). No significant differences were observed between the CRP, white blood cell, lymphocyte, or hematocrit levels of the groups (p>0.05).

In terms of comorbid diseases, the mortality rate was significantly higher in the patients with chronic obstructive pulmonary disease (COPD) (78.9% vs 48.0%; p=0.016). However, no significant association was found between mortality and the presence of hypertension (p=0.309), diabetes mellitus (p=0.649), or chronic heart failure (p=0.761). A comparison of clinical and laboratory parameters between survivors and non-survivors is presented in Table 1.

Table 1. Comparison of clinical and laboratory findings between survivors and non-survivors

	Survivors (n=43)	Non-survivors (n=51)	p
Age	67 (47-76)	71 (58-77)	0.290
PSI	125 (0-140)	155 (127-196)	**<0.001**
CURB-65	1 (0-3)	2 (2-4)	**<0.001**
CRP	100.4 (31.03-153.2)	91.9 (39.2-184.7)	0.306
Procalsitonin	0.13 (0.09-0.93)	0.77 (0.23-2.54)	**0.002**
Urea	35.5 (19.2-59.1)	62.8 (39.1-154.4)	**<0.001**
SOFA	6 (4-7)	8 (5-10)	**0.006**
APACHE II	19 (16-22)	24.5 (18.75-28.25)	**0.014**
WBC	8.26 (6.92-15.9)	11.9 (7.92-18.6)	0.084
Lymphocyte	1.03 (0.69-1.78)	0.76 (0.4-1.64)	0.174
Haematocrit	32.7 (28.1-36.7)	31.6 (26.1-35.2)	0.216

Values are presented as median (IQR). Comparisons between survivors and non-survivors were performed using the Mann–Whitney U test. A p-value <0.05 was considered statistically significant.

IQR: Interquartile range, PSI: Pneumonia severity index, CRP: C-reactive protein, SOFA: Sequential Organ Failure Assessment, APACHE II: Acute Physiology and Chronic Health Evaluation II, WBC: White blood cell

From the perspective of clinical scoring systems, PSI, CURB-65, SOFA, and APACHE II scores were significantly higher in non-survivors than in survivors (p<0.001, p<0.001, p=0.006, and p=0.014, respectively). These differences are shown in boxplots in Figure 1 and Figure 2.

Examination of laboratory parameters associated with mortality in this study revealed significantly higher urea and procalcitonin levels in non-survivors than in survivors (p<0.001 and p=0.002, respectively). These differences are shown in boxplots in Figure 3.

When mortality rates were analyzed according to the most frequently detected viral subtypes, 36.4% of the patients who tested positive for rhinovirus/enterovirus, 66.7% of those positive for SARS-CoV-2, and 50% of those positive for influenza A-H3 were non-survivors. However, these differences were not statistically significant (p = 0.215).

Discussion

Viral respiratory infections were detected in 95 of the 547 patients whose respiratory panels were investigated in the ICU of a tertiary university hospital in this study. This figure (17.4%) is consistent with previously reported rates and is similar to the 22.4% reported by Al-Dorzi et al.[9] in their Saudi Arabia-based study. The mortality rate in viral-positive patients was 54%.

Scores such as APACHE II, SOFA, PSI, and CURB-65, which were significantly associated with mortality in this study, were of prognostic value in this patient group. Similarly, Al-Dorzi et al. identified PSI scores as independent predictors of admission to intensive care and in-hospital mortality.

Despite the prevalence of comorbidities such as hypertension, heart failure, and malignancy in this study, the variables most closely linked to mortality were the PSI and SOFA scores. Immunosuppression and bacterial superinfections increase mortality, particularly in cases of sepsis associated with viral infections.[10]

Viral infections began to increase again with the lifting of protective measures, such as masks and isolation, in the wake of the COVID-19 pandemic. According to the Centers for Disease Control and Prevention data[11], influenza alone produces between 9 and 41 million cases and causes 4,900-51,000 deaths annually.

Another important finding of the present study is that mortality occurred in approximately half of the patients with viral infections. This may be attributed to the limited early diagnosis and effective treatment of viral infections. The limited number of antiviral agents and the fact that priority is usually attached to support therapy is one of the factors contributing to the high mortality in viral infections.

In this study, high mortality rates due to viral respiratory infections were observed in patients under follow-up in the intensive care unit. Similarly, in their extensive 10-year analysis, O'Halloran et al.[12] reported significantly higher influenza-associated hospital admission, intensive care requirement, and in-hospital mortality rates and

noted that these outcomes were more common among socially disadvantaged groups in particular. This shows that epidemiological risk factors should be considered in addition to clinical factors in the management of viral infections.

In our study, when laboratory parameters associated with mortality were evaluated, procalcitonin and urea levels were significantly higher in patients who died. This finding is important in terms of demonstrating the effects of systemic inflammation and organ dysfunction on mortality. Previous studies have shown that procalcitonin levels are associated not only with bacterial superinfection but also with severe inflammatory responses, such as cytokine storms, and are a valuable biomarker for predicting prognosis. The COVIDeF cohort study by Cancella de Abreu et al.[13] also found that elevated procalcitonin levels were independently associated with in-hospital deterioration (ICU admission, mechanical ventilation, ARDS development, or death). In the same study, urea levels were also shown to be significantly associated with poor clinical outcomes, because elevated urea levels may be associated with a severe course of the disease and fatal outcomes, especially through renal dysfunction and tissue perfusion insufficiency. In this context, procalcitonin and urea levels should be considered for early risk stratification and prognosis determination in intensive care unit patients with viral respiratory tract infections.

In subgroup analyses, numerical differences were observed between mortality rates according to viral agents; however, these differences were not statistically significant. This is probably due to the relatively low number of patients infected with each viral agent. Similarly, it has been reported that larger samples are needed to evaluate the effect of viral subtypes on mortality.[14,15]

This study had several limitations. In particular, owing to its single-center and retrospective design, the generalizability of the results may be limited. In addition, the relatively small number of patients may have reduced the statistical power of the comparisons between subgroups. Due to missing laboratory and clinical data in some patients, we were unable to obtain a complete dataset for all variables. Additionally, because the vaccination status of the patients was not known, we were unable to assess the potential effect of vaccination on disease severity or outcomes. In light of these limitations, the study's findings should be interpreted with care. Future multicenter prospective studies with larger samples will yield stronger evidence on this subject.

In conclusion, this study revealed that mortality rates are high in cases of viral respiratory infection followed up in the ICU and that the presence of COPD and clinical scores such as APACHE II, SOFA, PSI, and CURB-65 are significantly associated with mortality. Careful evaluation of clinical scoring systems and early laboratory markers is of critical importance for the early identification of high-risk patients and optimization of intensive care management. Our findings support the importance of risk classification in the management of viral pneumonia, and further prospective studies with larger sample sizes are needed.

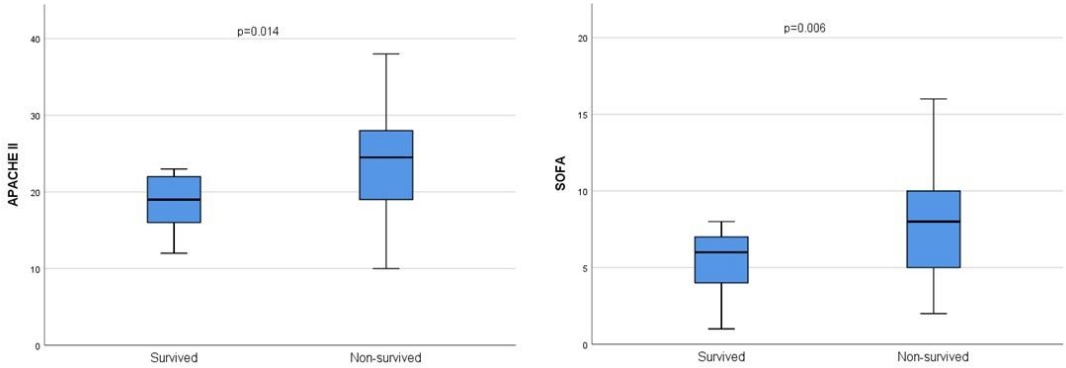


Figure 1. A comparison of APACHE II and SOFA scores in survivors and non-survivors

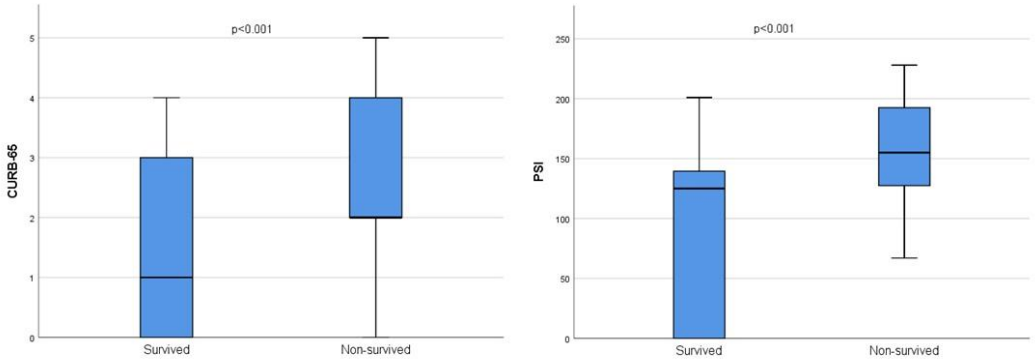


Figure 2. A comparison of CURB-65 and PSI scores in survivors and non-survivors

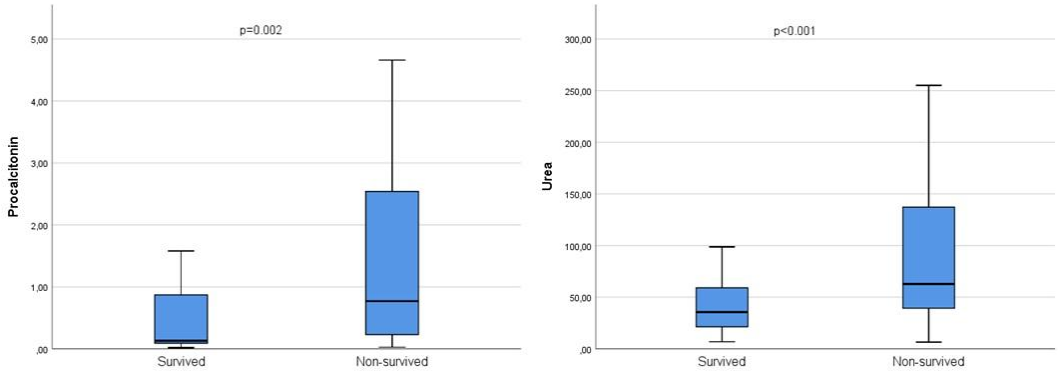


Figure 3. A comparison of procalcitonin and urea levels in survivors and non-survivors

Ethical Approval

Approval for the study was granted by the Kocaeli University Non-Interventional Clinical Research Ethics Committee (Approval number: GOKAEK-2025/05/33).

Conflict of Interest

There is no conflict of interest to declare.

Author Contributions

VA, ÖG: Conceptualization, methodology, formal analysis and investigation, writing-original draft preparation; SK, İİA: Data curation; VA, AK, NB, AK: Writing-review and editing; AK, NB, AK: Supervision

Financial Support

None

References

1. Fragkou PC, Moschopoulos CD, Karofylakis E, Kelesidis T, Tsiodras S. Update in viral infections in the intensive care unit. *Front Med (Lausanne)*. 2021;8:575580. doi:10.3389/fmed.2021.575580
2. Xu JQ, Zhang WY, Fu JJ, et al. Viral sepsis: diagnosis, clinical features, pathogenesis, and clinical considerations. *Mil Med Res*. 2024;11:78. doi:10.1186/s40779-024-00581-0
3. Kelesidis T, Mastoris I, Metsini A, Tsiodras S. How to approach and treat viral infections in ICU patients. *BMC Infect Dis*. 2014;14:321. doi:10.1186/1471-2334-14-321
4. Lin GL, McGinley JP, Drysdale SB, Pollard AJ. Epidemiology and immune pathogenesis of viral sepsis. *Front Immunol*. 2018;9:2147. doi:10.3389/fimmu.2018.02147
5. Hong HL, Hong SB, Ko GB, et al. Viral Infection is not uncommon in adult patients with severe hospital-acquired pneumonia. *PLoS One*. 2014;9(4):e95865. doi:10.1371/journal.pone.0095865
6. Piralla A, Rovida F, Girello A, et al. Frequency of respiratory virus infections and next-generation analysis of influenza A/H1N1pdm09 dynamics in the lower respiratory tract of patients admitted to the ICU. *PLoS One*. 2017;12(6):e0178926. doi:10.1371/journal.pone.0178926
7. Olsen SJ, Winn AK, Budd AP, et al. Changes in influenza and other respiratory virus activity during the COVID-19 Pandemic-United States, 2020-2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(29):1013-1019. doi:10.15585/mmwr.mm7029a1. PMID: 34292924
8. Telenti A, Arvin A, Corey L, et al. After the pandemic: perspectives on the future trajectory of COVID-19. *Nature*. 2021;596(7873):495-504. doi:10.1038/s41586-021-03792-w
9. Al-Dorzi HM, Alsafwani ZA, Alsalahi E, et al. Patients with influenza admitted to a tertiary-care hospital in Riyadh between 2018 and 2022: characteristics, outcomes and factors associated with ICU admission and mortality. *BMC Pulm Med*. 2024;24(1):464. doi:10.1186/s12890-024-03281-6
10. Verdier V, Lilienthal F, Desvergez A, Gazaille V, Winer A, Paganin F. Severe forms of influenza infections admitted in intensive care units: Analysis of mortality factors. *Influenza Other Respir Viruses*. 2023;17(7):e13168. doi:10.1111/irv.13168
11. Naquin A, O'Halloran A, Ujamaa D, et al. Laboratory-confirmed influenza-associated hospitalizations among children and adults-influenza hospitalization surveillance network, United States, 2010-2023. *MMWR Surveill Summ*. 2024;73(6):1-18. doi:10.15585/mmwr.ss7706a1
12. O'Halloran AC, Holstein R, Cummings C, et al. Rates of influenza-associated hospitalization, intensive care unit admission, and in-hospital death by race and ethnicity in the United States from 2009 to 2019. *JAMA Netw Open*. 2021;4(8):e2121880. doi:10.1001/jamanetworkopen.2021.21880
13. Cancella De Abreu M, Ropers J, Oueidat N, et al. Biomarkers of COVID-19 short-term worsening: a multiparameter analysis within the prospective multicenter COVIDeF cohort. *European Journal of Emergency Medicine*. 2024;31(6):429-437. doi:10.1097/MEJ.0000000000001175
14. Ambrosch A, Luber D, Klawonn F, Kabesch M. Focusing on severe infections with the respiratory syncytial virus (RSV) in adults: Risk factors, symptomatology and clinical course compared to influenza A / B and the original SARS-CoV-2 strain. *J Clin Virol*. 2023;161:105399. doi:10.1016/j.jcv.2023.105399
15. Surie D, Yuengling KA, DeCuir J, et al. Severity of respiratory syncytial virus vs COVID-19 and influenza among hospitalized US adults. *JAMA Network Open*. 2024;7(4):e244954. doi:10.1001/jamanetworkopen.2024.4954

doi: 10.53446/actamednicomedia.1626897

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

EVALUATION OF HEART RATE VARIABILITY IN CHILDREN PRESENTING WITH SYNCOPE

SENKOP İLE BAŞVURAN ÇOCUKLARDA KALP HIZI DEĞİŞKENLİĞİNİN DEĞERLENDİRİLMESİ

Bekir Yükcü[1*], Betül Diler Durgut[2], Emine Tekin[2], Fidel Ceren Yavuzyılmaz[3]

[1]Giresun Obstetric and Pediatric Disease Education and Research Hospital, Department of Pediatric Cardiology, Giresun, Türkiye. [2]Giresun Obstetric and Pediatric Disease Education and Research Hospital ocaeli University, Department of Pediatric Neurology, Giresun, Türkiye. [3]Giresun Obstetric and Pediatric Disease Education and Research Hospital, Department of Pediatrics, , Türkiye.

ABSTRACT

Objective: This study evaluated the diagnostic value of heart rate variability (HRV) parameters obtained from 24-hour Holter monitoring in pediatric patients with vasovagal syncope (VVS) and compared them to a control group. The study also analyzed time-domain and frequency-domain HRV parameters to explore the autonomic mechanisms underlying VVS and the diagnostic potential of HRV.

Methods: This retrospective study was conducted at Giresun Women's and Children's Health Training and Research Hospital. The study included 41 pediatric patients with syncope and 36 healthy controls who underwent 24-hour Holter electrocardiography (ECG) monitoring for suspected arrhythmia; however, no arrhythmia was found. Comprehensive cardiac, neurological, and demographic evaluations were performed for all participants. HRV parameters were analyzed from 24-hour Holter recordings, and group comparisons were performed using the Mann–Whitney U test. Spearman correlation and Receiver Operating Characteristic analyses were also conducted.

Results: Time-domain HRV parameters were significantly lower in the syncope group compared to those in the control group ($p < 0.05$). No significant differences were observed in frequency-domain parameters. The standard deviation of normal-to-normal RR intervals (SDNN) demonstrated the highest diagnostic accuracy, with a cut-off value of <163 ms (Area Under the Curve: 0.753, sensitivity: 72.2%, specificity: 75.6%).

Conclusion: HRV parameters obtained from 24-hour Holter monitoring provide valuable insights into autonomic imbalance in pediatric VVS. SDNN emerged as a strong diagnostic marker in this regard. Further studies with larger, more homogeneous populations are needed to establish normative HRV values and refine diagnostic criteria in pediatric populations.

Keywords: 24-hour Holter monitoring, autonomic imbalance, heart rate variability, vasovagal syncope

ÖZ

Amaç: Bu çalışmada, pediatrik vazovagal senkop (VVS) hastalarında 24 saatlik Holter monitörizasyonu ile elde edilen kalp hızı değişkenliği (HRV) parametrelerinin tanısal değerinin değerlendirilmesi ve kontrol grubu ile karşılaştırılması amaçlanmıştır. Zaman alanı ve frekans alanı HRV parametreleri incelenerek VVS'nin altta yatan otonom mekanizmaları ve HRV'nin tanısal potansiyeli araştırılmıştır.

Yöntem: Retrospektif tasarlanan bu çalışma, Giresun Kadın Doğum ve Çocuk Hastalıkları Eğitim ve Araştırma Hastanesi'nde gerçekleştirildi. Çalışmaya, senkop şikayeti ile başvuran 41 çocuk hasta ve aritmi şüphesi nedeniyle 24 saatlik Holter elektrokardiyografi (EKG) monitorizasyonu yapılan ancak herhangi bir aritmiye rastlanmayan 36 sağlıklı kontrol dahil edildi. Tüm hastalar kapsamlı kardiyak, nörolojik ve demografik değerlendirmelere tabi tutuldu. HRV parametreleri 24 saatlik Holter EKG kayıtlarından analiz edildi ve gruplar arası farklar Mann-Whitney U testi ile değerlendirildi. Ayrıca, Spearman korelasyon ve Alıcı Çalışma Karakteristiği (ROC) analizi yapıldı.

Bulgular: Zaman alanı HRV parametreleri, senkop grubunda kontrol grubuna göre anlamlı olarak düşük bulundu ($p<0,05$). Frekans alanı parametreleri açısından gruplar arasında anlamlı fark tespit edilmedi. Ardışık normal sinüs kalp atımları arasındaki sürenin standart sapmasının (SDNN) 163 ms altındaki değerler, vasovagal senkopu öngörmede en yüksek tanısal doğruluğu gösterdi (Eğrinin Altındaki Alan (AUC): 0,753, duyarlılık: %72,2, özgüllük: %75,6).

Sonuç: Yirmidört saatlik Holter monitörizasyonu ile elde edilen HRV parametreleri, pediatrik VVS hastalarında otonom disfonksiyonu değerlendirmede etkili bilgiler sağlayabilir. SDNN, güçlü bir tanısal belirteç olarak öne çıkmaktadır. Normatif HRV değerlerinin oluşturulması için daha geniş ve homojen popülasyonlarla yapılacak çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: 24 saat Holter monitörizasyonu, kalp hızı değişkenliği, otonomik dengesizlik, vazovagal senkop.

***Corresponding author/İletişim kurulacak yazar:** Bekir Yükcü; Giresun Obstetric and Pediatric Disease Education and Research Hospital Pediatric Cardiology Department, Giresun, Türkiye
Phone/Telefon: +90 (454) 310 20 20, **e-mail/e-posta:** byukcu@gmail.com
Submitted/Başvuru: 26.01.2025 • **Accepted/Kabul:** 01.03.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Syncope is a temporary and self-limiting loss of consciousness caused by decreased cerebral blood flow and marked by rapid onset, brief duration, and spontaneous recovery.[1,2] Syncope is a frequent clinical concern in children and adolescents, with approximately 15% having at least one episode by the end of childhood adolescence.[3,4] Vasovagal syncope (VVS) is the most common cause, typically triggered by long periods of standing, emotional stress, or environmental factors.[1] VVS is linked to autonomic imbalance, which may involve parasympathetic overactivity, sympathetic inhibition, or a combination of both, thereby resulting in hypotension and bradycardia.[1,5] While typically harmless, recurrent VVS considerably influences quality of life and impacts physical, psychological, and psychosocial activities.[6,7]

Heart rate variability (HRV) is defined as the variation in time between successive heartbeats.[8] Measuring this variability provides a non-invasive assessment of autonomic nervous system (ANS) activity. It indicates the balance between sympathetic and parasympathetic heart rate regulation and assesses autonomic balance, blood pressure, gas exchange, vascular tone, and functions such as gut activity and facial muscle movement regulation.[9,10] ANS activity enables us to better adjust to difficult environmental and psychological factors. Conversely, disruption of this system and the resulting decrease in HRV, a marker of autonomic imbalance, have been associated with negative effects on a range of cardiovascular and non-cardiovascular conditions in both adults and children.[9,11,12] Moreover, HRV has been used for various clinical conditions, including respiratory distress syndrome, significant patent ductus arteriosus, congenital heart defects, neonatal sepsis, necrotizing enterocolitis, anxiety disorders, obstructive sleep apnea, depression and neurological issues injuries.[13-21] But a higher HRV is not always positive; it can indicate a higher mortality risk, particularly in older adults, likely due to conduction issues and abnormalities.[22] Therefore, many studies have been conducted to establish optimal HRV norm values; however, studies on children in this field remain limited.[9,23,24] An ideal HRV is a sign of a robust, adaptable ANS capable of effective self-regulation.

In cases of syncope, lower HRV frequently indicates an autonomic imbalance, particularly in VVS, where there is excessive parasympathetic activity or diminished sympathetic response is predominant.[11,25] By analyzing time-domain and frequency-domain parameters, HRV provides valuable insights into the pathophysiological mechanisms underlying syncopal episodes, thereby making it an essential non-invasive tool in clinical investigations.[9,23] Despite its potential, only a limited number of pediatric studies have investigated HRV in VVS, thus leaving a gap in understanding baseline HRV values and their diagnostic utility in this population.[26]

This study aims to evaluate the predictive value of HRV parameters obtained from 24-hour Holter monitoring, which is regarded as more reliable and comprehensive than short-term HRV measurements, in distinguishing pediatric patients with VVS from healthy controls. By investigating both time-domain and frequency-domain HRV metrics, the study seeks to clarify the autonomic mechanisms underlying VVS and explore the potential of HRV as a diagnostic tool. These findings could help refine diagnostic criteria and enhance clinical management strategies for pediatric syncope.

Methods

The study was approved by the ethics committee of Giresun University (April 2023 / Decision No: 2) and were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments.

Study Population

This retrospective cross-sectional study was conducted between June 2022 and December 2024, with 92 patients aged 6–12 years who presented with acute loss of consciousness at the Pediatric Neurology and Pediatric Cardiology outpatient clinics of Giresun Women's and Children's Health Training and Research Hospital. The study focused exclusively on pediatric patients diagnosed with VVS. It was conducted according to the principles outlined in the Canadian Cardiovascular Society and Canadian Pediatric Cardiology Association Position Statement on the Approach to Syncope in the Pediatric Patient.[27] VVS is a syncopal syndrome usually triggered by standing for over 30 seconds or encountering pain, emotional stress. It presents symptoms such as warmth, sweating, pallor, and nausea; is linked to hypotension and relative bradycardia when present; and is characterized by post-event fatigue.

The control group consisted of patients who underwent 24-hour Holter monitoring for ventricular extrasystoles rather than palpitations, thus ensuring a more appropriate comparison with the patient group. Additionally, the patients were divided into two age groups: those aged 6–12 years were classified as the preadolescent group, and those aged 12–18 years were classified as the adolescent group.

Syncope Group Inclusion Criteria

- At least three syncopal episodes within the last year
- No serious cardiac finding (including ECG, 24-hour Holter monitoring, and echocardiography (may be accompanied by mild symptoms that do not require treatment or follow-up)
- Normal neurological evaluations (including EEG)
- No other identifiable etiology for syncope (anemia, psychological disease)
- No medication used for acute and chronic diseases

Control Group Inclusion Criteria

- No syncope history within the last year
- No cardiac, neurological, or psychological symptoms (may be accompanied by mild

symptoms that do not require treatment or follow-up)
- No medication used for acute and chronic diseases
- No arrhythmia on Holter monitoring.

Patient Selection

Patients presenting to the pediatric cardiology clinic with acute loss of consciousness and a preliminary diagnosis of cardiac syncope were initially evaluated with a detailed medical history and physical examination. Patients diagnosed with typical VVS during this evaluation were discharged with recommendations for lifestyle modifications and follow-up. Patients referred with murmurs, palpitations, chest pain, family history of cardiac disease, history of sudden death, prolonged loss of consciousness (>5 minutes), or recurrent syncope underwent further investigations, including electrocardiography (ECG), echocardiography, and 24-hour Holter monitoring.

Among these patients, those diagnosed with VVS formed the study group. Following exclusions for epileptic seizures (n = 6, generalized tonic-clonic), cardiac syncope (n = 2, pulmonary embolism, supraventricular tachycardia), and incomplete Holter data (n = 43), 41 patients diagnosed with VVS were included in the study. The control group included patients referred to the pediatric cardiology clinic due to a murmur but without complaints. Among these patients, those who underwent Holter monitoring because ventricular extrasystoles were detected on their ECG and had no ventricular extrasystoles or any other arrhythmias on Holter recordings were included.

Data Collection

A retrospective review was conducted on the medical records of patients diagnosed with VVS. Demographic data—including gender, age, weight, height, body mass index, socioeconomic status, and educational background—were obtained. Information regarding the pattern, timing, frequency, and duration of syncope, triggering factors, and history of cardiac or neurological diseases was recorded. Clinical findings from physical examinations, blood pressure measurements, ECGs, echocardiograms, and 24-hour Holter monitoring parameters were analyzed. Laboratory results were also collected, including hemoglobin, glucose, sodium, calcium, potassium, thyroid-stimulating hormone, vitamin B12, and vitamin D levels. Moreover, the Modified Calgary score of all patients included in the study was calculated and recorded.[28]

24-Hour ECG Monitoring

All participants underwent 24-hour ECG recordings using a GE Healthcare SEER 1000 Holter monitor (seven channels, California, USA). The Holter device was attached during daily activities, and the recorded data were analyzed using the device's proprietary software and with artifact corrections applied. The HRV parameters analyzed in this study included both time-domain and frequency-domain measures, described below.[11,25,29]

All time-domain parameters were calculated using the complete 24-hour Holter recordings. Minimum, maximum, and mean heart rates were analyzed to assess HRV over the recording period. The total number of QRS complexes and the total duration of Holter recordings were quantified. Mean and median RR intervals—representing the average and median times between successive R waves, respectively—were measured in milliseconds. The standard deviation of normal-to-normal (SDNN) RR intervals, served as a global marker of HRV. At the same time, the standard deviation of the averages of normal-to-normal (SDANN) RR intervals across all five-minute segments, thereby reflecting longer-term variability. The overall variability of RR intervals was further assessed using the standard deviation of RR (SDRR) intervals. Further, the HRV triangular index (HRV TI) quantified overall variability by dividing the total number of all NN intervals by the peak height of their histogram. Additionally, NN50, representing the count of interval differences greater than 50 milliseconds between successive NN intervals, and pNN50%, the percentage of such intervals, was calculated to assess short-term variability. The root mean square of successive RR interval differences (RMSSD) provided insight into parasympathetic activity. Lastly, the SDNN Index, derived as the mean of the five-minute standard deviations of NN intervals over 24 hours, provided a measure of variability attributed to cycles shorter than five minutes.[9]

Frequency-domain parameters were also analyzed to evaluate the power distribution across different frequency bands[9]:

- High-frequency (HF) power in normalized units: The relative power of the high-frequency band (0.15 Hz–0.4 Hz) in normal units.
- Low-frequency (LF) power in normalized units: The relative power of the low-frequency band (0.04 Hz–0.15 Hz) in normal units.
- The LF/HF ratio, calculated as the ratio of LF power to HF power, is a marker of autonomic balance.
- The Symindex, derived from logarithmic transformation, assesses the ratio of LF power to HF power in normalized units, thus offering another perspective on the balance between sympathetic and parasympathetic contributions to HRV.

Statistical Analysis

This study conducted all statistical analyses using SPSS version 25 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to assess the normality of continuous variables. Since the data did not follow a normal distribution, continuous variables were presented as median (minimum–maximum) values, while categorical variables were summarized as numbers and percentages (n, %). Patients were categorized into two groups: the patient group (individuals experiencing syncope) and the control group (individuals not experiencing syncope). The Mann–Whitney U test was used to compare continuous

variables across groups, and categorical variables were assessed using the chi-square test. For variables identified as statistically significant in the Mann–Whitney U test, receiver operating characteristic (ROC) analysis was conducted to determine threshold values for predicting syncope. Further, corresponding ROC curves were generated to visualize the discriminatory ability of these variables. The correlation matrix heatmap was generated to analyze the relationships between statistically significant HRV parameters. The heatmap was created with the assistance of the ChatGPT-4.0 model (OpenAI, San Francisco, USA), which facilitated the visualization of these relationships. The research utilized a 95% confidence interval, thereby guaranteeing strong and dependable statistical results. A significance level of $p < 0.05$ was deemed statistically significant for all tests.

Results

The median age of the study population was 13 years (25th–75th percentile: 11–15 years), and 55.8% of the participants were female. Normal sinus rhythm was observed in 80.5% of patients, while normal echocardiographic findings were noted in 64.9% of them. The median systolic and diastolic blood pressures were 110 mmHg (97.5–116.5) and 61 mmHg (56–68.5) (Table 1).

Table 1. Characteristics of Clinical and Demographic Data in Syncope and Control Groups

Parameters		**n (%)** **Median (25^{th}–75^{th} Percentiles)**
Gender	Male	34 (44.2%)
	Female	43 (55.8%)
Age (years)		13 (11–15)
Family History of Cardiac Disease	Yes	14 (18.2%)
	No	63 (81.8%)
Family History of Epilepsy	Yes	12 (15.6%)
	No	65 (84.4%)
Family History of Syncope	Yes	3 (3.9%)
	No	74 (96.1%)
Rhythm	Normal Sinus Rhythm	62 (80.5%)
	Sinus Arrhythmia	9 (11.7%)
	Sinus Bradycardia	4 (5.2%)
	Sinus Tachycardia	2 (2.6%)
Echocardiographic Findings	Normal	50 (64.9%)
	Mitral Insufficiency	18 (23.4%)
	PFO	4 (5.2%)
	Aortic Pathologies	1 (1.3%)
	MVP-MI	1 (1.3%)
	PDA	1 (1.3%)
	Coronary Chamber Fistula	2 (2.6%)
Number of Syncope (episodes)		2 (1–3)
RDW_SD (%)		37 (34.2–39.0)
RDW_CV (%)		13 (12.45–13.60)
Glucose (mg/dL)		78 (70–87)
TSH (µIU/ml)		2.06 (1.6–2.8)
Hemoglobin (g/dL)		13.2 (12.4–13.9)
Vitamin B12 (pg/ml)		345 (262–473)
Vitamin D (nmol/l)		18 (12.3–23.5)
Systolic Blood Pressure (mmHg)		110 (97.5–116.5)
Diastolic Blood Pressure (mmHg)		61 (56–68.5)
Mean Blood Pressure (mmHg)		75.3 (69.8–83.7)
QTc Interval (ms)		405 (391–419)
SF (%)		38 (36–42)
Holter HRV Minimum (ms)		55 (49–59)
Holter HRV Average (ms)		85 (77–89)
Holter HRV Maximum (ms)		143 (133–152)
Holter Duration (hours)		19.7 (18.6–21.1)

Abbreviations: B12: Vitamin B12, BP: Blood Pressure, CV: Coefficient of Variation, HRV: Heart Rate Variability, MI: Mitral Insufficiency, MVP: Mitral Valve Prolapse, PDA: Patent Ductus Arteriosus, PFO: Patent Foramen Ovale, QTc: Corrected QT Interval, RDW: Red Cell Distribution Width, SD: Standard Deviation, SF: Shortening Fraction, TSH: Thyroid-Stimulating Hormone

The median age of participants was 13 years (6–17) in the syncope group and 14 years (9–17) in the control group ($p = 0.095$). The Holter minimum heart rate was 57 bpm (43 bpm–76 bpm) in the syncope group and 51.5 bpm (40 bpm–81 bpm) in the control group ($p = 0.015$). The SDNN and SDANN values were significantly lower in the syncope group, with medians of 139 ms (97 ms–264 ms) and 113 ms (71 ms–237 ms) ($p < 0.001$ for both) (Table 2).

Table 2. Comparative Analysis of Clinical, Hemodynamic, and Heart Rate Variability Parameters Between the Pediatric Syncope and Control Groups

Parameters (Unit)	Syncope Group (N = 41) Median (Min–Max), n (%)	Control Group (N = 36) Median (Min–Max), n (%)	p
Age (years)	13 (6–17)	14 (9–17)	0.095[a]
Number of Syncope (episodes)	2 (1–6)	0 (0)	<0.001[a]
RDW SD (%)	35.6 (31.6–45.3)	38.5 (29–47.9)	0.001[a]
RDW CV (%)	12.6 (10.6–15.6)	13.4 (12.4–15.6)	<0.001[a]
Glucose (mg/dL)	76 (68–95)	75 (69–99)	0.234
Hemoglobin (g/dL)	13.2 (10–16.6)	13.1 (11.2–16.9)	0.890[a]
TSH (µIU/mL)	1.9 (0.6–4.8)	2.12 (0.64–6.1)	0.253[a]
Vitamin B12 (pg/ml)	342 (158–828)	370 (136–2630)	0.862[a]
Vitamin D (nmol/l)	16 (3.8–42)	19 (7.4–47.2)	0.279[a]
Systolic Blood Pressure (mmHg)	109 (80–134)	110 (85–138)	0.845[a]
Diastolic Blood Pressure (mmHg)	61 (54–82)	64 (57–86)	0.552[a]
Mean Blood Pressure (mmHg)	78 (72–91)	80 (70–94)	0.564[a]
Baseline Heart Rate (bpm)	80 (53–114)	80 (56–121)	0.732[a]
QTc Interval (ms)	410 (379–465)	396 (383–426)	0.038[a]
Shortening Fraction (SF) (%)	38 (31–49)	37 (32–48)	0.849[a]
Time Domain Parameters			
Holter Minimum Heart Rate (bpm)	57 (43–76)	51.5 (40–81)	0.015[a]
Holter Mean Heart Rate (bpm)	86 (73–107)	82.5 (60–105)	0.179[a]
Holter Maximum Heart Rate (bpm)	143 (122–168)	147 (101–178)	0.588[a]
Mean RR Interval (ms)	730 (575–998)	760 (588–1034)	0.147[a]
SDNN (ms)	139 (97–264)	182 (65–289)	<0.001[a]
SDANN (ms)	113 (71–237)	148.5 (43–265)	<0.001[a]
Median RR Interval (ms)	716 (556–996)	740 (556–1004)	0.526[a]
HRV TI	30 (18–50)	39 (18–52)	0.001[a]
SDRR (ms)	34 (20–139)	42.5 (20–73)	0.028[a]
PNN50 (%)	22 (1–54)	31 (6–69)	0.008[a]
RMSSD (ms)	57 (28–272)	67 (32–327)	0.038[a]
SDNNI (ms)	54 (28–149)	67 (30–198)	0.009[a]
NN50	22.119 (6.022–65.361)	29.565 (4.116–73.469)	0.011[a]
Frequency Domain Parameters			
LF (nu)	69.9 (27.9–89.4)	69.8 (14.8–91.8)	0.475[a]
HF (nu)	23.1 (7.5–68.3)	22 (7.7–65.6)	0.806[a]
LF/HF Ratio	2.8 (0.4–12.0)	3.42 (0.27–11.83)	0.775[a]
Symindex	0.99 (–0.9–2.48)	1.23 (–1.27–2.47)	0.581[a]
Gender **Female**	23 (56.1%)	20 (55.6%)	1.000[b]
Male	18 (43.9%)	16 (44.4%)	
Modified Calgary Score	3.5 (–1–6)	Not applicable -	

Table 2 notes: [a] Mann–Whitney U test, [b] Pearson Chi-square test

Abbreviations: bpm: Beats Per Minute, CV: Coefficient of Variation, HF: High Frequency, HRV: Heart Rate Variability, LF: Low Frequency, NN50: Number of Normal-to-Normal Intervals >50 ms, nu: Normalized Units, PNN50: Percentage of NN50, QTc: Corrected QT Interval, RDW: Red Cell Distribution Width, RMSSD: Root Mean Square of Successive Differences, SDANN: Standard Deviation of the Average of NN Intervals, SDNN: Standard Deviation of NN Intervals, SDNNI: Standard Deviation of Successive Differences of NN Intervals, SDRR: Standard Deviation of R-R Intervals, SF: Shortening Fraction, TSH: Thyroid-Stimulating Hormone.

Significant differences were observed in HRV parameters between preadolescent and adolescent patients in the syncope group. Adolescents had higher SDNN (p = 0.001), SDANN (p = 0.007), and HRV triangular index (p = 0.038) values. The mean RR interval (p = 0.009) and median RR interval (p = 0.012) were also higher in the adolescent group. In the frequency domain, the LF component was higher in adolescents (p = 0.039). The mean heart rate in the 24-hour Holter ambulatory was lower in adolescents than preadolescents, but this difference was not statistically significant (p = 0.064). (Table 3)

Adolescents had significantly higher SDNN and SDANN values than preadolescents in the control group (p < 0.05). The mean RR interval was significantly longer in the adolescent group, while the mean heart rate in the 24-hour Holter ambulatory was significantly lower (p < 0.05). In the frequency domain, the LF component, LF/HF ratio, and Symindex were significantly higher in adolescents, while the HF component was lower (p < 0,05). (Table 4)

The diagnostic performance of parameters was evaluated using ROC analysis. Among the parameters, SDNN demonstrated the highest diagnostic accuracy, with an area under the curve (AUC) of 0.753 (95% CI: 0.642–0.864), a cut-off value of <163 ms, sensitivity of 72.2%, and specificity of 75.6% (p < 0.001). (Table 5 and Figure 1)

The Spearman correlation coefficients revealed a significant positive correlation between SDNN Index (ms) and RMSSD (ms) (r = 0.92, p < 0.001). Additionally, the SDNN Index (ms) correlated strongly with PNN50 (%) (r = 0.90, p < 0.001) (Figure 2).

Table 3: Comparison of HRV Parameters Between Preadolescents and Adolescents in the Syncope Group.

Parameters (Unit)		Preadolescent Group (N = 20) Median (25th–75th Percentiles) or n (%)	Adolescent Group (N = 21) Median (25th–75th Percentiles) or n (%)	p
Gender	Female	10 (50%)	13 (61.9%)	0.536[b]
	Male	10 (50%)	8 (38.1%)	
Age (years)		10 (7.25–11)	15 (14–16)	<0.001[a]
Time Domain Parameters				
Holter Mean Heart Rate (bpm)		88 (79–96)	85 (79–87)	0.064[a]
Mean RR Interval (ms)		704 (640–765)	766 (724–811)	0.009[a]
SDNN (ms)		123.5 (110.0–136.5)	157 (141–191)	0.001[a]
SDANN (ms)		99 (86.3–117.5)	133 (103.5–155.5)	0.007[a]
Median RR Interval (ms)		688 (620–768)	756 (712–804)	0.012[a]
HRV TI		27 (23–33)	32 (27–37)	0.038[a]
SDRR (ms)		43 (32.5–58.3)	46 (36.5–64.5)	0.523[a]
PNN50 (%)		16 (10.5–27.8)	25 (16.5–32)	0.215[a]
RMSSD (ms)		54,5 (40–74.3)	60 (48–78.5)	0.489[a]
SDNNI (ms)		50.5 (40.3–68)	64 (45–86.5)	0.106[a]
NN50		19730 (11573–28597)	22975 (15779–31340)	0.335[a]
Frequency Domain Parameters				
LF (nu)		61.54 (44.93– 72.30)	72.15 (57.95–79.50)	0.039[a]
HF (nu)		25.52 (18.84–45.58)	20.41 (14.63–29.33)	0.137[a]
LF/HF Ratio		2.50 (1.03–3.83)	3.38 (2.06–5.57)	0.130[a]
Symindex		0.91 (0.03–1.34)	1.19 (0.65–1.57)	0.241[a]

Table 3 notes: [a] Mann–Whitney U test, [b] Pearson Chi-square test

Abbreviations: bpm: Beats Per Minute, HF: High Frequency, HRV: Heart Rate Variability, LF: Low Frequency, LF/HF Ratio: Ratio of Low Frequency to High Frequency, NN50: Number of Normal-to-Normal Intervals >50 ms, nu: Normalized Units, PNN50: Percentage of NN50, RMSSD: Root Mean Square of Successive Differences, SDANN: Standard Deviation of the Average of NN Intervals, SDNN: Standard Deviation of NN Intervals, SDNNI: Standard Deviation of Successive Differences of NN Intervals, SDRR: Standard Deviation of R-R Intervals.

Table 4. Comparison of HRV Parameters Between Preadolescents and Adolescents in the Control Group.

Parameters (Unit)		Preadolescent Group (N = 14) Median (25th–75th Percentiles) or n (%)	Adolescent Group (N=22) Median (25th–75th Percentiles) or n (%)	p
Gender	Female	7 (50%)	13 (59.1%)	0.734[b]
	Male	7 (50%)	9 (40.9%)	
Age (years)		12 (10.8–12)	15 (14–16.3)	<0.001[a]
Time Domain Parameters				
Holter Mean Heart Rate (bpm)		90 (74–98.3)	82 (72–86)	0.041[a]
Mean RR Interval (ms)		697.5 (632–833)	772 (716–856)	0.038[a]
SDNN (ms)		157 (123-190)	197 (167.5–227.3)	0.025[a]
SDANN (ms)		131 (104.8–148.3)	166 (141.3–207.3)	0.011[a]
Median RR Interval (ms)		676 (636–836)	752 (690–838)	0.061[a]
HRV TI		32.5 (28–41.8)	40 (33.8–47)	0.071[a]
SDRR (ms)		62 (40.3–91.3)	47.5 (38.5–72.8)	0.160[a]
PNN50 (%)		31.5 (12.5–43.3)	31 (20.5–32.3)	0.962[a]
RMSSD (ms)		79.5 (49–109.5)	64 (51.5–91.3)	0.689[a]
SDNNI (ms)		75.5 (51–103.8)	67 (55.8–98.5)	0.885[a]
NN50		30897 (17538–40830)	27711 (20854–34718)	0.450[a]
Frequency Domain Parameters				
LF (nu)		51.88 (47.58–71.79)	78.40 (61.45–81.63)	0.012[a]
HF (nu)		35.9 (22.47–43.74)	17.59 (14.16–29.40)	0.017[a]
LF/HF Ratio		1.40 (1.10–3.58)	4.39 (2.08–5.73)	0.021[a]
Symindex		0.33 (0.10–1.22)	1.48 (0.73–1.75)	0.021[a]

Table 4 notes: [a] Mann–Whitney U test, [b] Pearson Chi-square test

Abbreviations: bpm: Beats Per Minute, HF: High Frequency, HRV: Heart Rate Variability, LF: Low Frequency, LF/HF Ratio: Ratio of Low Frequency to High Frequency, NN50: Number of Normal-to-Normal Intervals >50 ms, nu: Normalized Units, PNN50: Percentage of NN50, RMSSD: Root Mean Square of Successive Differences, SDANN: Standard Deviation of the Average of NN Intervals, SDNN: Standard Deviation of NN Intervals, SDNNI: Standard Deviation of Successive Differences of NN Intervals, SDRR: Standard Deviation of R-R Intervals.

Table 5. Diagnostic Performance of Heart Rate Variability Parameters in Identifying Pediatric Syncope

Test Result Variable (Unit)	Area	Cut-Off	95% CI	Sensitivity (%)	Specificity (%)	p-value
SDNN (ms)	0.753	<163	0.642–0.864	72.2	75.6	<0.001
SDANN (ms)	0.752	<133.5	0.643–0.862	69.4	70.7	<0.001
HRV TI	0.719	<33.5	0.603–0.834	66.7	68.3	0.001
SDRR (ms)	0.645	<38.5	0.519–0.772	66.7	63.4	0.029
PNN50 (%)	0.675	<27.5	0.554–0.796	66.7	68.3	0.008
SDDNI (ms)	0.672	<64.5	0.550–0.794	61.1	63.4	0.01
RMSSD (ms)	0.638	<61	0.514–0.762	61.1	58.5	0.038
NN50	0.668	<26005	0.545–0.791	63.9	63.4	0.011

Abbreviations: CI: Confidence Interval, HRV TI: Heart Rate Variability Triangular Index, NN50: Number of Normal-to-Normal Intervals >50 ms, PNN50: Percentage of NN50, RMSSD: Root Mean Square of Successive Differences, SDANN: Standard Deviation of the Average of NN Intervals, SDDN: Standard Deviation of NN Intervals, SDRR: Standard Deviation of R-R Intervals, SDNNI: Standard Deviation of Successive Differences of NN Intervals.

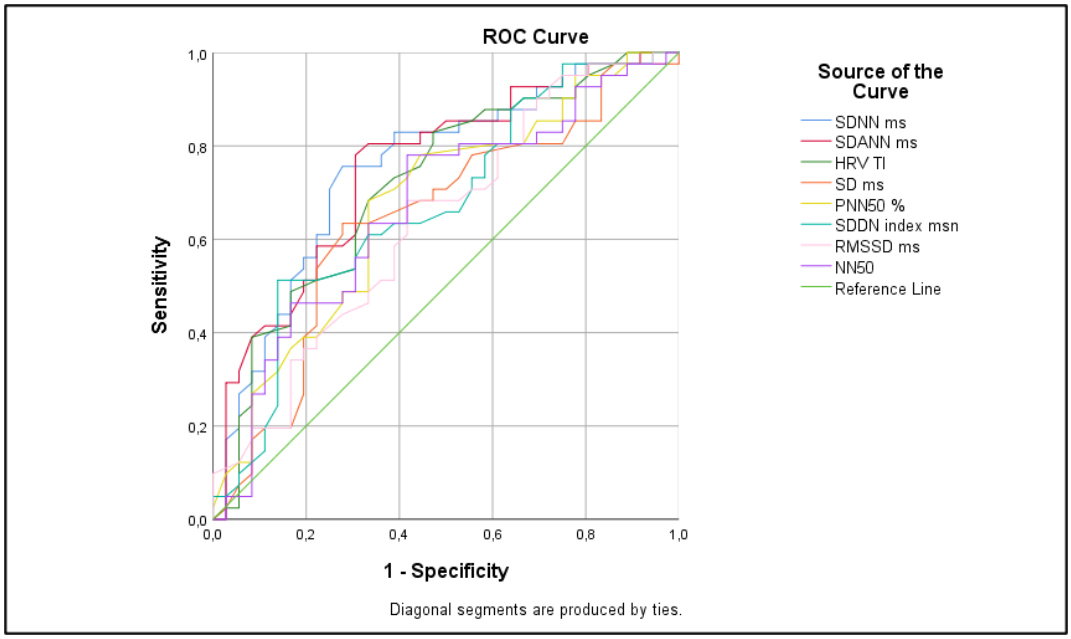


Figure 1. The ROC curve of heart rate variability parameters for predicting vasovagal syncope. (See Table 3). ROC: Receiver operating characteristic.

Discussion

Our study compared pediatric patients with syncope to an asymptomatic control group with ventricular extrasystoles, and we observed that only time-domain HRV parameters—including SDNN, SDANN, pNN50%, RMSSD, SDNN Index, and NN50—were significantly reduced in the syncope group during 24-hour Holter monitoring. These findings are consistent with previous studies that demonstrated decreased HRV in children with syncope. A unique aspect of our study is that it is the first to include HRV-TI and Symindex as HRV parameters in pediatric patients with syncope. While a significant difference was identified in HRV-TI values between the syncope and control groups, no significant difference was observed in Symindex.

Syncope constitutes a significant proportion of hospital admissions in the pediatric population and is a source of considerable anxiety for both children and their families. Thus, it is crucial to eliminate the most concerning etiologies, such as neurological and cardiac causes. The high prevalence of syncope in the community has led to the development of cost-effective approaches, with guidelines published for adult and pediatric populations. According to the guideline, obtaining a detailed medical history and performing a thorough physical examination are essential initial steps, followed by using the Modified Calgary Syncope Score when appropriate.[27,28] Routine investigations—such as ECG, echocardiography, exercise testing, and 24-hour Holter monitoring—are not universally recommended.[27] However, additional cardiological evaluations are advised in cases in which specific red flags are present, including the detection of murmurs suggestive of structural heart disease, syncope triggered by exercise or sound, the absence of prodromal symptoms before the event, a family history of sudden cardiac death or heart disease, or a prolonged recovery period following syncope.[27] The tilt test, commonly used

in adults to differentiate syncope etiologies, has limited utility in children due to challenges in interpretation and its inability to reliably exclude other causes. Consequently, this test is not routinely recommended for pediatric populations.[27,30,31] In our hospital, the absence of tilt testing capabilities prevented its use in our patient cohort. Instead, the diagnosis of VVS was established using the Modified Calgary Syncope Score.[27] This was supplemented by comprehensive cardiological and neurological evaluations and appropriate diagnostic testing to exclude any underlying organic pathology that could explain the syncope episodes.

The primary pathology of VVS is believed to result from excessive parasympathetic activity, inhibition of the sympathetic system, or a complex interplay between these two systems.[5] The responses of these systems can be triggered and exacerbated by factors such as pain, periods of illness, fasting, prolonged standing, crowded or poorly ventilated environments, and sleep deprivation, ultimately leading to syncope.[5,32] HRV is considered a valuable diagnostic tool for predicting this autonomic imbalance and, due to its non-invasive nature, is easy to implement in clinical practice. Reduced HRV, which reflects autonomic imbalance, has been associated with poor prognosis in various conditions, particularly in adults.[33] Notably, reduced HRV has been correlated with increased mortality rates.[34] Studies in pediatric populations have also demonstrated that decreased HRV is linked to various cardiac and non-cardiac events.[35,36] HRV can be assessed using both time-domain and frequency-domain parameters. Among the time-domain metrics, the most used ones are mean heart rate, RMSSD, and SDNN; in the frequency domain, LF, HF, and the LF/HF ratio are frequently analyzed. Increased SDNN and LF indicate heightened sympathetic activity, whereas elevated RMSSD and HF are more closely associated with enhanced parasympathetic activity. In our study, we also utilized normalized values of LF and HF, which are considered more meaningful than their raw values for assessing autonomic regulation.[1,37] The LF/HF ratio is particularly valuable for determining the balance between sympathetic and parasympathetic nervous system activity.[37]

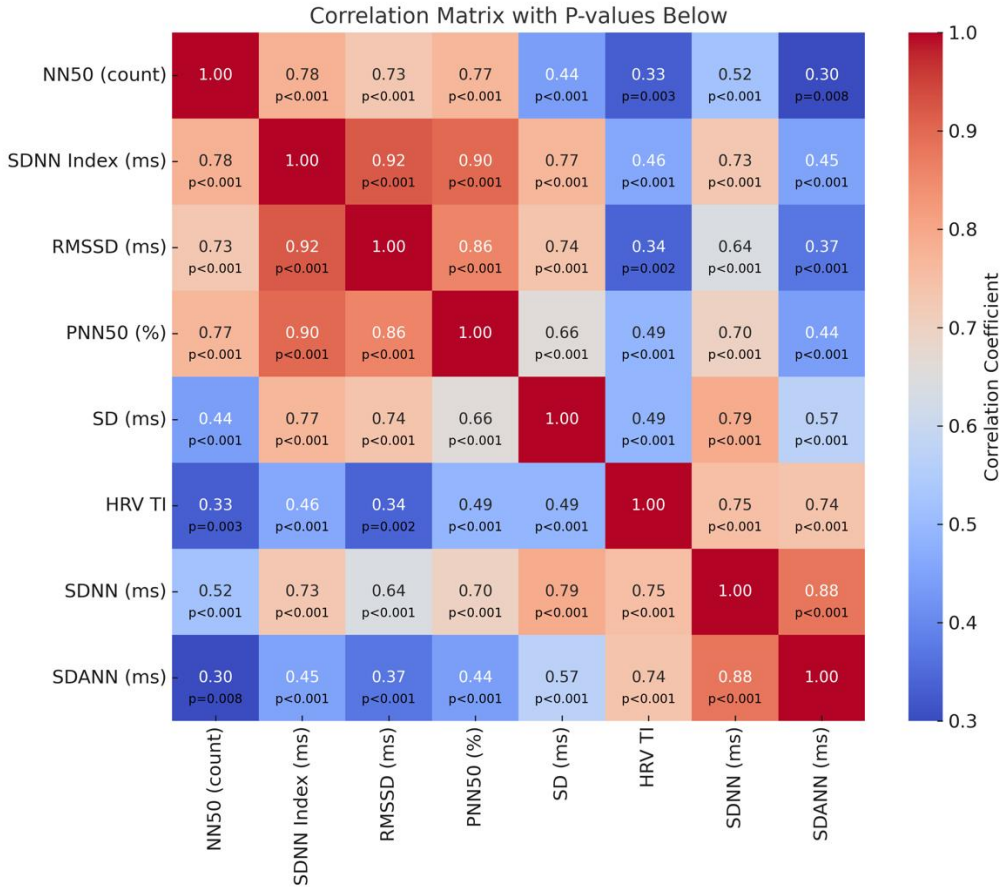


Figure 2. Spearman correlation coefficients matrix map of heart rate variability parameters with p-values. The colors represent the strength and direction of the correlations: red indicates strong positive correlations, blue indicates strong negative correlations, and lighter shades signify weaker relationships.

There is only a limited number of studies focusing on normative HRV values in children. In this context, Gasior et al.[23] conducted a study on healthy children aged 6–13 years and identified heart rate as the most influential factor affecting HRV. They published normative HRV values by categorizing participants based on their heart rates. Using the results of this study to classify our patients, the mean heart rate in the syncope group during Holter monitoring was 86 bpm, which corresponds to the Q3 category (heart rate: >84.3 bpm–92.0 bpm). Within this category, we observed that the time-domain parameter SDNN was increased, while RMSSD and pNN50 (%) were within normal ranges. Additionally, among frequency-domain parameters, an increase in LF (nu) and the LF/HF ratio—alongside a decrease in HF (nu)—was noted. These findings indicate a predominance of sympathetic activity in the syncope group, which contrasts with general literature that suggests parasympathetic dominance in syncope. In the control group, the mean heart rate was 82.5 bpm, which aligns with the Q2 category (heart rate: >77.8 bpm–84.3 bpm) from the same study. When categorized accordingly, it was observed that the control group clustered in a similar manner as the syncope group. The discrepancies between our study and the referenced study may be attributable to differences in methodology, as the referenced study utilized short-term ECG monitoring (five minutes) and was conducted with healthy, non-athletic Caucasian children as participants. Future studies should aim to form age- and physiologically matched cohorts to address these inconsistencies and enable more accurate comparisons. This approach would provide a more robust understanding of normative HRV values in pediatric populations and allow for the better evaluation of autonomic imbalance in conditions such as syncope.

The high prevalence of syncope during adolescence is attributed to the predominant role of vagal tone during this period.[1,38,39] This phenomenon is believed to result from the relatively low blood volume that cannot keep pace with the rapid growth phase in adolescents, thereby leading to increased sympathetic activity.[1] In response, parasympathetic overactivity occurs, culminating in vasovagal syncope.[1] Supporting this information, the study by Sun Hee Shim et al.[1] demonstrated that parasympathetic activity increased when patients were categorized into adolescent and preadolescent groups, while sympathetic activity decreased in the adolescent group. Similarly, our study divided the syncope and control groups into adolescent and preadolescent subgroups. Among adolescents with syncope, significant increases were observed in parameters indicative of heightened sympathetic activity—such as SDNN, LF (nu), and the LF/HF ratio—compared to the preadolescent group. Changes consistent with increased parasympathetic activity were also observed in average heart rate and mean RR intervals. Moreover, significant differences were noted in the adolescent control group in the SDNN, SDANN, LF (nu), and the LF/HF ratio—all of which are associated with sympathetic activity—compared to the preadolescent group. Decreases in parasympathetic parameters were also observed in this group. Based on these findings, our study suggests that sympathetic activity, rather than vagal tone, plays a more dominant role in adolescents, particularly those with syncope.

In the study by Hosaka et al.,[40] which analyzed 24-hour ambulatory ECG recordings, found no significant differences in mean RR, SDNN, or SDANN values. However, HRV parameters indicative of parasympathetic dominance—such as the SD index, RMSSD, and pNN50—were significantly higher in the neuro-mediated syncope group compared to the control group. Similarly, Kochiadakis et al.[41] reported that baseline HRV indices that reflected parasympathetic dominance were increased in patients with syncope compared to healthy controls. Our study observed significant differences between the syncope and control groups in time-domain parameters, including SDNN, SDANN, HRV-TI, SDRR, pNN50, RMSSD, SDNNI, and NN50. However, no significant differences were noted in frequency-domain parameters. These findings suggest that, as described in the literature, both sympathetic and parasympathetic systems are affected in patients with syncope, thereby indicating autonomic imbalance. In syncope patients, increased sympathetic activity is counterbalanced by increased parasympathetic activity to maintain autonomic equilibrium.

In the study by Sun Hee Shim et al.[1], it was found that SDNN and RMSSD parameters were significantly higher in patients with syncope. Additionally, compared to the control group, normalized LF values and the LF/HF ratio were non-significantly lower and normalized HF values were higher in the syncope group. These findings indicate an increase in parameters suggestive of parasympathetic dominance in patients with syncope. In contrast, our study demonstrated significant differences in parameters that reflect the involvement of both sympathetic and parasympathetic systems in the study group compared to the control group.

Studies that investigate normative HRV values based on 24-hour Holter monitoring in children are limited. Kovalchuk et al.[26] conducted a study involving 56 children with syncope and 41 healthy controls aged 8–17 years, analyzing differences in time- and frequency-domain HRV parameters, such as SDANN, RMSSD, pNN50, LF index, HF index, LF/HF ratio, as well as total power across daytime, nighttime, and 24-hour periods. In the syncope group, compared to the control group, significant reductions were observed in SDANN (during the entire 24-hour period and nighttime), RMSSD (during the entire 24-hour period and daytime), and pNN50 (during daytime). In the frequency-domain analysis, only a significant increase was found in the LF/HF ratio during nighttime. Based on these findings, the authors concluded that pediatric patients with VVS exhibit autonomic imbalance characterized by increased sympathetic activity. In our study, which did not stratify 24-hour Holter monitoring results by time periods, we similarly observed reductions in SDNN, pNN50, and RMSSD values, which are consistent

with the findings of Kovalchuk et al.[26] However, unlike their study, we observed a non-significant increase in LF/HF ratio.

Longin et al.[5] compared the frequency-domain parameters of (HRV) in short-term (five-minute) ECG recordings between children and adolescents aged 5–15 years with neurocardiogenic syncope and their healthy counterparts. In the pediatric group (ages 5–11), significant increases in total power, very low frequency (VLF, 0.01–0.05 Hz), and low-frequency (LF) band values were observed compared to the control group. Significant increases were noted among adolescents in VLF and peak VLF band values. These findings suggest that baseline sympathetic activity is elevated in both children and adolescents with syncope, as shown in the study by Longin et al.[5] However, the study did not include baseline data for parameters indicative of parasympathetic activity, thus making it impossible to conclude parasympathetic regulation. In our research, normalized LF and HF parameters were used, thereby preventing a direct comparison with the results of Longin et al. Nevertheless, the observed changes in our study, which also indicate increased sympathetic activity, align with the findings of Longin et al. and support a similar interpretation regarding the autonomic imbalance in pediatric syncope.

Study Limitations

This study has several limitations that warrant consideration. The relatively small sample size and short follow-up period limit the generalizability of the findings, while the exclusion of patients with incomplete Holter data likely affected the homogeneity of the study population. Additionally, the absence of head-up tilt testing, although not routinely recommended in pediatric syncope, restricted our ability to assess autonomic changes during syncope episodes. Further, lack of stratification by syncope subtypes, triggering factors, or symptom severity may have limited identifying specific HRV patterns. Furthermore, intrinsic factors influencing HRV—such as baseline heart rate, physical activity, and respiratory patterns—were not controlled, potentially introducing bias. Future studies with larger, more homogeneous populations and standardized methodologies are needed to establish normative HRV values and refine diagnostic criteria in pediatric syncope.

Conclusion

This study evaluated differences in HRV parameters between pediatric patients with syncope and a control group using 24-hour Holter ECG monitoring. Significant reductions were observed in time-domain parameters—including SDNN, SDANN, HRV-TI, SDRR, pNN50, RMSSD, SDNNI, and NN50in the syncope group compared to controls. At the same time, no meaningful differences were identified in frequency-domain parameters. Notably, the SDNN was the most predictive parameter for identifying syncope in this population. These findings highlight the potential utility of HRV as a diagnostic tool in pediatric syncope. However, the lack of established normative HRV values and limited research in children emphasizes the need for large-scale, multicenter, and prospective studies. Such investigations should aim to standardize HRV measurements, account for confounding factors such as baseline heart rate and physical activity, and further clarify the clinical significance of HRV parameters in pediatric populations.

Compliance with Ethical Standards

The Clinical Research Ethics Committee of Giresun University approved this study on April 4, 2023 (Decision No: 2). Before the study, the parents of all participants provided written informed consent.

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

Conception and Design of Study: BY, BDD, and ET. Data Acquisition: BY and FCY. Data Analysis: BY and FCY. Drafting Manuscript: BY. Critical Revision of Manuscript: BY, BDD, and ET. Final Approval: BY, BDD, ET, and FCY. Supervision: BY.

Financial Disclosure

None.

Acknowledgments

We would like to express our heartfelt gratitude to Mustafa Altun for his invaluable help in collecting data for this study.

References

1. Shim SH, Park S-Y, Moon SN, et al. Baseline heart rate variability in children and adolescents with vasovagal syncope. *Korean journal of pediatrics*. 2014;57(4):193. doi: 10.3345/kjp.2014.57.4.193
2. Johnsrude CL. Current approach to pediatric syncope. *Pediatr Cardiol.* 2000;21(6):522-531. doi:10.1007/s002460010130
3. Wieling W, Ganzeboom KS, Saul JP. Reflex syncope in children and adolescents. *Heart*. 2004;90(9):1094-1100. doi:10.1136/hrt.2003.022996
4. Shen WK, Sheldon RS, Benditt DG, et al. 2017 ACC/AHA/HRS Guideline for the Evaluation and Management of Patients With Syncope: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. *Circulation*. 2017;136(5):e60-e122. doi:10.1161/cir.0000000000000499
5. Longin E, Reinhard J, von Buch C, Gerstner T, Lenz T, König S. Autonomic function in children and adolescents with neurocardiogenic syncope. *Pediatr Cardiol*. 2008;29(4):763-770. doi:10.1007/s00246-008-9198-z
6. Ng J, Sheldon RS, Ritchie D, Raj V, Raj SR. Reduced quality of life and greater psychological distress in vasovagal syncope patients compared to healthy individuals. *Pacing*

Clin Electrophysiol. 2019;42(2):180-188. doi:10.1111/pace.13559
7. Zhang Q, Sun Y, Zhang C, Qi J, Du J. Vitamin D Deficiency and Vasovagal Syncope in Children and Adolescents. *Front Pediatr*. 2021;9:575923. doi:10.3389/fped.2021.575923
8. McCraty R, Shaffer F. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Global advances in health and medicine.* 2015;4(1):46-61. doi.org/10.7453/gahmj.2014.07
9. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health.* 2017;5:258. doi:10.3389/fpubh.2017.00258
10. Paul HA. Biofeedback: A Practitioner's Guide. In: Schwartz M, Andrasik F, ed. Child & Family Behavior Therapy. 4th Edition. New York, NY: The Guilford Press; 2016:161-170.
11. Dash RR, Samanta P, Das S, et al. Heart Rate Variability in Unexplained Syncope Patients Versus Healthy Controls: A Comparative Study. *Cureus.* Jul 2023;15(7):e41370. doi:10.7759/cureus.41370
12. Ernst G. Hidden Signals-The History and Methods of Heart Rate Variability. *Front Public Health.* 2017;5:265. doi:10.3389/fpubh.2017.00265
13. Bersani I, Piersigilli F, Gazzolo D, et al. Heart rate variability as possible marker of brain damage in neonates with hypoxic ischemic encephalopathy: a systematic review. *Eur J Pediatr*. 2021;180(5):1335-1345. doi:10.1007/s00431-020-03882-3
14. Kero P. Heart rate variation in infants with the respiratory distress syndrome. *Acta Paediatr Scand Suppl.* 1974;(250):1-70.
15. Prietsch V, Maier RF, Schmitz L, Obladen M. Long-term variability of heart rate increases with successful closure of patent ductus arteriosus in preterm infants. *Neonatology.* 1992;61(3):142-149.
16. Griffin MP, Moorman JR. Toward the early diagnosis of neonatal sepsis and sepsis-like illness using novel heart rate analysis. *Pediatrics.* 2001;107(1):97-104. doi:10.1542/peds.107.1.97
17. Stone ML, Tatum PM, Weitkamp JH, et al. Abnormal heart rate characteristics before clinical diagnosis of necrotizing enterocolitis. *J Perinatol*. 2013;33(11):847-850. doi:10.1038/jp.2013.63
18. Biswas AK, Scott WA, Sommerauer JF, Luckett PM. Heart rate variability after acute traumatic brain injury in children. *Crit Care Med*. 2000;28(12):3907-3912. doi:10.1097/00003246-200012000-00030
19. Ucak S, Dissanayake HU, Sutherland K, de Chazal P, Cistulli PA. Heart rate variability and obstructive sleep apnea: Current perspectives and novel technologies. *Journal of Sleep Research*. 2021;30(4):e13274.
20. Cheng YC, Su MI, Liu CW, Huang YC, Huang WL. Heart rate variability in patients with anxiety disorders: A systematic review and meta-analysis. *Psychiatry and Clinical Neurosciences*. 2022;76(7):292-302.
21. Correia AT, Lipinska G, Rauch HL, Forshaw PE, Roden LC, Rae DE. Associations between sleep-related heart rate variability and both sleep and symptoms of depression and anxiety: A systematic review. *Sleep Medicine*. 2023;101:106-117. doi.org/10.1016/j.sleep.2022.10.018
22. Stein PK, Domitrovich PP, Hui N, Rautaharju P, Gottdiener J. Sometimes higher heart rate variability is not better heart rate variability: results of graphical and nonlinear analyses. *Journal of Cardiovascular Electrophysiology*. 2005;16(9):954-959. doi.org/10.1111/j.1540-8167.2005.40788.x
23. Gąsior JS, Sacha J, Pawłowski M, et al. Normative Values for Heart Rate Variability Parameters in School-Aged Children: Simple Approach Considering Differences in Average Heart Rate. *Front Physiol.* 2018;9:1495. doi:10.3389/fphys.2018.01495
24. Bobkowski W, Stefaniak ME, Krauze T, et al. Measures of heart rate variability in 24-h ECGs depend on age but not gender of healthy children. *Frontiers in Physiology*. 2017;8:311. doi.org/10.3389/fphys.2017.00311
25. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Review. *Frontiers in Public Health.* 2017;5:258. doi:10.3389/fpubh.2017.00258
26. Kovalchuk T, Boyarchuk O, Pavlyshyn H, Balatska N, Luchyshyn N. Analysis of heart rate variability in paediatric patients with vasovagal syncope. *Pediatria Polska-Polish Journal of Paediatrics.* 2019;94(6):357-367. doi.org/10.5114/polp.2019.92965
27. Sanatani S, Chau V, Fournier A, Dixon A, Blondin R, Sheldon RS. Canadian Cardiovascular Society and Canadian Pediatric Cardiology Association Position Statement on the Approach to Syncope in the Pediatric Patient. *Canadian Journal of Cardiology.* 2017;33(2):189-198. doi:10.1016/j.cjca.2016.09.006
28. Yang J, Zhu L, Chen S, et al. Modified Calgary score in differential diagnosis between cardiac syncope and postural orthostatic tachycardia syndrome-associated syncope in children. *Cardiol Young*. 2013;23(3):400-404. doi:10.1017/s1047951112001266
29. ELECTROPHYSIOLOGY, Task Force of the European Society of Cardiology the North American Society of Pacing. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation.* 1996, 93.5: 1043-1065. doi.org/10.1161/01.CIR.93.5.1043
30. Boysen A, Lewin MA, Uhlemann F. Common patterns of response to the head-up tilt test in children and adolescents. *Cardiol Young.* 2006;16(6):537-539. doi:10.1017/s1047951106000886
31. Batra AS, Balaji S. Usefulness of tilt testing in children with syncope: a survey of pediatric electrophysiologists. *Indian Pacing Electrophysiol J.* 2008;8(4):242-246.
32. Sutton R. Reflex syncope: Diagnosis and treatment. *J Arrhythm.* 2017;33(6):545-552. doi:10.1016/j.joa.2017.03.007
33. Ernst G. Heart-Rate Variability-More than Heart Beats? *Front Public Health.* 2017;5:240. doi:10.3389/fpubh.2017.00240
34. Kleiger RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol.* 1987;59(4):256-262. doi:10.1016/0002-9149(87)90795-8
35. Bakari S, Koca B, Öztunç F, Abuhandan M. Heart rate variability in patients with atrial septal defect and healthy children. *Journal of Cardiology.* 2013;61(6):436-439. doi.org/10.1016/j.jjcc.2013.01.014
36. Birch SL, Duncan MJ, Franklin C. Overweight and reduced heart rate variability in British children: an exploratory study. *Preventive Medicine*. 2012;55(5):430-432. doi.org/10.1016/j.ypmed.2012.09.015
37. Lazzeri C, La Villa G, Barletta G, Franchi F. 24-hour heart rate variability in patients with vasovagal syncope. *Pacing Clin Electrophysiol.* 2000;23(4):463-468. doi:10.1111/j.1540-8159.2000.tb00828.x
38. Benditt DG, van Dijk JG, Sutton R, et al. Syncope. *Curr Probl Cardiol.* 2004;29(4):152-229. doi:10.1016/j.cpcardiol.2003.12.002

39. Kenny RA, Bhangu J, King-Kallimanis BL. Epidemiology of syncope/collapse in younger and older Western patient populations. *Prog Cardiovasc Dis.* 2013;55(4):357-363. doi:10.1016/j.pcad.2012.11.006
40. Hosaka H, Takase B, Katsushika S, Ohsuzu F, Kurita A. Altered fractal behavior and heart rate variability in daily life in neurally mediated syncope. *Biomed Pharmacother.* 2003;57:77-82. doi:10.1016/j.biopha.2003.08.009
41. Kochiadakis GE, Kanoupakis EM, Rombola AT, Igoumenidis NE, Chlouverakis GI, Vardas PE. Reproducibility of tilt table testing in patients with vasovagal syncope and its relation to variations in autonomic nervous system activity. *Pacing Clin Electrophysiol*. 1998;21(5):1069-1076. doi:10.1111/j.1540-8159.1998.tb00152.x

doi: 10.53446/actamednicomedia.1628412

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article |Araştırma Makalesi

OPTIMIZING SERUM RNA ISOLATION: A COMPARATIVE ANALYSIS OF COMMERCIAL KITS FOR YIELD, PURITY, AND CONTAMINATION CONTROL

SERUM RNA İZOLASYONUNUN OPTİMİZE EDİLMESİ: VERİM, SAFLIK VE KONTAMİNASYON KONTROLÜ İÇİN TİCARİ KİTLERİN KARŞILAŞTIRMALI ANALİZİ

Esra Duman[1*], Ozge Ozmen[2]

[1]Kocaeli University, Institute of Gastroenterology and Hepatology, Department of Molecular Gastroenterology and Hepatology, Kocaeli, Türkiye. [2]Ankara University, Faculty of Veterinary Medicine, Department of Genetics, Ankara, Türkiye.

ABSTRACT

Objective: Isolation of RNA from serum samples has gained importance, especially in studies on the use of small RNA molecules such as miRNA as biomarkers. Selection of the optimal kit is critical for the accuracy of downstream processing. The aim of this study was to compare the performance of different commercial kits in terms of efficiency, RNA purity and contamination control during the isolation process.

Method: Three different RNA isolation kits were used for 5 sheep serum samples: 1. miRNeasy Serum/Plasma Kit (Cat. No: 217184, Qiagen, USA), 2. Norgen Plasma/serum RNA purification kit (Cat. No: 55000, Norgen, Canada), 3. Nucleogene RNA isolation kit (Cat. No: NG044, Nucleogene, Turkey). The purity and intensity of the obtained RNAs were evaluated by measuring A260/280 ratios with a nanodrop spectrophotometer.

Results: When the concentrations and A260/280 ratios obtained from the kits were evaluated by One Way Anova Test using GraphPad Prism (V10.4.0), it was observed that there was a statistically significant difference between the concentrations and A260/280 ratios of the 3 kits (p≤0.05 and p≤ 0.001).

RNAs obtained from Norgene showed the lowest concentration and the lowest A260/280 ratiowhere as Nucleogene had the highest RNA concentration and A260/280 ratio of 2.0or higher among the three kits (p≤0.05).

Conclusion: Among the kits used for serum RNA isolation, the Nucleogene kit stands out with the highest RNA yield and suitable A260/280 values in general. However, the Norgene and Qiagen kits may still be preferred under specific experimental conditions.

Keywords: RNA isolation, serum, A260/280 ratio, commercial reagent kits

ÖZ

Amaç: Serum örneklerinden RNA izolasyonu, özellikle miRNA gibi küçük RNA moleküllerinin biyo-belirteç olarak kullanımına yönelik çalışmalarda önem kazanmıştır. Optimal kitin seçimi sonraki işlemlerin doğruluğu için kritik öneme sahiptir. Bu çalışmanın amacı izolasyon sürecinde farklı ticari kitlerin verimlilik, RNA saflığı ve kontaminasyon kontrolü açısından performanslarını kıyaslamaktır.

Yöntem: Çalışmada koyun venöz kanından elde edilen 5 serum örneği için üç farklı RNA izolasyon kiti kullanılmıştır: 1. miRNeasy Serum/Plazma Kit (Kat. No: 217184, Qiagen, ABD), 2. Norgen Plazma/serum RNA saflaştırma kiti (Kat. No: 55000, Norgen, Kanada), 3. Nucleogene RNA izolasyon kiti (Kat. No: NG044, Nucleogene, Türkiye). Elde edilen RNA'ların saflık ve yoğunluğu nanodrop spektrofotometre ile A260/280 oranları ölçülerek değerlendirilmiştir.

Bulgular: Kitlerden elde edilen konsantrasyonlar ve A260/280 oranları GraphPad Prism (V10.4.0) kullanılarak One Way Anova Testi ile değerlendirildiğinde, 3 kitin konsantrasyonları ve A260/280 oranları arasında istatistiksel olarak anlamlı bir fark olduğu görülmüştür (p≤0,05 ve p≤ 0,001).

Norgene'den elde edilen RNA'lar en düşük konsantrasyona ve en düşük A260/280 oranına sahipken, Nucleogene üç kit arasında en yüksek RNA konsantrasyonuna ve 2 ve üzeri A260/280 oranına sahipti (p≤0,05).

Sonuç: Serum RNA izolasyonu için kullanılan kitler arasında Nucleogene kiti genel olarak en yüksek RNA verimi ve uygun A260/280 değerleriyle öne çıkmaktadır. Bununla birlikte Qiagen ve Norgene kitleri bazı spesifik durumlarda tercih edilebilir.

Anahtar Kelimeler: RNA izolasyonu, serum, A260/280 oranı, ticari reaktif kitler

***Correspondingauthor/İletişim kurulacak yazar:** Esra Duman; Kocaeli University, Institute of Gastroenterology and Hepatology, Department of Molecular Gastroenterology and Hepatology, Kocaeli, Türkiye.
Phone/Telefon: +90 (530) 1146818, **e-mail/e-posta:**esra.duman@kocaeli.edu.tr
Submitted/Başvuru:28.01.2025 ● **Accepted/Kabul:**15.05.2025 ● **Published Online/Online Yayın:** 30.06.2025

Introduction

Ribonucleic Acid (RNA) isolation is an important step for analyzing gene expression in molecular biology research. However, the fact that RNA is an unstable molecule, has a very short half-life and can be easily degraded by RNases in the environment brings some difficulties in RNA isolation.[1] Intensive denaturation methods are employed during isolation to prevent the activity of RNases that degrade RNA commonly present in blood, tissues, and various environmental bacteria and fungi.[2] RNA expression analysis from blood samples is an important non-invasive method due to its potential as a biomarker in many pathologies, especially cancer.[3] In addition, many non-coding RNAs are also expressed in a tissue- or organ-specific manner, suggesting that have high specificity and are applicable as biomarkers.[4]

Since serum collection is non-invasive and uses blood remaining from routine examinations, it aligns well with ethical protocols. Additionally, obtaining serum is easier and more accessible than tissue sampling. For these reasons, serum is often preferred in studies. However, the high protein and lipid content in blood increases the risk of contamination, and the RNA concentration is lower compared to tissue samples, making RNA isolation from serum more challenging.[5] Various techniques and commercial kits are available for RNA extraction from biofluids; however, comprehensive data identifying the most appropriate method or kit for each specific biofluid remains insufficient. Methods such as Real Time Polymerase Chain Reaction (RT PCR) or RNA-sequencing (RNA-Seq) after RNA extraction requires high density and quality RNA. The lower concentration of RNAs in plasma compared to tissue poses a handicap in RNA extraction and quality from serum. [6]

The aim of this study was to compare 3 different commercial RNA isolation kits in terms of RNA concentration and quality to determine the most efficient and suitable one for RNA isolation from serum samples.

Methods

The study was performed with 5 serum samples obtained from sheep, stored at -80 and left over from the study approved by the ethics committee of Fırat University Animal Experiments Ethics Committee numbered 2012/06/65.

Three different commercial RNA isolation kits were used in the study: 1. miRNeasy Serum/Plasma Kit (Cat.No: 217184, Qiagen, USA), 2.Norgen Plasma/serum RNA purification kit (Cat. No: 55000, Norgen, Canada), 3. Nucleogene RNA isolation kit (Cat. No: NG044, Nucleogene, Turkey). All the steps of isolation stages took place at Kocaeli University Stem Cell and Gene Research Center (KÖGEM). The protocols of the kits used are as described below.

1. miRNeasy Serum/Plasma Kit Protocol

- Transfer 150 ml of serum /plasma into a 1.5 mL centrifuge tube
- 2ul proteinase K 10 min. incubation at room temperature. Add 750 µL (x5 volumes) pf QIAzol Lysis Reagent and Vortex 5 s.
- Incubate for 10 min at room temperature
- Add 100 µL chloroform. Vortex vigorously for 30 s. And incubate for 3 min. at room temperature.
- Centrifuge sample for 15 min at 14,000 x RPM at 4^{o}C.
- Transfer the 400 ul upper aqueous phase to a new 1.5 mL centrifuge tube. Avoid the white interphase.
- Carefully measure the aqueous phase and add 600 ul (1.5 x volumes) of 100% ethanol. Do not vortex. Mix gently and thoroughly. Do not centrifuge and do not delay moving on the next step.
- Assemble a MinElute spin column in a new collection tube. Load up to 700 uL of the mixture in spin column, including any precipitate that may have formed, on to column.
- Spin for 30 s at 3000 R at room temperature, discard flow-through.
- Spin for 30s at 10.000 RPM at room temperature. Add 700 µl Buffer RWT to the RNeasy MinElute spin column. Centrifuge for 30 s at 10.000 RPM at room temperature to wash the column. Discard the flow-through.
- Pipet 500 µl Buffer RPE onto the RNeasy MinElute spin column. Centrifuge for 30s at 10.000 RPM to wash the column. Discard the collection tube with the flow-through.
- Open lid, dry for 10 min at room temperature. Transfer the RNeasy MinElute spin column into a new 2mL collection tube.
- Open the lid of the spin column and centrifuge at full speed 15.000 RPM for 5 min to dry the membrane.
- Discard the collection tube with flow-through. Dry for 10 min at room temperature, open lid.
- Transfer the RNeasy MinElute spin column into a new 1.5 ml collection tube.
- Add 15 µL RNase-free water directly to the center of the spin column membrane.
- Wait 10 min. After 5 min. centrifuge for 1 min at 100 *x g* (500 RPM)
- Centrifuge for 1 min at full speed (15.000 RPM) to eluate the RNA.
- Repeat step 16, 18.

2. Nucleogene RNA Isolation Kit Protocol

Perform all steps at room temperature and centrifugation at 10,000-16,000 x *g* for 1 min, unless specified.

- Ensure that the sample and Lysis Buffer and 4 ul Enhancer are fully mixed and 10 minutes incubation at room temperature, later centrifuge for 2 minutes at 14,000 x *g* as solid particles will clog the column.

- Carefully withdraw the supernatant and transfer it to a new (1.5-2 ml) microcentrifuge tube.
- Add an equal volume ethanol (95-100%) (e.g. transferred supernatant 250 ul+250 ul ethanol) to a sample lysed in Lysis Buffer and mix (vortex 1 min or pipetting) thoroughly.
- Transfer the mixture into a Spin Column in a Collection Tube and 11.000 g 30 second centrifuge. Empty the collection tube and place the spin column back in the collection tube.
- Add 400 µl of Wash I Buffer to the Spin Column and centrifuge at 11,000 *g* for 30 seconds.
- Add 400 µl of Wash I Buffer to the Spin Column and centrifuge at 11,000 x *g* for 30 seconds. Empty the collection tube and place the spin column back in the collection tube.
- Add 700 µl of Wash II Buffer to the Spin Column and centrifuge at 14,000 x *g* for 1 min. Discard the collection tube and place the spin column in a new nuclease-free microcentrifuge tube (1.5-2 ml).
- To elute the RNA, add 30 µl of Elution Buffer directly to the center of the spin column and incubate 2 min, after the incubation centrifuge at 11,000 x *g* for 2 min.

3. Norgen Plasma/Serum RNA Purification Kit Protocol

- Place 200 µL of plasma/serum sample in a 2 mL tube and add 600 µL of Lysis Buffer. Mix well by. Vortexing for 10 seconds.
- Add 800 µL of 96-100% ethanol. Mix well by vortexing for 10 seconds.
- Transfer 650 µL of the mixture from Step 2 into a Micro Spin column. Centrifuge for 2 minutes at 3,300 *x g (*~6,000 RPM). Discard the flow through and reassemble the spin column with its collection tube.
- Repeat Step 3 two more times until all the mixture from Step 2 has been transferred to the Micro Spin column.
- Apply 400 µL of Wash Solution A to the column and centrifuge for 30 seconds at 3,300 *xg(*~6,000 RPM). Discard the flow through and reassemble the spin column with its collection tube.
- Repeat step 5 two more times, for a total of three washes.
- Spin the column, empty, for 2 minutes at 13,000 *x g* (~14,000 RPM). Discard the collection tube.
- Transfer the spin column to a fresh 1.7 mL Elution tube. Apply from 10 µL up to 25 µL of Elution Solution A to the column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 x *g* (~2,000 RPM), followed by 2 minutes at 5,800 x g (~8,000 RPM).
- For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 x *g* (~2,000 RPM), followed by 2 minutes at 5,800 x *g* (~8,000 RPM).

RNAs obtained according to kit protocols were measured by nanodrop spectrophotometer (Thermo, ND2000) to determine their purity and intensity.

Statistical Analysis

Concentrations and A260/280 ratios obtained from the kits were evaluated by One Way Anova Test using GraphPad Prism V10.4.0.

Results

Concentrations and A260/280 Ratios of the isolated RNAs are as given in the Table 1.

Table 1. Concentration and A260/280 values of RNAs obtained from the kits

	Concentration			A260/280 Ratio		
	Norgene	Qiagen	Nucleogene	Norgene	Qiagen	Nucleogene
1	10,30	23,4	64,4	1,05	1,43	1,62
2	11,30	25,7	30,1	1,00	1,43	2,12
3	10,01	48,8	27,5	0,95	1,33	2,22
4	10,06	33,2	18,4	1,00	1,44	2,69
5	20,01	17,4	28,8	1,01	1,45	1,69

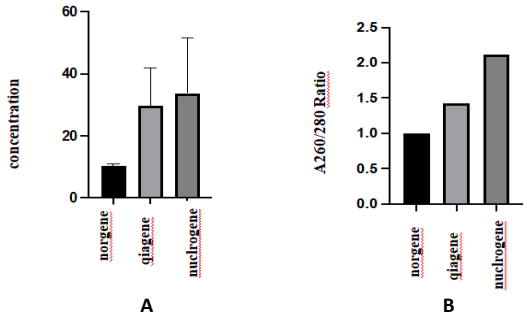


Figure 1. A. Concentration and **B.**A260/280 value graphics of RNAs obtained from the kits according to the kit protocols.

It was observed that there was a statistically significant difference between the concentrations and A260/280 ratios of the 3 kits ($p \leq 0.05$ and $p \leq 0.001$).

RNAs obtained from Norgene had the lowest concentration and the lowest A260/280 ratio, while Nucleogene had the highest RNA concentration and A260/280 ratio of 2.0 and above among the three kits ($p \leq 0.05$).

Discussion

The search for novel biomarkers for the early detection of human diseases has intensified in recent years. The use of small RNAs such as microRNAs as potential markers has been the focus of much attention.[7] Blood is a complex fluid that is in contact with all the tissues of the body. As such, it provides unique information about different parts of the body. Blood sampling is considered a non-invasive procedure and is therefore widely used to assess biomarkers associated with

disease. Various methods have been developed to isolate and stabilize RNA from blood, with the advent of personalized medicine for the treatment and diagnosis of chronic diseases and the development of individualized treatmentstrategies.[7,8]

For the isolation of small RNA from biological fluids, there are currently two main approaches. The first approach uses column-based technology for binding and elution of small RNAs, while the second approach uses the long-established protocol using phenol and guanidinium thiocyanate reagents.[8]Methods such as RT PCR or RNA-seq after RNA extraction requires high density and quality RNA. The lower concentration of RNAs in plasma compared to tissue poses a handicap in RNA extraction and quality from serum.

It is important to maximize the yield of microRNA isolated. Since the abundance of microRNA in serum is significantly lower than in solid tissues, low RNA yields will result in low abundance microRNA signatures not being detected.[9]

There are studies comparing commercial RNA isolation kits available in the market in terms of RNA quality and concentration, and while Qiagen is mostly prominent in these studies, there is no comparison study on the Nucleogen kit and in this sense, it is aimed to contribute to the literature.[6,10-12]

Guatam et al.[12] compared two TRIzol methods (TRIzol Reagent and TRIzol LS reagent) using different carriers and compared miRNeasy mini kit (Qiagen, USA) and miRVANA miRNA isolation kit (Thermofisher, USA). As a result, they reported that miRNeasy mini kit yielded 2-3 times better quality RNA than miRVANA. Similarly, Li et al.[6] compared miRNeasy mini kit, miRVANA and Norgene total RNA isolation kit (Norgene, Canada) and reported that the RNA quality of miRNeasy and miRVANA kit was better than Norgen. In our comparisons, Norgen ranked last in terms of RNA quality and quantity.

Among the kits used for serum RNA isolation, the Nucleogene kit stands out with the highest RNA yield and suitable A260/280 values in general. However, Norgene and Qiagen kits may be preferred in some specific cases.As a conclusion between the three kits we tested, the Nucleogene kit yielded the highest concentration of microRNA and can be chosen as the first choice among the three kits for RNA isolation from serum.

Compliance with Ethical Standards

Approval for this study was covered under the ethical approval numbered 2012/06/65, which had been obtained before the start of the original study. The present work was conducted using remaining biological materials from that ethically approved research.

Conflict of Interest

The authors have no material or immaterial conflict of interest with the subject and/or any other author.

Author Contributions

ÖÖ and ED collaborated during the design of the study, data collection and analysis, literature review and manuscript writing.

Financial Disclosure

No financial support was used in the study.

References

1. Tanriverdi K, Kucukural A, Mikhalev E, et al. Comparison of RNA isolation and associated methods for extracellular RNA detection by high throughput quantitative polymerase chain reaction.*Anal Biochem*. 2016;501:66-74. doi:10.1016/j.ab.2016.02.019
2. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present [published correction appears in J Biomed Biotechnol. 2013;2013:628968]. *J BiomedBiotechnol*. 2009;2009:574398. doi:10.1155/2009/574398
3. Wong RKY, MacMahon M, Woodside JV, Simpson DA. A comparison of RNA extraction and sequencing protocols for detection of small RNAs in plasma. *BMC Genomics*. 2019;20(1):446. doi:10.1186/s12864-019-5826-7
4. Roest HP, IJzermans JNM, van der Laan LJW. Evaluation of RNA isolation methods for microRNA quantification in a range of clinical biofluids. *BMC Biotechnol*. 2021;21(1):48. doi:10.1186/s12896-021-00706-6
5. Sriram H, Khanka T, Kedia S, et al. Improved protocol for plasma microRNA extraction and comparison of commercial kits. *BiochemMed (Zagreb)*. 2021;31(3):030705. doi:10.11613/BM.2021.030705
6. Li Y, Kowdley KV. Method for microRNA isolation from clinical serum samples. *Anal Biochem*. 2012;431(1):69-75. doi:10.1016/j.ab.2012.09.007
7. Chamberlain F, Grammatopoulos D. Methodology for Isolation of miRNA From the Serum of Women Investigated for Pre-eclampsia. *Cureus*. 2023;15(9):e46181. doi:10.7759/cureus.46181
8. Khoury S, Ajuyah P, Tran N. Isolation of small noncoding RNAs from human serum. *J Vis Exp*. 2014;(88):e51443. doi:10.3791/51443
9. Wilfinger WW, Eghbalnia HR, Mackey K, Miller R, Chomczynski P. Whole blood RNA extraction efficiency contributes to variability in RNA sequencing data sets. *PLoS One*. 2023;18(11):e0291209. doi:10.1371/journal.pone.0291209
10. Brunet-Vega A, Pericay C, Quílez ME, Ramírez-Lázaro MJ, Calvet X, Lario S. Variability in microRNA recovery from plasma: Comparison of five commercial kits. *Anal Biochem*. 2015;488:28-35. doi:10.1016/j.ab.2015.07.018
11. Chu CP, Nabity MB. Comparison of RNA isolation and library preparation methods for small RNA sequencing of canine biofluids. *Vet Clin Pathol*. 2019;48(2):310-319. doi:10.1111/vcp.12743
12. Gautam A, Kumar R, Dimitrov G, Hoke A, Hammamieh R, Jett M. Identification of extracellular miRNA in archived serum samples by next-generation sequencing from RNA extracted using multiple methods. *Mol Biol Rep*. 2016;43(10):1165-1178. doi:10.1007/s11033-016-4043-6

doi: 10.53446/actamednicomedia.1641437

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Olgu Sunumu | Case Report

TEDAVİ SÜRECİNE UYUM SAĞLAYAMAYAN BİR ERGEN İÇİN MULTİDİSİPLİNER ÇALIŞMA VE ÇOCUK KORUMA SİSTEMİ KAPSAMINDA KURUMLAR ARASI KOORDİNASYON: BİR OLGU SUNUMU

A MULTIDISCIPLINARY STUDY FOR AN ADOLESCENT WHO CANNOT ADAPT TO THE TREATMENT PROCESS AND INTER-INSTITUTIONAL COOPERATION WITHIN THE SCOPE OF THE CHILD PROTECTION SYSTEM: A CASE REPORT

Yunus Dursun[1*], Gülçin Ünverdi[2], Nursu Çakın Memik[1]

[1]Kocaeli Üniversitesi, Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Anabilim Dalı, Kocaeli, Türkiye. [2]KÇanakkale Devlet Hastanesi, Çanakkale, Türkiye.

ÖZ

Çocuklarda ruh sağlığını etkileyen ve sorunların ortaya çıkmasına neden olan genetik, sosyal ve çevresel pek çok etmen bulunmaktadır. Çocuğun bireysel özelliklerinin yanı sıra kaotik aile yapısı, ebeveynlerinin bireysel ve ruhsal sorunları, kendi ebeveynlerine bağlanma sorunları, ebeveyn tutumlarındaki ciddi problemler ile eşler arasındaki ilişki sorunları gibi aile kaynaklı unsurlar çocukta var olan ruhsal sorunların şiddetini artırmasına, başka ruhsal sorunların ortaya çıkmasına ve tedavi sürecinin olumsuz etkilenmesine neden olabilmektedir. Bu nedenle çocuk psikiyatri kliniklerinde yürütülen tedavide sadece çocukla çalışmak ya da aileye önerilerde bulunmak yeterli olmamakta olup aile temelli ele alınan kapsamlı, çok boyutlu ve multidisipliner çalışmayı ve ihtiyaç halinde çocuk koruma sisteminin devreye sokulmasını gerektiren bir süreç olabilmektedir.

Bu olgu sunumunda ebeveynlerin olumsuz tutum ve davranışları neticesinde daha fazla ve riskli davranış sorunları geliştiren, tedavisi aksatılan ve tedaviden fayda göremeyen, bakım, sağlık, eğitim ve diğer temel ihtiyaçları kapsamında ihmal edilen ve istismara açık hale gelen, bu nedenle çocuk koruma sistemi kapsamında değerlendirilen, kurum bakımına yerleştirilen 14 yaşındaki çocuğun farmakolojik tedavisi, multidisipliner çalışma ve kurumlar arası koordinasyonu içeren çok boyutlu tedavi süreci ile bu süreçte çocuk koruma sistemi kapsamında yapılan iş ve işlemler tartışılacaktır.

Anahtar Kelimeler: Ergenlik dönemi, korunmaya ihtiyacı olan çocuk, davranış sorunları, anne-baba tutumları

ABSTRACT

There are many genetic, social and environmental factors that affect mental health in children and cause the emergence of mental problems. In addition to the individual characteristics of the child, family-related factors such as chaotic family structure, individual and mental problems of the parents, attachment problems to their own parents, serious problems in parental attitudes and relationship problems between spouses may increase the severity of existing mental problems in the child, lead to the emergence of other mental problems and negatively affect the treatment process. Family-related factors may interrupt the child's mental development and even cause neglect and abuse. For this reason, in the treatment carried out in child psychiatry clinics, it is not enough to work only with the child or to make suggestions to the family; it can be a process that requires comprehensive, multidimensional and multidisciplinary work that is family-based and the child protection system to be activated when necessary.

In this case report, a 14-year-old child who developed more and risky behavioral problems as a result of the negative attitudes and behaviors of the parents, whose treatment was delayed and could not benefit from the treatment, who was neglected in terms of care, health, education and other basic needs and became vulnerable to abuse, and who was evaluated within the scope of the child protection system and placed in institutional care will be evaluated. in the article, the multidimensional treatment process including pharmacological treatment, multidisciplinary work and coordination between institutions, and the work and procedures carried out within the scope of the child protection system in this process will be discussed.

Keywords: Adolescence, child in need of protection, behavioral problems, parental attitudes

***İletişim kurulacak yazar/Corresponding author:** Yunus Dursun; Kocaeli Üniversitesi, Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Anabilim Dalı, Kocaeli, Türkiye.
Telefon/Phone: +90 (532) 440 07 37 **e-posta/e-mail:**yunusuzman@hotmail.com
Başvuru/Submitted: 17.02.2025 ● **Kabul/Accepted:** 16.06.2025 ● **Online Yayın/Published Online:** 30.06.2025

Giriş

Aile, bir çocuğun biyo-psiko-sosyal gelişimi açısından ilk, en temel ve önemli yapıtaşı olmasıyla kritik bir öne sahiptir. Bu nedenle çocuğun sağlıklı olabilmesi için sağlıklı işlevlere sahip bir ailede büyümesi gerekmektedir. Sağlıksız işlevlere sahip olan ailede yetişen çocuklarda ruhsal sorunların ortaya çıkması ve var olan sorunların ise şiddetini artırması beklenen bir durumdur. Bu nedenle yaşadığı ruhsal sorun nedeniyle tedaviye getirilen çocuktaki sorunun tespit edilebilmesi ve sağaltımı için aile sistemi temelli kapsamlı bir değerlendirme yapılarak aileden kaynaklı sorunların da tedavide ele alınması gerekmektedir. Çocukta meydana gelen ruhsal sorunlarının olası tüm boyutlarını öngörmedeki sınırlılıklar nedeniyle, Psikiyatristler, Klinik Psikologlar, Sosyal Hizmet Uzmanlarının birbirleriyle ve hastalığın tedavi ve yönetiminde diğer yardımcı profesyonellerle işbirliği yaptığı multidisipliner yaklaşımı daha da önemli hale getirmekte ayrıca karmaşık vakaları tartışmaları için bir platform sağlamaktadır[1]

Tedavi sürecinde sorunun tespit edilmesi kadar çözümü için çocuk ile birlikte ebeveynlerinin de tedaviye uyumu da oldukça önemlidir. Tüm müdahalelere rağmen bazı olgularda ebeveynlerin tedavi sürecinde gösterdikleri direnç sorunun çözülmesini engellemektedir. Bu durumda kurumlararası koordinasyon ile çocuk koruma sisteminin devreye alınması gerekebilmektedir. 2005 yılında yayımlanarak yürürlüğe giren Çocuk Koruma Kanunu korunma ihtiyacı olan ve suça sürüklenen çocukların korunması, yargılanması ile hak ve esenliklerinin korunmasını ilke edinir. Bu amaçla hazırlanan yasanın 5. maddesi koruyucu ve destekleyici tedbirleri içermektedir. Yasanın temel amacı ilgili kurumların uzmanlık alanları doğrultusunda çocuğun doğup büyüdüğü kendi ailesinin yanında kalarak koruyucu, önleyici ve iyileştirici faaliyet ve desteklerin aile bütünlüğü temelinde profesyonel açıdan sağlanmasıdır. Çocuğun ailesi yanında kalmasının onun biyo-psiko-sosyal gelişimini olumsuz etkilemesi, ailenin çocuğa zarar vermesi ve bu zararın diğer tedbir kararları ile desteklenerek ortadan kaldırılamayacağı kanaatine varılması durumunda çocuk hakkında bakım tedbir kararı alınarak çocuk ailenin yanından alınıp kurum bakımına yerleştirilebilmektedir.

Bu olgu sunumunda dikkat eksikliği hiperaktivite bozukluğu, davranım bozukluğu, obsesif kompulsif bozukluk ve internet bağımlılığı tanılarıyla takip edilen olgunun tedavisi ile bu süreçte uygulanan multidisipliner çalışma, kurumlar arası koordinasyon ve çocuk koruma sistemi kapsamında zorunlu olarak çocuğun aile ortamından uzaklaştırılmasının tedavi sürecine etkisi tartışılmaktadır.

Değerlendirilmeye alınan bu olgu sunumunda ebeveynlerin olumsuz tutum ve davranışları neticesinde daha fazla ve riskli davranış sorunları geliştiren, ailesi yanından alınarak kurum bakımına yerleştirilen 14 yaşındaki çocuğun farmakolojik tedavisi, disiplinler ve kurumlar arası koordinasyonu içeren çok boyutlu tedavi süreci ele alınmıştır. Çalışmada yukarıdaki belirtilen sorunların ortaya çıkma nedenlerinin kuramsal olarak incelenmesi ve sorunun çözümüne yönelik yapılan müdahalelerin bir olgu ile somut olarak örneklendirilmesi amaçlanmıştır. Bu amaç doğrultusunda olguda, örnek olay incelemesi ya da vaka çalışması tekniği olarak da isimlendirilen nitel araştırma yöntemlerinden durum çalışmasından yararlanılmıştır[2].

Olgu

14 yaşında, 9. sınıf öğrencisi erkek hasta ilk olarak babası eşliğinde hastane polikliniğimize getirilmiştir. Yapılan görüşmede hastanın evde babasına şiddet uygulaması sonucu babanın el parmaklarında kırık oluştuğu, annesinin de çocuktan fiziksel şiddet gördüğü için savcılıktan çocuğa uzaklaştırma kararı çıkarttığı ve tayinini başka bir ile aldırıp orada yaşamaya başladığı öğrenilmiştir. Hasta iki çocuklu bir ailenin ikinci çocuğudur. Babasının 58 yaşında üniversite mezunu olup emekli olduğu ve halen çalıştığı, annesinin ise 52 yaşında, üniversite mezunu olup halen çalıştığı, 20 yaşında şehir dışında okuyan üniversite öğrencisi abisinin olduğu öğrenilmiştir. Anne ve babanın 12 yıl önce olgu yaklaşık 3 yaşında iken boşandığı ancak olgu ile anne tek başına ilgilenmekte zorlandığı için anne ve babanın 6 ay önce tekrar birlikte yaşamaya başladıkları bilgileri edinilmiştir.

Olgu, erken çocukluk döneminden itibaren fazla hareketli, dikkati dağınık, takıntıları olan zor bir çocuk olarak tanımlanmıştır. 7 yaşında dikkat eksikliği hiperaktivite bozukluğu tanısı almış ancak takiplere düzenli olarak gitmemiştir. İlerleyen dönemlerde takıntılı davranışları artmaya başlayan olgu istediği olmadığı takdirde sinirlenir anneye şiddet uygularmış. Anne-baba arasında sorunlar şiddetlenince boşanma gerçekleşmiş ve velayet anneye verilerek olgu ve abisi annesinin yanında kalmaya başlamış. Son 6 aydır annesinden istediği şekilde karşılık almadığında kapıları, eşyaları kırar, anne ve babasına fiziksel şiddet uygularmış. Anne bu nedenle olgu'yu babasının yanında bırakarak farklı bir şehre taşınmış, baba ile yalnız kaldığında öfke kontrolünde daha çok zorlanmaya başlamış, baba da olgunun ona zarar vermesinden korktuğu için onu evde yalnız bırakıp, kendi arkadaşlarında kalırmış. Ebeveynlerin denetimi tamamen ortadan kalkınca olgu, bütün gün internette oyun oynayarak vakit geçirir olmuş. Okula gitmeyi bırakmış.

Zekâsı klinik olarak normal izlenimi veren olgunun WISC-R'da sözel puanı 98, performans puanı 127, toplam zekâ puanı 114 bulunmuştur. Polikliniktekı takip süreçlerinde yapılan değerlendirme ve gözlemlerde aile içi sorunların mevcut olduğu, uygunsuz anne-baba tutumlarının olduğu ve ebeveynlerin yüksek eğitim düzeylerine rağmen çocuğu sağlık ve eğitim açısından ihmal ettikleri anlaşılmıştır. Bu kapsamda klinikte görevli sosyal hizmet uzmanı tarafından hazırlanan ayrıntılı durum değerlendirme raporu ile çocuğun korunmaya muhtaçlık durumunun değerlendirilmesi ve gerekli tedbir kararlarının alınması için Aile ve Sosyal Hizmetler İl Müdürlüğüne (ASHİM) bildirimde bulunulmuştur. ASHİM tarafından yapılan sosyal inceleme neticesinde çocuğun

sağlık takibinin yapılması, eğitimine devam etmesi ve aile ilişkilerinin düzenlenmesi açısından 5395 sayılı Çocuk Koruma Kanunu kapsamında sağlık, eğitim ve danışmanlık tedbiri kararı alınmıştır. Tedbir kararları olmasına rağmen kontrol randevularına düzenli olarak gelmeyen hastanın babası kontrol randevu tarihlerinde polikliniğe gelerek çocuğunun odasına kapandığını, kendisine şiddet uyguladığını, temel barınma ve hijyen ihtiyaçlarını belirtmiştir. Olgu sağlık tedbir kararı kapsamında yataklı tedavi kurumuna sevk edilerek iki ay yataklı çocuk psikiyatrisi kliniğinde tedavi görmüş, taburculuğun hemen sonrasında ise gündüz kliniğinde tedavi altına alınmıştır

Gündüz kliniğimizde olgu'ya ruhsal bozuklukları ile ilgili psikoeğitim verilmiş, psikofarmakolojik tedavisi düzenlenmiş, ilaç tedavisine ek olarak bilişsel davranışçı terapiye başlanmıştır. Olgu internet kullanım süresi olarak günlük belirlenen kullanım sürelerine uyum sağlamıştır. İlgi alanlarına yönelik sosyal aktivitelere başlayan hastanın olumlu yanları desteklenerek güçlerini fark etmesi sağlanmaya çalışılmıştır. Günlük bireysel görüşmeler yapılarak tedavide öğrenilenleri günlük yaşantıda uygulama konusunda destek olunmuştur. Haftada en az bir gün anne ve baba ile ayrıntılı görüşmeler yapılarak ergenlikte kimlik gelişimi, bireyselleşme süreci ile ilgili bilgi aktarılarak çocuğun özerkleşmesine engel teşkil edecek ebeveynlik tutumları ele alınmıştır. Sınıf öğretmenleri ve okul ile görüşülmüştür. Gündüz kliniğinde olgunun sınıf içi ve dışı tutum ve davranışları, akran iletişimi, akademik becerileri ile ilgili gözlemler değerlendirilerek tedavi şekillendirilmiştir. Ancak ilerleyen süreçte hastanın kurallara uyum sağlamakta zorlandığı, diğer hastalara müdahalelerde bulunduğu, uyarılara karşı geldiği, kliniğe mazeretsiz devamsızlık yaptığı, anne babanın sınır koyamadığı gözlenmiştir. Çocuğun tedavisinden sorumlu doktor ve birimde görevli sosyal hizmet uzmanı tarafından çocuğun yaşadığı ev ve çevre koşullarının değerlendirilmesi amacıyla sosyal inceleme amaçlı ev ziyareti yapılması planlanmıştır. Ev ziyareti için babanın onayı alınmış ve kendileri ile randevulaşılan gün ve saatte ev ziyareti gerçekleştirilmiştir. Ev ziyareti esnasında odaların dağınık ve tadilat gerektiren bir görüntüsünün olduğu, ev kapılarının olgu eşyalara zarar verdiği için çıkartılmış olduğu, eşyaların oldukça eski, yer yer kullanışsız ve ailenin ihtiyaçlarını yeterince karşılamayacak durumda olduğu, evin hijyen koşullarını sağlamadığı, oldukça dağınık olduğu, olgunun kendisine ait bir odasının bulunmadığı, salondaki oldukça eski bir çekyatı yatak olarak kullandığı, yaşanılan konutun işyerlerinin yoğun olduğu merkezi bir konumda olması nedeniyle olgunun sosyal olanaklardan mahrum kaldığı, yaşanılan apartman ve etraftaki diğer binalarda genellikle bekar ya da yaşlı insanların yaşadığı, arkadaşlarının olmadığı, dışarıya çıkmadığı gözlemlenmiştir.

Olgunun takiplerinde ruhsal açıdan kötüleşme olduğu, internet bağımlılığı, takıntılar, öfke nöbetlerinin tekrar geliştiği, ebeveynlerin ise yapılan aile görüşmelerinde sorunları dışsallaştırma eğilimde oldukları, tutum değişimi konusunda direnç gösterdikleri, kendilerinde bir sorun olmayıp tüm sorunun çocuklarında olduğuna dair söylemlerinin olduğu, ailenin kendilerine yönelik davranışsal önerileri ihmal ettikleri, tedavi ekibinden de çocuktaki sorunların tedavi edilmesine dair beklentilerinin olduğu, olgunun kliniğe düzenli ve devamlı gelmesini sağlamadıkları, yalnız bırakmaya devam ettikleri gözlemlenmiştir

Sosyal hizmet uzmanı tarafından danışmanlık tedbir kapsamında vakadan sorumlu kurum danışmanı ile görüşme yapılmış, yapılan görüşmede danışmanlık tedbir sürecinde yeterince ilerleme sağlanamadığı bilgileri edinilmiştir. Okul idarecileri ve rehberlik birimi ile yapılan görüşmede de çocuğun okula gitmediği bilgileri edinilmiştir.

Olgu bir aylık takip sürecinden sonra gündüz kliniğine uyum sağlamaması ve devam etmek istememesi üzerine taburcu edilerek takiplerinin poliklinikten yapılmasına karar verilmiştir. Sık aralıklarla poliklinikten takip edilmeye başlayan ve sağlık tedbir kararı kapsamında olan olgunun bir süre sonra poliklinik takiplerini de aksattığı gözlenmiştir.

Takip için kontrol randevularını aksatan olgudaki davranış sorunlarının artmaya başladığı, hastaneye gelmeyi kabul etmediği, anne ve babaya fiziksel şiddet uyguladığı bu nedenle anne ve babanın olgu'yu evde yalnız bırakarak evi terk etmek zorunda kaldıkları bilgileri edinilmiştir. Sağlık tedbir kararı kapsamında da poliklinik takiplerine gelmeyen olgu hakkında baba sık sık hastaneye gelerek olgunun sorunlarının artarak devam ettiğini, kendilerinin çaresiz kaldıklarını belirtmiştir.

Gündüz kliniği tedavi ekibi olgu hakkında vaka toplantısı yapmıştır. Yapılan tüm değerlendirmeler neticesinde çocukta yaşanan sorunların giderilmesi sürecini anne-babanın yönetemediği, aksine anne-baba tutumlarının çocuktaki sorunları daha da artırdığı ve bu tutumların onu kışkırtmaya yönelik olduğu, anne-babaya verilen önerilerin yerine getirilmediği anlaşılmış olup çocuğun artan şiddet davranışlarının kendisinde ve ebeveyne yönelik ciddi ve hayati sonuçlar doğurabileceği, babanın evi terk etmesi, çocuğun evde tek başına dışarı çıkmadan vakit geçirdiği ve kapıyı kimseye açmamasının da tehlike arz ettiği, bu nedenle çocuğun kurum bakımına alınmasının gerekliliği konusunda fikir birliğine varılmıştır. Çocuğun tedavi süreci ile çocuk ve ebeveynleri arasındaki sorunun varlığı ve çocuğun güncel durumu ile ilgili sosyal hizmet uzmanı tarafından durum değerlendirme raporu hazırlanarak çocuğun kurum bakımına alınmasının ivedilikle değerlendirilmesi ve ilgili kurumlardan çocuk ile çalışma yapan temsilciler ile olgu hakkında değerlendirilme yapılabilmesi amacıyla çocuk koruma alt komisyonunun toplanması için ASHİM'e bildirimde bulunulmuştur. ASHİM tarafından yapılan kapsamlı sosyal inceleme neticesinde çocuğun babasının şiddet nedeniyle evi terk ettiği, uzun süredir tek başına evde kaldığı uzun zamandır da okula gitmediği, okulda olduğu sınırlı zamanlarda okuldaki arkadaşlarına da şiddet uyguladığı bilgileri edinilmiştir. İlgili kurum ve kuruluşların temsilcilerinin de katılım sağladığı çocuk koruma alt komisyonunda yapılan kapsamlı değerlendirme de çocuğun yataklı tedavi kurunda tedavi

görmesi ve taburculuk sonrasında kurum bakımına alınmasının uygun olacağı yönünde karar alınmıştır. Babası evden ayrılan ve evde yalnız başına kalan ve kapıyı kimseye açmayan olgu için mahkemeden zorunlu tedavi kararı çıkarılmıştır. Yataklı tedavi ve kurumum bakımını ilk başta reddeden olgu kendisine yapılan kapsamlı açıklamalar sonrasında tedavi kurumuna gitmeyi ve sonrasında da kurumda kalmayı kabul etmiş ve kendisi ile sözlü kontrat yapılmıştır. Olgu ve baba ile yapılan görüşmeler neticesinde olgu yataklı bir çocuk psikiyatri kliniğine yatırılmış, taburculuk sonrasında ise aile yanına verilmeden kurum bakımına alınmıştır.

Sağlık tedbir kararı kapsamında takipleri hastanemizde yapılan ve önceleri takiplerine düzenli olarak getirilmeyen çocuk, kurumda korunma altına alındıktan sonra kurum personeli tarafından düzenli olarak kontrol randevularına getirilmiştir. Olgu kurum bakımına alındıktan sonra yakın takibe alınmış ve kısa süre aralıklarla tedavisi düzenlenmiştir. Olgu ve kurum meslek elemanları ile yapılan düzenli görüşmelerde çocuğun tedavi sürecinden fayda gördüğü, ailesi yanında ilaç kullanımını reddeden olgunun kurumda ilaçlarını düzenli olarak aldığı, kurum danışmanı ile rutin görüşmelere katıldığı ve onunla olumlu iletişim halinde olduğu, kuruma ilk geldiği zamanlarda bilgisayara kullanımı süresinde kendisine esnek davranıldığı ancak ilerleyen zamanlarda bu sürelere ilişkin kurallara uyum sağladığı, kurumun kendine yönelik hazırlamış olduğu tüm kurallara uyum sağlamaya çalıştığı, kurumdaki arkadaşlarına ve personele yönelik herhangi bir şiddet davranışı sergilemediği, ebeveynlerinin yanında iken bırakmış olduğu okuluna düzenli olarak devam ettiği, sosyal aktivitelere de isteyerek katıldığı, ebeveynlerinin kendisini kurumun belirlediği zaman diliminde kurumda ziyaret ettiği, ilk başlarda kurum danışmanının gözetiminde görüşme yaptığı, ebeveynleri ile olumlu iletişim halinde olduğu, ilerleyen zamanlarda resmi tatil ve bayram günlerinde ebeveynlerinin yanına kısa süreli izinli olarak gidebileceği bilgileri edinilmiştir. Uzun aralıklı takiplerde de olguda herhangi bir olumsuz durum gözlemlenmemiştir.

Tartışma

Psikiyatrik tedavi süreci, hastayı, ailesi, çevresi ve toplumu da içine alacak seviyede bütüncül olarak ele alınmasını ve bununla birlikte disiplinler arası ekip çalışmasını gerektirmektedir[3]. Kliniklerde ruhsal sorun yaşayan bireylerin kapsamlı tedavi hizmeti alabilmeleri için kliniklerde psikiyatri uzmanı ve psikiyatri hemşiresi, psikolog, sosyal hizmet uzmanı ve mesleki terapistlerden "psikiyatri ekipleri" oluşturmaları tavsiye edilmektedir[4]. Bu nedenle multidisipliner yaklaşım çağın bir ihtiyacıdır. Multidisipliner yaklaşımdaki işbirlikçi ilişki, ekip çalışmasını teşvik eder ve farklı uzmanlık alanlarının yüksek uzmanlık becerileri en kaliteli hizmeti sunmak için birlikte çalışır. Ekipteki üye sayısı uzmanlıklar arasındaki koordinasyona bağlıdır. Tedavi sürecinde çocuğun tedavisinden sorumlu psikiyatrist sadece tanı koyup farmakoterapi reçete etmekle kalmaz, aynı zamanda farklı risk faktörlerini, psiko-sosyal yönleri ve diğer karmaşık sorunların belirlenmesi için diğer ruh sağlığı uzmanlarıyla koordinasyon sağlar[5]. Olgunun tedavi gördüğü gündüz kliniğinde çocuk ve ergen psikiyatristi, sosyal hizmet uzmanı, psikolog, psikiyatri hemşiresi görev yapmakta ve olgular ile disiplinler arası ekip çalışması yapılmaktadır.

Bireyin sağlıklı bir kişi olabilmesi onun biyo-psiko-sosyal yönden sağlıklı olmasına bağlıdır. Her çocuk gelişim sürecinde birtakım zorluklar ve sorunlar yaşayabilmektedir. Bu sorunlar bireyin kendi özelliklerinden kaynaklanabileceği gibi ailesel ya da çevresel nedenlerden de kaynaklanabilmektedir. Ergenlik, çocuk olarak yaşanılan bir hayattan erişkin olarak yaşanılacak bir hayata geçişte önemli bir dönemidir. İnsan hayatında biyolojik, psikolojik ve toplumsal açıdan hızlı ve önemli değişmelerin meydana geldiği ergenlik dönemi, pek çok sorunun da ortaya çıktığı ve yaşandığı bir dönem olması nedeniyle oldukça önemlidir. Bireyde meydana gelen biyolojik, psikolojik değişme ve gelişmeler sonucunda ortaya çıkan sorunlara, aile, okul ve arkadaş çevresinde yaşanan toplumsal sorunların da eklenmesi, bu dönemi daha da sıkıntılı bir dönem haline getirebilmektedir[6]. Bu dönemde yaşanan ruhsal sorunlar kişinin çaresiz hissetmesine, içine kapanmasına ya da bir takım davranış sorunları geliştirmesine neden olabilir. Bununla birlikte anne babanın tutarsızlığı, çocuk üzerindeki denetim zayıflığı ve fiziksel içerikli ceza uygulamaları da çocukta davranış sorunları geliştirmesine neden olabilmektedir[7]. Aile bireyin sağlıklı olmasına imkân oluşturan en temel kurumdur. Bireyin işlevselliği üzerinde en çok etkiye sahip olan sistem de aile sistemidir. Bu sistem bireylerin gereksinimlerinin karşılandığı en temel sistem olup bireyin işlevselliği açısından yaşamış olduğu sorunların kaynağı da genel olarak aile sistemi kaynaklıdır[8]. Bu açıdan bakıldığında ruhsal tedavide kısa ve uzun süreli etkileri açısından ergen, okul ve toplum temelli müdahaleleri kullanan aile odaklı yaklaşım en etkili tedavi yönetimi olarak kabul edilmektedir[7]. Dikkat Eksikliği Hiperaktivite Bozukluğu (DEHB) dikkat, konsantrasyon hareketli olma hali ve dürtü kontrolünün eşlik ettiği, çocukların akademik ve sosyal hayatını olumsuz olarak etkileyen ve çocukluk döneminde sık karşılaşılan psikiyatrik bozukluk olup uzun süreli bütüncül olarak uygulanan tedavi ile hastalıkta fark edilir bir düzelme olmaktadır[9]. Bu hastalığın tedavi sürecinde ebeveyn tutumları ve eğitimi özel önem arz etmekte olup tedavi edilmemesi durumunda başka psikiyatrik sorunların eklenmesi, aile ve arkadaş çevresi ile sorunlar yaşama, sigara, alkol ve madde kullanımına yönelme ve adli olaylara karışma gibi problemler ile karşılaşılabilmektedir[10]. Olgunun ilk tedaviye başlama yaşı 7'dir. Dikkat eksikliği hiperaktivite bozukluğu tanısı alan çocuk düzenli takiplerine getirilmemiş, ebeveynleri tarafından uzun süre tedaviye ara verilmiş, ergenlik dönemi ile birlikte artan şikayetleri kapsamında ancak tedaviye getirilmiştir. Zamanında tedavisi aksatılan çocuğun ebeveynlerinin kendi aralarındaki iletişim sorunları ve çatışmaları neticesinde meydana gelen

boşanma süreci ile boşanma öncesi ve sonrası çocuğa yönelik sorunlu tutum ve davranışları nedenleriyle olgunun ruhsal şikayetleri artmış daha farklı sorunlar da geliştirmiştir.

Ebeveynler arasındaki şiddetli çatışma boşanma ile sonuçlanabilmekte ve boşanma sonrası bu çatışma ortamından uzak kalması çocukların yararına olabilmektedir. Ancak boşanma sonrası çocuğa yönelik devam eden olumsuz tutum ve davranışlar çocuğun yine bu süreçten olumsuz etkilenmesine neden olabilmektedir. Bu nedenle bu tür ailelerde anne-babanın boşanması ya da tek ebeveynli olması da çocuk ihmal ve istismarında risk etmenlerindendir[11]. Sosyal devletler, çeşitli sosyal politika ve yasal düzenleme gibi unsurlar ile ailenin güçlendirilmesini ve desteklenmesini sağlayarak çocukların öncelikli olarak ailesi yanında bakılmalarının sağlanmasını amaçlamaktadır ancak tüm bu sistemler sayesinde bir çocuğun ailesi yanında korunup bakımı imkânsız hale geldiğinde alternatif bakım seçenekleri devreye girebilmektedir[12].

24.12.2006 tarihinde yayımlanarak yürürlüğe giren 26386 sayılı Çocuk Koruma Kanununa Göre Verilen Koruyucu ve Destekleyici Tedbir Kararlarının Uygulanması Hakkında Yönetmelik çocuk koruma kanununda belirtilen koruyucu ve destekleyici tedbir kararlarının hangi kurumlar tarafından ve nasıl uygulanması gerektiği konularında detaylı hükümleri içermektedir. İlgili yönetmeliğin 20. ve 21. maddeleri ile tedbir kararlarının etkinliğinin değerlendirilmesi için il ve ilçelerde vaka bazında değerlendirme yapılabilmesi amacıyla Çocuk Koruma Alt Komisyon Kurullarının oluşturulması hükme bağlamıştır. Yaşanan sorun ve aksaklıklar nedeniyle diğer kurumların da hakkında tedbir kararı uyguladıkları olgunun durumunun komisyon nezaretinde değerlendirilebilmesi amacıyla hastane sosyal hizmet uzmanı tarafından Çocuk Koruma İl Koordinasyon Kurulu alt komisyon toplantısının düzenlenmesi talep edilmiştir. Toplantıya hastaneden çocuk psikiyatri doktoru ve sosyal hizmet uzmanı katılmış, Sosyal Hizmetler İl Müdürlüğünden çocuk hakkında danışmanlık tedbir kararını uygulayan sosyal hizmet uzmanı, Milli Eğitim İl Müdürlüğü'nden eğitim tedbirini uygulayan rehber öğretmen ve İl Sağlık Müdürlüğü'nden ise sağlık tedbir kararının uygulanmasında koordinasyon sorumlusu çocuk gelişimcisi katılım sağlamıştır.

Olgunun anne babasının boşanması, boşanma sonrasında aralarındaki iletişim sorunlarının artarak devam etmesi, çocuklarının iyiliği için gerekli olan ortak söylem ve davranış geliştirememeleri, kendilerine verilen tüm önerilere ve eğitimlere rağmen çocuklarına yönelik sorunlu tutum ve davranışlarına devam etmeleri, üzerilerine düşen sorumlulukları yeterince yerine getirememeleri, yaşanan sorunlarda çocuğu hedef ve suçlu olarak göstermeleri gibi durumlar çocuğun sorunlarının çeşitlenmesine, şiddetini artırmasına ve tedaviye uyumunu olumsuz etkilemesine neden olmuş ve daha önce olgu hakkında alınan sağlık, eğitim ve danışmanlık tedbir kararlarının uygulanamaması ve amacına ulaşılamaması ve tedbir kararlarının uygulanmamasında ebeveynlerinin önemli derecede etkilerinin olması, tüm müdahalelere rağmen ebeveynlerinin süreci kolaylaştırmaktan ziyade daha da içinden çıkılamaz hale getirmeleri nedeniyle son çare olarak çocuk hakkında bakım tedbiri alınarak kurum bakımına alınmasının gerekliliği üzerinde fikir birliğine varılmıştır. Bu kapsamda olgunun kurum bakımına alınması Çocuk Koruma İl Koordinasyon Kurulu alt komisyon toplantısında kararlaştırılmıştır.

Çocukların gelişimleri açısından yetişebilecekleri en uygun ortam sağlıklı aile ortamıdır ancak her çocuk bu ortama sahip olamamakta ve aile kaynaklı ciddi sorunlar nedeniyle çocuklar kurum bakımına alınabilmektedir. Kurum bakımının çocukların biyo-psiko-sosyal gelişimleri üzerinde pek çok olumsuz etkileri olduğu bilinmektedir[13]. Milosavljević-Đukić (2020)'nin Belgrad'da kurum bakımında kalan çocukların ruhsal özelliklerini ele aldığı 486 çocuğun ele alındığı çalışmada, bu çocukların %19,8'ine (n=96) ruhsal tanı konulduğunu, kuruma yerleştirilen çocuk ve ergenlerin, olumsuz etkiler ve ilk çocukluktan itibaren olumlu duygusal uyaranların yokluğu göz önünde bulundurulduğunda, savunmasız bir nüfus olduğunu ve ruhsal bozukluk geliştirme riskinin önemli ölçüde daha yüksek olduğunu belirterek kurumda kalmaya bağlı ruhsal sonuçların çocuğun yaşına, kurumda kalma süresine, önceki aile deneyimlerine ve çocuğun geçirdiği yaşam değişikliklerine bağlı olduğunu belirtmiştir[14].

Bu nedenle çocukların kurum bakımına alınmaları en son çare olarak değerlendirilmelidir. Ailenin desteklenerek çocukların korunmaya ihtiyaç olma durumlarının engellemesi durumunda çocukların kurum bakımına alınmasına gerek duyulmamaktadır. Ancak ilgili kurumlarının tüm müdahalelerine rağmen çocuğun aile sisteminde zarar gördüğünün değerlendirildiği durumlarda bu seçenek zorunlu olarak devreye sokulmaktadır.

Olguda ailenin yanında kaldığı süreçte gerek Sosyal Hizmetler İl Müdürlüğünün gerekse de psikiyatri kliniğinin tüm müdahale ve çalışmalarına rağmen iyileşmenin olmadığı, çocuğun tedaviyi aksattığı, kendisine önerilen ilaçları kullanmadığı, okulunu bıraktığı, kendisini odasına kapattığı, beslenme gibi zorunlu ihtiyaçlarını dahi aksattığı, sürekli bilgisayar başında vakit geçirdiği, haftalarca evinden dışarı çıkmadığı bilgileri edinilmiş, anne ve babanın bu süreci yönetemediği ve hatta çocuğun bu sürece gelmesinde kendilerinden kaynaklı tutum sorunları nedeniyle etkilerinin olduğu, çocuğu kendi halinde başıboş bıraktıkları anlaşılmış ve ciddi olarak ihmal edilen, yaşamsal risk yaşayan çocuğun öncelikle zorunlu yatış tedavisinin sağlanması ve sonrası kurum bakımına alınması için mahkeme kararının çıkarılması sağlanmıştır. Yataklı tedavi sonrası kurum bakımına alınan olgu kurumlar arası multidisipliner bir yaklaşım ile değerlendirilerek tedaviden fayda sağlamış, sosyal aktivitelere katılım sağlamış, ara verdiği eğitimine düzenli olarak devam etmiş ve ebeveynleri ile de görüşerek sağlıklı iletişim kurmuştur.

Ergenin sağlıklı bir gelişim gösterebilmesi için aile yanında akran ilişkileri de önem kazanmaktadır. Ergenlik dönemi

çocuğun ailesinden uzaklaşıp kendi yaşıtları içerinde kabul görmek ve iletişim kurmak istediği bir dönemdir, bu dönemde olumlu arkadaş ilişkileri çocuğun olumlu davranış geliştirmesinde kilit role sahiptir. Çocuk evlerinde kalan ergenlik çağındaki çocukların ruhsal dayanıklılık, öz yeterlilik ve sosyal duygusal öğrenme becerilerinin değerlendirildiği yakın tarihli bir çalışmada çocuğun yakın arkadaşlarının bulunmasının onların öz yeterlilik ve duygusal öğrenme düzeylerini artırdığı bulunmuştur[15]. Olgunun kurum bakımına alınmadan önce hem ailesi ile ciddi sorunlar yaşaması hem de yakın arkadaş çevresinin olmaması onun davranış sorunu geliştirmesinde etkili olmuştur. Kurum bakımına alındıktan sonra kontrol randevularında olgu ve kurum görevlileri ile yapılan görüşmede olgunun kurumda diğer çocuklar tarafından kabul gördüğü, olgunun da onlarla kısa süre içerisinde olumlu iletişim kurduğu, onlarla birlikte kurum sosyal aktivitelerine katılım sağladığı, kurum aktivite dışı zamanlarda da arkadaşlarıyla paylaşımlarda bulunduğu, yakın iletişim içerinde bulunduğu kişiler ile okuluna gittiği, bu durumun olguda olumlu etki oluşturduğu bilgileri edinilmiştir. Yataklı tedavi sonrası çatışma ortamında olduğu evi yerine hemen kurum bakımına alınan olgunun kurumda hemşire gözetiminde ilaçlarını düzenli olarak alması, kurum danışmanı ve sosyal serviste görevli meslek elemanlarının olgu ile rutin görüşme yapmaları, kurumda kendi yaşıtı olan kişiler ile sosyal aktivite programlarına katılması, çocuğun yaş ve gelişimine uygun tutum ve davranışlarda bulunulması, kurum hemşiresi ve danışmanının tedaviden sorumlu doktor ile sık iletişimde olması ve tedavi kapsamında kendilerine çocuk için verilen önerileri yerine getirmeleri, olgunun okuluna devam etmesinin olguda sorunlu davranışların azalmasında ve tedaviye uyumunu artırmasında etkili olduğu düşünülmektedir.

Bu olguda tedaviye dirençli bir hastanın multidisipliner yaklaşımın kapsayıcılığı ile artan tedavi etkinliği, farklı meslek gruplarının tedavi üzerindeki katkıları ve uzun süreli takipte yaşanan süreçlere ve bu süreçte çocuk koruma sisteminin devreye alınması durumuna dikkat çekilmek istenmiştir. Çocuk ruh sağlığı alanında karşılaşılan tedavi dirençli olguları multidisipliner yaklaşımla ve gerektiğinde kurumlar arası koordinasyon ile ele almak tedavi etkinliğini artırmaktadır. Olgumuzda yataklı servisten taburculuk sonrası gündüz kliniğine başlayarak yakın takibe alınmıştır. Olgunun parçalanmış aile yapısına sahip olsa da anne ve babasının eğitimli olması, maddi yoksunluk içerisinde olmamaları ve çocuğu tedaviye getirme motivasyonlarının olması nedenleriyle öncelikle onun ailesinin yanında kalarak desteklenmesi ve tedavi edilmesi amaçlanmış ve çocuğun ve ailenin bu sürece aktif katılımlarının sağlanması ve desteklenmesi amacıyla da Çocuk Koruma Kanunu kapsamında çocuk hakkında danışmanlık, eğitim ve sağlık tedbir kararları alınmıştır. Ancak süreç içerisinde bahse konu olan çocuğun yaşamış olduğu sorunların daha da arttığı, bunda daha çok ailesinin çocuğa yönelik tutumlarının etkisinin olduğu, ebeveyn tutumlarındaki bu sorunların çocuğun sağlık durumunu daha da kötüleştirdiği anlaşıldığından çocuğun kurum bakımına alınması sağlanmıştır. Olgu hakkında bakım tedbir kararının geç uygulanması ruhsal sorununun daha da artmasına neden olduğundan bu tür vakalarda sorun fazla kronikleşmeden daha erken harekete geçilmesi ayrıca önem arz etmektedir. Ebeveynlerin iyi sosyo-ekonomik düzey ve yüksek eğitim seviyesine sahip olmaları çocukların ihmal ve istismarı açısından koruyucu faktör olmasına karşın tek başına yeterli değildir. Bu durumlar sosyal inceleme sürecinde sosyal hizmet uzmanının çocuğun korunmaya ihtiyacı olan çocuk kapsamında değerlendirmesinde bakım tedbiri kapsamında korunmaya ihtiyacı olmadığına dair bir tür yanılsama yaşamasına neden olabilir. Olgudan da anlaşılacağı üzere çocukların ailelerin yanında olması onların biyo-psiko-sosyal gelişimi ve sağlığı açısından her zaman olumlu bir durum oluşturmamakta hatta kimi zamanı çocukların iyilik hallerini tehlikeye atabilmektedir. Bu nedenle bu tür olgularda son seçenek olan kurum bakım tedbirinin uygulanmasının çocuk üzerinde bir takım olumsuz etkileri olabileceği ancak kaotik aile yapısının ve uygun olmayan tutumların meydana getirdiği olumsuz etkilere göre daha yararlı sonuçları olacağını düşündürmektedir.

Etik Standartlara Uygunluk

Hastanın gizliliği korunarak etik unsurlara dikkat edilmiştir.

Yazar Katkısı

YD:Fikir; YD, GÜ, NÇM: Tasarım; YD, GÜ, NÇM: Yazım; YD, GÜ, NÇM: Denetleme; YD, GÜ, NÇM: Literatür taraması; YD, GÜ, NÇM:Yorum; YD, NÇM: Eleştirel inceleme

Çıkar Çatışması

Bu çalışmada herhangi bir potansiyel çıkar çatışması bulunmamaktadır.

Finansal Destek

Herhangi bir finansal destek alınmamıştır.

Kaynaklar

1. Tenea-Cojan ŞT, Dinescu VC, GheormanV, Dragne IG, Gheorman V, Forțofoiu MC, Dobrinescu AG. ExploringMultidisciplinary Approaches to Comorbid Psychiatric and Medical Disorders: A Scoping Review. Life. 2025;15(2):251. doi: 10.3390/life15020251
2. Subaşı M, OkumuşK. Bir araştırma yöntemi olarak durum çalışması. Atatürk Üniversitesi Sosyal Bilimler Enstitüsü Dergisi. 2017;21(2):419-426.
3. Saruç S, Duyan V.Psikiyatride Ekip Çalışması ve Sosyal Hizmet Bakış Açısı. Kriz Dergisi, 2009;17(1):37-44.
4. Mental Health Commission. Multidisciplinary Team Working: From Theory To Practice: Discussion Paper (1.84 MB). -2006; 2489.
5. Kumar D, Sinha UK, Khanna A, Kar SK. Multidisciplinary approach in child and adolescent depression: Experience froma tertiary mental health institution in India. Open Journal of Psychiatry. 2013;3(3):8-14. doi:10.4236/ojpsych.2013.33A002

6. Avcı M. Ergenlikte Toplumsal Uyum Sorunları. Atatürk Üniversitesi Sosyal Bilimler Enstitüsü Dergisi. 2006;7(1), 39-63.
7. Tonyali A, Goker Z, Uneri OS. Psychosocial Interventions in the Treatment of Child and Adolescent ConductDisorder/Cocuk ve Ergen Davranim Bozuklugu Tedavisinde Psikososyal Mudahaleler. Psikiyatride Guncel Yaklasimlar/CurrentApproaches to Psychiatry, 2019;11(3):284-304. doi:10.18863/pgy.425225
8. Duyan V. Aileye Yönelik Planlı Müdahale Sürecinin Aşamaları, Toplum ve Sosyal Hizmet. 2003; 14(1):41-61.
9. Özbay A, Kayhan Z. Dikkat Eksikliği ve Hiperaktivite Bozukluğunun (DEHB) Nedenleri ve Tedavi Yöntemleri. Elektronik Sosyal Bilimler Dergisi, 2024; 23(89), 394-406. doi:10.17755/esosder.1283141
10. Ercan ES. Dikkat Eksikliği ve Hiperaktivite Bozukluğu, Çocuk Ve Erişkinlerde Hiperaktivite, Erken Tanı, Tedavi, Doğan Kitap Mega Basım. İstanbul, 2008
11. Kayma D. Aile Hukuku Davalarında Çocuk İhmal ve İstismarı: Ebeveyne Yabancılaşma Sendromu. Çocuğun İstismarı ve İhmali (pp.147-171), Ankara: Nika Yayınevi. 2023.
12. Karataş K.Türkiye'de Çocuk Koruma Sistemi ve Koruyucu Aile Uygulamaları Üzerine Bir Değerlendirme, Toplum ve Sosyal Hizmet, 2007;18(2):7-19
13. Afyonoğlu M F, Kesen NF, İşler G.Geçici Süreyle Kurum Bakımını Deneyimlemiş Çocuklar: Korunma Altına Alınma Sebepleri ve Deneyimleri/Temporarily Institutionalized Children: The Reasons for their Protection and Their Experiences. Nitel Sosyal Bilimler. 2021;3(2):258-279. doi:10.47105/nsb.1000546
14. Milosavljević-Đukić I. Settıng–Mental Health Characterıstıcs Of Chıldren From The Center For Protectıon Of Infants, ChıldrenAnd Youth In Belgrade Deca I Mladı U Instıtucıonalnom Setıngu–Karakterıstıke Mentalnog Zdravlja Dece U Centru Za ZaštıtuOdojčadı, Dece I.2023, doi: 10.2298/VSP191205092M
15. Toraman Ç, Sarıgedik E, Toraman MÇ, Noyan C O. Kurum bakımında kalan ergenlerin, psikolojik dayanıklılık, öz yeterlilik ve sosyal duygusal öğrenme becerilerinin değerlendirilmesi. Toplum ve Sosyal Hizmet. 2023;34(1):169-184. doi:10.33417/tsh.1062003

doi: 10.53446/actamednicomedia.1696468

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Case Report | Olgu Sunumu

FORMATION VARIATION OF THE MEDIAN NERVE: A CADAVERIC CASE REPORT

N.MEDIANUS'UN OLUŞUM VARYASYONU: BİR KADAVRA OLGU SUNUMU

Mehtap Erdogan[1*], Keziban Karacan[1], Huseyin Baylan[1], Ebru Mihriban Guven[1]

[1]Sakarya University, Faculty of Medicine, Department of Anatomy, Sakarya, Türkiye.

ABSTRACT

The median nerve, the longest branch of the brachial plexus, is formation in axillary fossa by a fusion of two nerves including the radix lateralis nervi mediani and radix medialis nervi mediani. A variant of that formation was observed during routine dissection at anatomy department of our faculty. It was found that the left median nerve was formed by three branches (two lateral and one medial). One of the roots originated from the fasciculus medialis; the other two roots originated from the fasciculus lateralis. This variation should be known and has clinical importance especially in surgical interventions, surgical interventions of traumatic cases and radiologic evaluations in order not to mislead the clinician.

Keywords: Anatomical variation, dissection, median nerve, brachial plexus

ÖZ

Plexus brachialisin en uzun dalı olan n. medianus; fossa axillariste, radix lateralis nervi mediani ile radix medialis nervi mediani'nin birleşmesi ile oluşmaktadır.

Sakarya Üniversitesi Tıp Fakültesi Anatomi Anabilim Dalı'nda tıp öğrencileri için yapılan rutin diseksiyon sırasında, N.medianus'un varyant bir oluşumu gözlenmiştir. Sol aksiller bölgede, normalden farklı olarak N.medianus'un iki lateral ve bir medial dalın birleşimiyle oluştuğu tespit edilmiştir. Köklerin biri, fasciculus medialisten; diğer iki kök ise fasciculus lateralisten kaynaklanmaktaydı. Bu varyasyon, özellikle cerrahi girişimlerde, travmatik olguların cerrahi girişimlerinde ve radyolojik değerlendirmelerde klinisyeni yanıltmaması için bilinmelidir ve klinik öneme sahiptir.

Anahtar Kelimeler: Anatomik varyasyon, diseksiyon, n. medianus, plexus brachialis

***Corresponding author/İletişim kurulacak yazar:** Mehtap Erdogan; Sakarya University, Faculty of Medicine, Department of Anatomy, Sakarya, Türkiye.
Phone/Telefon: +90 (553) 112 47 46, **e-mail/e-posta:** mehtaperdogan@sakarya.edu.tr
Submitted/Başvuru: 11.05.2025 • **Accepted/Kabul:** 17.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

The plexus brachialis is a complex nerve network that provides motor and sensory fibers to the upper extremity through the fusion of the ramus ventralis arising from the C5-T1 segments. The median nerve is usually formed anterior or lateral to the a.axillaris by the union of the radix lateralis (LR) from the faciculus lateralis and the radix medialis (MR) from the faciculus medialis (Figure 1). However, as a result of variations during embryonic development, the median nerve may form with three or more roots. Knowing this variation reduces the risk of complications in clinical and surgical practices.[1-3]
Anatomical variations of the brachial plexus have crucial importance in the diagnosis of nerve blockages, peripheral nerve compression syndromes and surgical interventions.[1,4] Although the median nerve is a union of two branches emerging from the lateral and medial faciculi of the brachial plexus.[2] However, many variations on this classical formation exist in literature. The presence of abnormal branches between musculocutaneous nerve and median nerve is among common variations.[4,5]

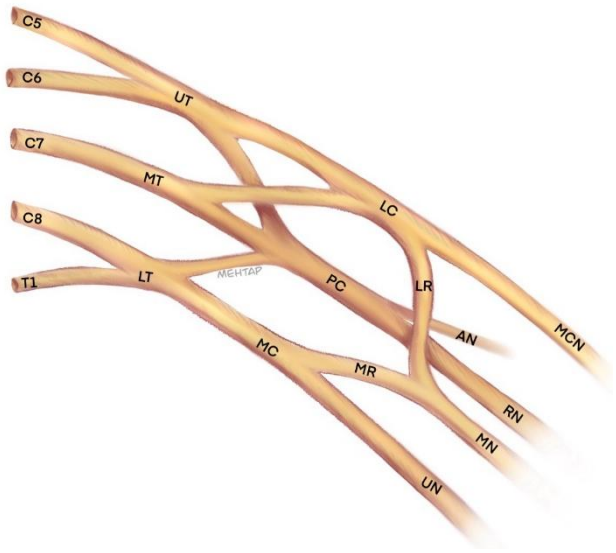


Figure 1. Diagram of brachial plexus (Image credits: Mehtap Erdogan)
(UT: Upper Trunk; MT: Middle Trunk; LT: Lower Trunk; MC: Medial Cord; PC: Posterior Cord; LC: Lateral Cord; MCN: musculocutaneous nerve; MN: median nerve; UN: ulnar nerve; LC: Lateral cord of brachial plexus; LR: Lateral root of the median nerve; MR: medial root of median nerve)

The embryological origin of these anatomical variations is based on the segmental organization and the environmental factors.[6] This variability during the embryonic period may affect the formation and distribution of the median nerve.[7] Variations may increase risks in surgical procedures and repair of nerve injuries and affect the efficacy of peripheral nerve blocks.[1,3]

This case aims to contribute to clinical practice and the prevention of surgical complications by better understanding the variants of the median nerve.

Case Report

A variant formation of the median nerve (MN) was observed in the left axilla and arm region of an adult male cadaver during dissection in Sakarya University Faculty of Medicine Anatomy Laboratory.
In the cadaver we examined, the median nerve was formed by the merger of three roots: the first lateral root of the median nerve (LR1), the second lateral root of the median nerve (LR2) and the medial root of the median nerve (MR). LR1 and LR2 originated from the lateral cord and MR from the medial cord. After LR1, LR2 and MR met anterior to the axillary artery, MN then continued along the arm in its normal course, passing anterior to the brachial artery (Figure 2). On the other hand the other side MN (of the right upper extremity) had no variation different from the population. No previous surgical intervention or anomaly was found in the cadaver.

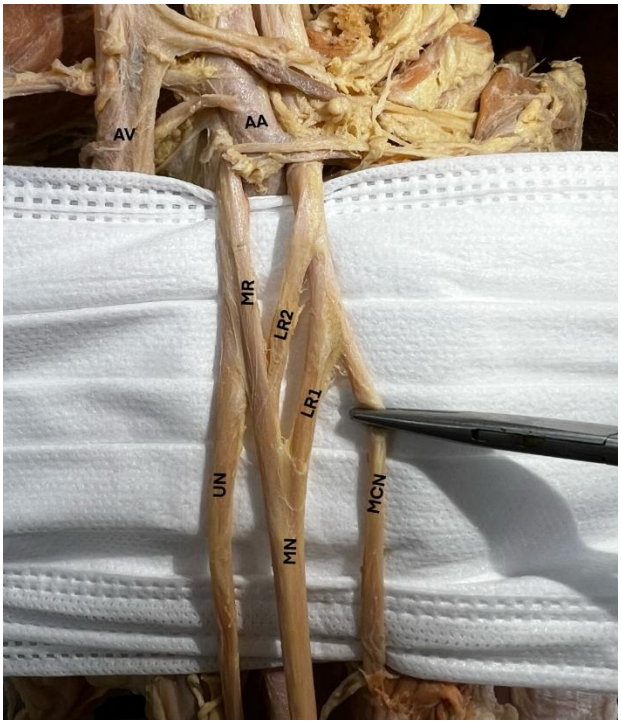


Figure 2. Dissection of the anterior compartment of left axilla showing variant formation of median nerve
(MCN: musculocutaneous nerve; MN: median nerve; UN: ulnar nerve; LC: Lateral cord of brachial plexus; LR1: First lateral root of the median nerve; LR2: Second lateral root of median nerve; MR: medial root of median nerve; AA: Axillary artery; AV: Axillary vein)

Remarkably, the high level of origin of the deep brachial artery observed in some literature cases was not detected in this cadaver.

Discussion

Although the anatomists, clinicians may come across many variations in the formation of the MN, MN is mostly reported to consist of 2 roots (1 lateral and 1 medial). Like we detected, there are also variations in which it may consist of 2 lateral and 1 medial root.

MN variations result from segmental changes in the embryonic development of the plexus brachialis. The three-rooted median nerve formation observed in our study is similar to previously described variations in the literature. However, in this case, no accompanying vascular anomaly was observed and the vascular structures were found to conform to classical anatomy. Like our finding, Sontakke et al.[8] Sontakke et al. also reported the formation of a median nerve with three roots, including two lateral and one medial roots[8]. Besides no variation of the deep brachial artery and the vascular structures were found in our case.

Pandey[1] and Uzun[5] described many variations related to brachial plexus formation and stated that most variations are due to the connections between the musculocutaneous nerve and MN.[1,5] Uzun et al. emphasised the presence of conduction branches between the musculocutaneous nerve and MN, and stated that this may increase the risk of surgical complications.[5] The presence of such transmission branches was not observed in this case, but this may vary in different populations.[3]

In a case reported by Morimoto et al. 2015, proximal entrapment of the median nerve and course variations of the axillary artery were observed together and it was emphasised that such variations may lead to nerve compression[9] Nonthasaen et al. have analysed the variational relationships between the axillary artery and median nerve in detail and emphasised the importance of considering these relationships in surgical interventions.[10]

In conclusion, the median nerve variations should be considered in clinical applications, especially in surgical procedures, nerve blockades and peripheral nerve injuries. Embryological development and segmental changes affect the median nerve formation leading to clinically significant consequences.[6,7] These variations may pose a risk for nerve compression syndromes, surgical complications and nerve injuries and should be considered in clinical diagnosis and treatment processes.[1,3]

In the light of all these findings, consideration of brachial plexus variations in clinical practice reduces the risk of complications, especially in cases such as nerve blocks, peripheral nerve surgery and upper extremity trauma.

The clinical significance of such variations is especially important in axillary surgeries, post-traumatic reconstruction and nerve block applications. The position of the roots and their relationship with the axillary artery and its branches may predispose to vascular or neurological compression syndromes.

Compliance with Ethical Standards

The Institutional Review Board of Kocaeli University granted ethical permission for this investigation (Approval Code: KOÜ GOKAEK-2019, Project Identifier: 2019/269). All procedures were conducted in compliance with the principles outlined in the Declaration of Helsinki.

Conclusion

Variant formations of the median nerve should be considered in upper extremity surgery, radiological diagnosis and evaluation of peripheral nerve injuries. Three-rooted median nerve variations are rarely detected in routine dissections and may lead to results that may affect clinical practice.

Compliance with Ethical Standards

This case report is based on an anatomical variation observed in a formalin-fixed cadaver during routine dissection practice. According to institutional and national guidelines, this type of study does not require ethical committee approval. Nevertheless, all procedures were conducted in accordance with ethical and professional standards.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

ME: Study design, dissection, photography, and literature review; ME and HB: Writing the first draft of the article and manuscript revision; KK and HB: Review of the manuscript with focus on formatting and layout corrections; EMG and KK: Critical reading and providing feedback on the manuscript.

Financial Disclosure

None.

References

1. Pandey SK, Shukla VK. Anatomical variations of the cords of brachial plexus and the median nerve. *Clin Anat.* 2007;20(2):150-156. doi:10.1002/ca.20365
2. Budhiraja V, Rastogi R, Asthana AK. Anatomical variations of median nerve formation: embryological and clinical correlation*. J Morphol Sci.* 2011;28(4):283-286.
3. Ghosh B, Dilkash MNA, Prasad S, Sinha SK. Anatomical variation of median nerve: cadaveric study in brachial plexus. *Anat Cell Biol.* 2022;55(2):130-134. doi:10.5115/acb.22.022
4. Choi D, Rodríguez-Niedenführ M, Vázquez T, Parkin I, Sañudo JR. Patterns of connections between the musculocutaneous and median nerves in the axilla and arm. *Clin Anat.* 2002;15(1):11-17. doi:10.1002/ca.1085
5. Uzun A, Seelig LL Jr. A variation in the formation of the median nerve: communicating branch between the musculocutaneous and median nerves in man. *Folia Morphol (Warsz).* 2001;60(2):99-101.
6. Larsen WJ. Human Embryology. 3rd Edition. New York, USA: Churchill Livingstone; 2001.

7. Sanes JR, Reh TA, Harris WA. Development of the Nervous System. 3rd Edition. Amsterdam, Netherlands: Elsevier; 2012.
8. Sontakke BR, Tarnekar AM, Waghmare JE, Ingole IV. An unusual case of asymmetrical formation and distribution of median nerve. *Int J Anat Var.* 2011;4:57-60.
9. Morimoto D, Isu T, Kim K, Sugawara A, Isobe M, Morita A. Proximal entrapment neuropathy of the median nerve above the elbow-case report. *J Nippon Med Sch.* 2015;82(6):287-289. doi:10.1272/jnms.82.287
10. Nonthasaen P, Chaimongkhol T, Chobpenthai T, Mahakkanukrauh P. Anatomical variations and surgical implications of axillary artery branches: an anatomical study of the coracoid process region. *Anat Cell Biol*. 2025;58(1):35-43. doi:10.5115/acb.24.215

doi: 10.53446/actamednicomedia.1474539

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Review | Derleme

A GENETIC DISEASE BEHIND OBESITY AND ITS NUTRITIONAL TREATMENT; PRADER-WILLI SYNDROME

OBEZİTENİN ARKASINDAKİ GENETİK HASTALIK VE BESLENME TEDAVİSİ; PRADER-WİLLİ SENDROMU

Gokcen Dogan[1], Aylin Bulbul[2*]

[1]Lokman Hekim University, Health Sciences Faculty, Department of Nutrition and Dietetics, Türkiye. [2]Ankara University, Health Sciences Institute, Department of Nutrition and Dietetics, Ankara, Türkiye.

ABSTRACT

Obesity is a health problem that reduces quality of life and is associated with many chronic diseases. It has multiple causes, including physiological, socio-cultural, psychological, and genetic factors. Prader-Willi Syndrome (PWS) is the most common genetic disorder associated with obesity. It is characterized by severe anorexia in infancy and hyperphagia during childhood. The hyperphagia that begins in childhood is the main cause of obesity in PWS. Hyperphagia occurs because of an imbalance in the hunger-satiety metabolism. Due to elevated levels of the hormone ghrelin, a sense of satiety cannot be achieved. The aim of the treatments applied is to prevent obesity. Medical nutrition therapy is considered the most effective treatment method. To prevent obesity in individuals with PWS, lifelong adherence to nutritional therapy is required.

Keywords: Prader-Willi Syndrome, obesity, medical nutrition therapy

ÖZ

Obezite yaşam kalitesini azaltan, birçok kronik hastalıkla ilişkilendirilen bir sağlık sorunudur. Obezitenin fizyolojik, sosyo-kültürel, psikolojik, genetik birçok sebebi bulunmaktadır. Prader-Willi Sendromu (PWS) obezite ile ilişkilendirilen en yaygın genetik hastalıktır. Bebeklik döneminde şiddetli anoreksi, çocukluk döneminde hiperfaji ile karakterizedir. Çocukluk dönemi itibariyle başlayan hiperfaji PWS'de obezite oluşumunun temelidir. Hiperfaji açlık-tokluk metabolizmasındaki dengenin bozulması sonucu oluşmaktadır. Artan grelin hormonu seviyeleri sebebiyle tokluk hissi oluşamamaktadır. Uygulanan tedavilerin amacı obezitenin önlenmesine yöneliktir. En etkili tedavi yönteminin tıbbi beslenme tedavisi olduğu belirtilmektedir. PWS'de obezitenin önüne geçilebilmesi için yaşam boyu beslenme tedavisine devam edilmesi gerekmektedir.

Anahtar Kelimeler: Prader-willi sendromu, obezite, tıbbi beslenme tedavisi

***Corresponding author/İletişim kurulacak yazar:** Aylin Bulbul; Ankara University, Health Sciences Institute, Department of Nutrition and Dietetics, 06610, Ankara, Türkiye.
Phone/Telefon: +90 (539) 647 52 10, **e-mail/e-posta:**aylinbulbul98@gmail.com
Submitted/Başvuru: 27.04.2024 • **Accepted/Kabul:** 16.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Prader-Willi Syndrome (PWS) is a genetic disorder characterized by feeding difficulties and severe hypotonia in early infancy, followed by excessive eating and loss of control overeating behavior in in boys during childhood. The prevalence of the disease is reported to range between 1/10,000 and 1/30,000. Individuals with PWS exhibit delayed development of language and motor skills, along with mild cognitive impairment. Anger outbursts and manipulative behaviors are commonly observed phenotypic features accompanying these symptoms. Clinical manifestations include hypogonadism, genital hypoplasia, delayed pubertal development, and infertility affecting both sexes. Additionally, short stature due to growth hormone deficiency is commonly seen. The clinical features of PWS were first described first described in 1887 as increased adipose tissue. In 1956, Prader, Labhart, and Willi, who named the disease, made a definition that included clinical features such as cognitive impairment and hypogonadism along with obesity.[1,2] Prader-Willi Syndrome is associated with developmental disabilities and genetic abnormalities, such as lack of expression of genes inherited from the paternal chromosome 15q11-q13 region.[3] This lack of gene expression contributes to the unique clinical features of PWS, including hypotonia, short stature, and obesity.[4]

The excessive eating behavior that develops in conjunction with Prader-Willi syndrome leads to the formation of obesity. Consequently, comorbidities of obesity, such as insulin resistance, type II diabetes mellitus, and sleep disorders, are frequently observed in individuals with Prader-Willi syndrome.[5] Obesity formation in Prader-Willi syndrome is attributed to disruptions in hypothalamic control pathways and imbalance in hunger-satiety metabolism hormones. Despite research efforts investigating the genetic basis of obesity, there are relatively few studies exploring the relationship between Prader-Willi syndrome and obesity. This review aims to investigate the relationship between Prader-Willi syndrome—the most common genetic cause of obesity—and obesity itself.[6]

Clinical Findings in Prader-Willi Syndrome

Characteristic facial features such as almond-shaped eyes, low-set ears, and a small mouth are observed in individuals with PWS. In addition to these facial features, clinical findings include infantile central hypotonia, feeding difficulties in infancy, growth retardation, hypogonadism, hyperphagia (excessive eating), and rapid weight gain after infancy.[7] The age-dependent clinical findings in PWS are presented in Table 1.

Hypotonia: In PWS, signs of hypotonia can be detected as early as the fetal period. It is often associated with decreased fetal movements and an increased likelihood of cesarean delivery. In infancy, it results in reduced reflexes, lack of mobility, decreased response to spontaneous stimuli, feeding difficulties, and failure to achieve necessary weight gain. Mild to moderate hypotonia persists throughout the life of individuals with Prader-Willi syndrome.[8]

Table 1. Clinical Findings in Prader-Willi Syndrome.

Age Range (year)	Clinical Findings
0-2	• Inadequate sucking reflex • Hypotonia
2-6	• Inadequate sucking reflex • Hypotonia
6-12	• Hypotonia • Growth retardation • Obesity due to excessive eating
≥13	• Cognitive impairment • Uncontrolled eating behavior • Hypothalamic hypogonadism and/or typical behavioral issues

Hypogonadism: Congenital hypogonadism is a commonly encountered clinical manifestation in PWS. It results from inadequate secretion of gonadal steroids due to deficiency in pituitary gonadotropins, including Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). Cryptorchidism is found in the majority of male children with Prader-Willi syndrome.[9,10]

Growth Retardation: Individuals with Prader-Willi syndrome (PWS) commonly experience developmental delays in motor skills. The prevalence of this delay is reported to range between 90-100%. In the majority of children with PWS, the acquisition of sitting, walking, and speaking skills occurs much later compared to their peers. When examining the IQ scores of PWS individuals, it is noted that the majority have a mild level of intellectual disability (IQ: 60-70). Regardless of IQ levels, children with PWS often struggle with learning difficulties, and their academic skills are generally weak.[11]

Diagnostic Criteria

Classic neonatal findings of PWS include central hypotonia and decreased deep tendon reflexes. Additional features are feeding difficulty, weak crying, decreased fetal movements, and characteristic facial features. Diagnostic criteria were developed by Holm et al. in 1993. Minor and major criteria are shown in Table 2. For diagnosing PWS, major criteria are assigned 1 point each, while minor criteria are assigned 0.5 points. Between the neonatal period and age 3, a total score of 5 or more, including at least four major criteria, is considered diagnostic. Between ages 3 and adolescence, a score above 8, including at least five major criteria, is diagnostic.[12]

Prader-Willi Syndrome and Obesity

Obesity is the most significant health issue in PWS. It is a risk factor for many diseases such as heart disease, atherosclerosis, and diabetes. Obesity develops in conjunction with excessive eating after anorexia experienced in infancy.[13] In Prader-Willi syndrome, individuals often exceed the ideal body weight

significantly, and if left untreated, obesity-related complications such as cardiopulmonary disorders, hypertension, and diabetes mellitus can result in significant morbidity and mortality. In PWS, the body fat percentage can reach 40–50%, markedly exceeding normal ranges.[13]

Table 2. Diagnostic Criteria for PWS.

Criteria	Score
Major Criteria • Infantile central hypotonia • Characteristic facial findings (dolichocephaly, narrow bifrontal diameter, almond eyes, downward-turned lips, small mouth) • Feeding difficulties in infancy • Developmental delay • Hypogonadism (hypoplasia of the scrotum, undescended testicle, small penis or testicle in boys; hypoplasia of the labia minora or clitoris in girls) • Hyperphagia • Rapid weight gain between the ages of 1-6	1-point for each
Minor Criteria • Decreased fetal movements and lethargy in the intrauterine period. • Esotropia, myopia • Small hands and feet • Short stature (compared to family members) • Hypopigmentation • Sleep disorder or apnea • Articulation defect • Viscous secretion • Behavior problems	0.5 point for each

In PWS, obesity arises due to hyperphagia, reduced satiety perception, and loss of appetite control. The combination of excessive caloric intake, reduced energy expenditure, and insufficient physical activity leads to morbid obesity. The use of appetite suppressant medications to control hyperphagia has not yet been proven effective in preventing obesity in PWS.[14] The physiological mechanisms involved in the formation of obesity include disturbances in limbic-hypothalamic pathways responsible for satiety control and changes in hormones regulating food intake.[15]

A persistent increase in plasma ghrelin is associated with an increase in appetite and food intake through central regulatory mechanisms in the hypothalamus. Decreased plasma Pancreatic Polypeptide (PP) and Peptide YY (PYY) play a role in the loss of satiety control. Growth hormone deficiency and hypogonadism lead to a decrease in muscle mass and an increase in body fat. Central hypothyroidism results in decreased energy expenditure.[16] Figure 1 illustrates the physiological mechanisms involved in the formation of obesity in PWS.

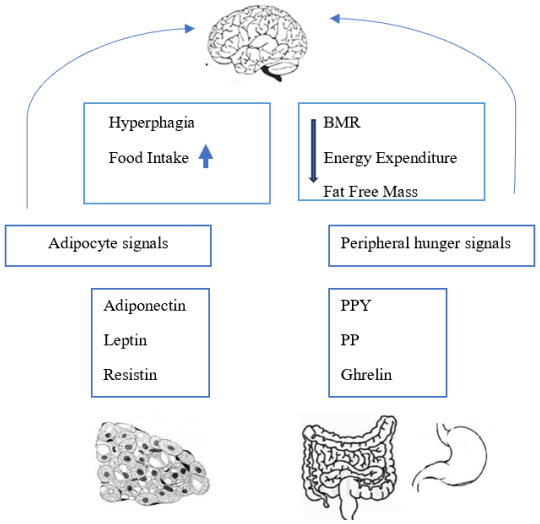


Figure 1. Physiological mechanisms of obesity in PWS.

Central Hormones and Peptides Playing a Key Role in Obesity Formation in Prader-Willi Syndrome

Ghrelin: Ghrelin, known as the hunger hormone, is secreted by the stomach. The release of ghrelin stimulates appetite and leads to food intake, after which blood ghrelin levels typically decrease. In Prader-Willi syndrome, it has been determined that ghrelin levels are above normal and show a continuous increase.[17-19] Additionally, A study evaluating ghrelin levels before and after food intake in individuals with PWS found no postprandial decrease in ghrelin levels.[20] Elevated ghrelin levels in PWS contribute to hyperphagia and are believed to play a fundamental role in the development of obesity.[19]

Pancreatic Polypeptide (PP) and Peptide YY (PYY): Pancreatic Polypeptide and Peptide YY are anorexigenic hormones released by the intestine that promote satiety and inhibit food intake.[21] In children with PWS, decreased levels of PP have been identified, indicating a lack of satiety.[22] Moreover, studies have found no difference in the brain expression patterns of ghrelin, PYY, or their receptors between individuals with and without PWS.[23]

Leptin: Leptin is a peptide produced by adipose tissue and plays a role in regulating appetite and fat storage. Released by adipocytes in response to signals of satiety, leptin inhibits neuropeptide Y neurons, reducing food intake and energy metabolism.[24] In a study by Goldstone et al, fasting leptin levels in individuals with PWS (n=42, aged 7 months to 5 years) were found to be significantly higher compared to age, gender, and body mass index-matched controls (n=9).[22] However, another study did not find a significant relationship between leptin levels and obesity in individuals with PWS or healthy controls.[25] The impact of leptin on appetite metabolism in PWS has not been conclusively established, and there are studies with varying results on this topic.

Adiponectin: Adiponectin is a peptide produced by adipose tissue and plays a role in regulating fat storage.

It has significant effects on increasing insulin sensitivity. In a study, serum adiponectin levels were found to be significantly lower in individuals with PWS who were obese compared to those who were lean.[26] It has been determined that adiponectin increases insulin sensitivity in Prader-Willi syndrome.[27] In another research, it was concluded that children with PWS have a lower likelihood of developing diabetes compared to a healthy control group with similar body mass index levels, attributed to the higher secretion of adiponectin in individuals with PWS.[28]

Nutritional Therapy in Prader-Willi Syndrome

Nutritional therapy for patients with PWS is typically structured as a standard two-stage process, based on the clinical course. The first stage (up to 18 months of age) addresses the period characterized by poor nutrition, often accompanied by hypotonia and growth retardation. In the second stage, the focus shifts to addressing obesity resulting from hyperphagia. During this stage, caloric intake is restricted to 60–80% of the Recommended Dietary Allowance (RDA) to prevent obesity caused by hyperphagia in children.[29]

The lack of satiety and hyperphagia is one of the most significant challenges affecting individuals with Prader-Willi Syndrome and their families. Individuals with PWS face numerous chronic diseases that pose a life-threatening risk due to the potential for obesity. Achieving and maintaining proper body weight is crucial in the treatment of Prader-Willi Syndrome. It is suggested that approximately 60% of the energy intake requirement is sufficient to achieve the necessary weight loss in these children.[13]

During adulthood, the recommended calorie intake to maintain weight varies among individuals but is generally advised to be between 1000-1200 kcal/day. For children, the recommended dietary treatment to achieve weight loss should be around 75-80% of their age-specific requirements. In cases of hyperphagia, energy intake needs to be restricted. The content of implemented diets should be balanced, including complex carbohydrates and being rich in fiber.[13] Without early, intensive nutritional therapy along with behavioral modification, PWS patients develop severe obesity associated with type 2 diabetes, obstructive sleep apnea, right-side heart failure, and other obesity-related metabolic complications.[30]

Dietary variety should be increased, with a particular emphasis on regular consumption of vegetable dishes and salads, while maintaining portion control with fruits. Increased water consumption is essential. Portion sizes should be reduced, and smaller plates should be used for serving to enhance visual appeal. The introduction of packaged products (biscuits, candies, etc.) to children with PWS should be delayed as much as possible. Food restrictions should not be used as a punitive measure, and no food item should be given as a reward to the child. PWS children should be encouraged to increase physical activity, including exercise, dance, and other calorie-burning activities. They should be kept away from stimuli that might encourage overeating. Establishing a daily routine that aids weight control is crucial, and its continuity should be ensured. Relatives and friends of individuals with PWS should be informed about the implemented diet plan, and they should be prevented from directing PWS individuals toward non-compliant eating habits. It should be communicated to the child with Prader-Willi Syndrome that giving them food "secretly" is not an expression of love; on the contrary, it negatively impacts the child's dietary regimen and health. While implementing dietary therapy, children should be educated in detail about what to consider in food choices, and if there is a deviation from the daily routine, the child should be informed in advance. Various activities should be organized to reduce repetitive food requests.[31]

The effectiveness of pharmacological treatments for Prader-Willi Syndrome has not been fully proven. Placebo-controlled studies of anorexigenic agents have shown no significant impact on hyperphagia. The effectiveness of endocannabinoid receptor agonists and various treatment approaches is still under investigation.[32,33]

Carbohydrate

Carbohydrates are organic compounds composed of carbon, oxygen, and hydrogen, serving as a primary source of energy for the body and being the most abundant nutrient in our diet. In a study focusing on carbohydrate intake ranging from 50% to 70%, with 12 grams or less of fiber, it was revealed that individuals with Prader-Willi Syndrome (PWS) could benefit from a well-balanced, energy-restricted diet with lower carbohydrate consumption and higher dietary fiber intake. It is known that individuals with PWS tend to prefer simple carbohydrates. However, excessive intake of simple carbohydrates, which are high in energy but low in nutrients, can lead to obesity. Therefore, while emphasizing the need to reduce energy intake in individuals with PWS, a healthy and balanced approach is recommended by providing complex carbohydrates. Considering the potential fluctuations in plasma glucose levels caused by simple carbohydrates, it is also suggested in the nutrition program to prefer carbohydrates with low glycemic index and load to avoid such fluctuations.[34]

In a study conducted by Irizarry et al., one group was given a low-carbohydrate diet (15% carb; 65% fat; 20% protein), while the other group received a low-fat diet (65% carb, 15% fat, 20% protein). In the study, subjects consuming the LF diet had lower postprandial insulin concentrations ($p<0.05$); and higher fasting GLP-1 ($p<0.05$).[35]

Dietary fiber is thought to be beneficial in body weight control due to its effect on the intestinal microbiota. Studies also emphasize dietary fiber intake in PWS.[36,37] In a study conducted by Zhang et al. on 38 obese children with PWS, they showed that a diet rich in indigestible carbohydrates had significant contributions to both weight loss and dysbiosis.[37]

Protein

Protein intake is crucial for individuals with Prader-Willi syndrome due to the unique challenges they face, such as obesity and altered energy metabolism. Adequate protein intake has an important role in increasing satiety. Proper protein intake is important in PWS patients with lean mass deficiencies.[38]

Butler et al.[32] conducted study that a comparison of two different obesity treatments for children with Prader-Willi syndrome. In this study, a group of 33 individuals followed a diet consisting of 45% of energy intake from carbohydrates (with a minimum of 20 grams of fiber per day), 25% from protein, and 30% from fats. The diet of another group of 30 individuals included 50%-70% carbohydrates (≤12 grams of fiber per day), 10%-23% fats, and 15%-20% proteins. Significant and higher losses in both body fat and body weight were observed in the first group of children.

The study, based on the premise that meals with high protein content increase energy expenditure, examined the postprandial energy expenditure of individuals with Prader-Willi syndrome consuming different meals. Isocaloric breakfasts were planned for 5 individuals with Prader-Willi syndrome aged between 10 and 25. One of these breakfasts contained 15% protein, while the other contained 50% protein. Upon examining the energy expenditure after consuming these two different meals, no significant difference was found.[39]

Fat

Fat intake, which is one of the essential nutrients for human life, ensures the intake of fat-soluble vitamins in the diet and the consumption of essential fatty acids that contribute to the structure of phospholipids in cell membranes. Different dietary models with varying levels of fat content are also being studied for Prader-Willi syndrome.[40]

Teke Kısa et al.[41] conducted a study investigating the impact of a ketogenic diet on weight management in children with Prader-Willi syndrome. In this study, 10 children followed a ketogenic diet for a minimum of 6 months. The diet was composed of 75%-85% of energy from fats, 15%-25% from protein, and carbohydrates. The median BMI SD score before diet intervention was 3.05 [−0.21–3.72], whereas it was 0.41 [−0.87–1.57] at the final evaluation ($p = 0.002$). While larger population studies are needed to determine the effectiveness of the ketogenic diet on body weight, it should be noted that it may have different side effects, such as hypercholesterolemia.

Bariatric surgery

The efficacy of restrictive bariatric procedures, such as gastric banding or bypass, on hyperphagia and long-term weight loss in individuals with PWS remains inconclusive.[42,43] While successful weight loss has been achieved with the use of biliopancreatic diversion, complications arising from intestinal absorption issues have been observed. Bariatric surgical procedures are generally recommended only in cases where excessive weight poses a life-threatening risk. Postoperative dietary control after bariatric surgery requires continuous and careful monitoring to ensure long-term success.[44]

New Approaches to Therapy in Prader-Willi Syndrome

Using belaronide to prevent hyperphagia

The PWS research community is highly interested in a medical agent that can decrease hyperphagia. Beloranib, an irreversible inhibitor of methionine aminopeptidase 2 (MetAP2), has garnered considerable attention as a potential treatment. MetAP2 inhibitors were previously utilized in cancer treatment due to their capability to slow endothelial cell growth and reduce angiogenesis. At lower doses, below those needed to inhibit angiogenesis and tumor growth, MetAP2 inhibitors have been shown to reduce food intake, body weight, and adipose tissue mass. Although the precise mechanism behind weight loss and reduced appetite are not fully understood, MetAP2 inhibitors lead to triglyceride lipolysis, fatty acid oxidation, ketogenesis, and suppression of food intake via alterations in the extracellular signal-related kinase stress kinase pathway.[45]

Using Co-enzyme Q10 (CoQ10) and Carnitine to increase energy expenditure

PWS is distinguished by infantile hypotonia, sarcopenia, and reduced resting energy expenditure. Some of these characteristics are shared with other conditions characterized by low levels of co-enzyme Q10 (CoQ10), an essential component of the mitochondrial respiratory chain and an electron carrier. Studies have indicated lower CoQ10 levels in both PWS and obese children compared to healthy non-obese controls. While CoQ10 is frequently used as a supplementary treatment in PWS without reported adverse effects, its effectiveness in enhancing motor development and metabolic function remains uncertain. Some reports suggest that CoQ10 supplementation may increase daytime alertness.[46]

Similar to CoQ10 deficiency, carnitine deficiency is linked to hypotonia, inadequate growth, and easy fatigability. Interestingly, unlike CoQ10, carnitine levels are higher in PWS individuals compared to healthy controls, indicating impaired carnitine utilization in PWS. Evidence regarding the beneficial effects of carnitine supplementation remains inconclusive. A recent study administered carnitine at a dosage of 25 mg/kg twice daily to twenty subjects; thirteen reported improved exercise tolerance and daytime alertness, while seven reported no benefits.[47]

Conclusions

Prader-Willi Syndrome is the most common genetic disorder underlying obesity, primarily due to impaired satiety signaling, which leads to hyperphagia. The increased rates of mortality and morbidity in PWS are attributed to coexisting diseases accompanying obesity. Therefore, preventing obesity is crucial for improving quality of life and preserving health in individuals with PWS. The most effective method used in the treatment

of obesity in PWS is nutritional therapy. Creating a balanced diet plan and ensuring its continuity are essential for controlling the disease.

Detecting endocrine disorders and applying replacement therapy for hormone deficiencies are crucial. Additionally, increasing physical activity significantly enhances the effectiveness of dietary interventions. A multidisciplinary treatment approach should be implemented for individuals with PWS, with dietary therapy supervised by a qualified dietitian. Caregivers, teachers, and family members should be thoroughly educated about PWS and the treatment strategies being implemented.

Conflicts of Interest

There is no conflict of interest between the authors.

Financial support

This study was not supported by any organization.

Author Contribution

GD, AB: Design, literature review and data collection, writing of the study; GD, AB: Analysis and interpretation, literature review, writing of the study.

References

1. Woodcock K, Oliver C, Humphreys GW. The relationship between specific cognitive impairment and behavior in Prader–Willi syndrome. *J Intellect Disabil Res.* 2011;55(2):152-171. doi:10.1111/j.1365-2788.2010.01368.x
2. Butler MG, Bittel DC, Kibiryeva N, Garg U. C-reactive protein levels in subjects with Prader-Willi syndrome and obesity. *Genet Med.* 2006;8(4):243-248. doi:10.1097/01.gim.0000204469.30913.67
3. Butler MG, Miller J, Forster JL. Prader-Willi syndrome—clinical genetics, diagnosis and treatment approaches: an update. *Curr Pediatr Rev.* 2019;15(4):207-244. doi:10.2174/1573396315666190716120925
4. Costeff H, Holm V, Ruvalcaba R, Shaver J. Growth hormone secretion in Prader-Willi syndrome. *Acta Paediatr.* 1990;79(11):1059-1062. doi:10.1111/j.1651-2227.1990.tb11383.x
5. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med.* 2012;14(1):10-26.
6. Muscogiuri G, Formoso G, Pugliese G, Ruggeri RM, Scarano E, Colao A. Prader-Willi syndrome: an uptodate on endocrine and metabolic complications. *Rev Endocr Metab Disord.* 2019;20(2):239-250.
7. Driscoll DJ, Miller JL, Schwartz S, Cassidy SB. Prader-Willi syndrome. *GeneReviews®*. https://www.ncbi.nlm.nih.gov/books/NBK1330/
8. Gunay-Aygun M, Schwartz S, Heeger S, O'Riordan MA, Cassidy SB. The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. *Pediatrics.* 2001;108(5):92.
9. Vogels A, Moerman P, Frijns J-P, Bogaert GA. Testicular histology in boys with Prader-Willi syndrome: fertile or infertile? *J Urol.* 2008;180(4S):1800-1804.
10. Angulo M, Butler M, Cataletto M. Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. *J Endocrinol Invest.* 2015;38(12):1249-1263.
11. Whittington J, Holland A, Webb T, et al. Academic underachievement by people with Prader–Willi syndrome. *J Intellect Disabil Res.* 2004;48(2):188-200.
12. Holm VA, Cassidy SB, Butler MG. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics.* 1993;91:398-402.
13. Butler MG. Prader-Willi syndrome: current understanding of cause and diagnosis. *Am J Med Genet.* 1990;35(3):319-332.
14. Chen KY, Sun M, Butler MG, Thompson T, Carlson MG. Development and validation of a measurement system for assessment of energy expenditure and physical activity in Prader-Willi syndrome. *Obes Res.* 1999;7(4):387-394.
15. Hill JO, Kaler M, Spetalnick B, Reed G, Butler MG. Resting metabolic rate in Prader-Willi syndrome. *Dysmorphol Clin Genet.* 1990;4(1):27.
16. Muscogiuri G, Barrea L, Faggiano F, et al. Obesity in Prader–Willi syndrome: physiopathological mechanisms, nutritional and pharmacological approaches. *J Endocrinol Invest.* 2021;44(10):2057-2070.
17. Butler MG, Bittel DC, Talebizadeh Z. Plasma peptide YY and ghrelin levels in infants and children with Prader-Willi syndrome. *J Pediatr Endocrinol Metab.* 2004;17(9):1177-1184.
18. Feigerlová E, Diene G, Conte-Auriol F, et al. Hyperghrelinemia precedes obesity in Prader-Willi syndrome. *J Clin Endocrinol Metab.* 2008;93(7):2800-2805.
19. Prodam F, Bellone S, Grugni G, et al. Influence of age, gender, and glucose tolerance on fasting and fed acylated ghrelin in Prader-Willi syndrome. *Clin Nutr.* 2009;28(1):94-99.
20. Gumus Balikcioglu P, Balikcioglu M, Muehlbauer MJ, et al. Macronutrient regulation of ghrelin and peptide YY in pediatric obesity and Prader-Willi syndrome. *J Clin Endocrinol Metab.* 2015;100(10):3822-3831.
21. Small CJ, Bloom SR. Gut hormones and the control of appetite. *Trends Endocrinol Metab.* 2004;15(6):259-263.
22. Goldstone A, Holland A, Butler J, Whittington J. Appetite hormones and the transition to hyperphagia in children with Prader-Willi syndrome. *Int J Obes.* 2012;36(12):1564-1570.
23. Talebizadeh Z, Kibiryeva N, Bittel DC, Butler MG. Ghrelin, peptide YY and their receptors: gene expression in brain from subjects with and without Prader-Willi syndrome. *Int J Mol Med.* 2005;15(4):707-711.
24. Pijl H, Toornvliet A, Meinders A. Serum leptin in normal-weight and obese humans. *N Engl J Med.* 1996;334(23):1544.
25. Goldstone AP, Brynes AE, Thomas EL, et al. Resting metabolic rate, plasma leptin concentrations, leptin receptor expression, and adipose tissue measured by whole-body magnetic resonance imaging in women with Prader-Willi syndrome. *Am J Clin Nutr.* 2002;75(3):468-475.
26. Bittel DC, Butler MG. Prader–Willi syndrome: clinical genetics, cytogenetics and molecular biology. *Expert Rev Mol Med.* 2005;7(14):1-20.
27. Haqq AM, Muehlbauer MJ, Newgard CB, et al. The metabolic phenotype of Prader-Willi syndrome (PWS) in childhood: heightened insulin sensitivity relative to body mass index. *J Clin Endocrinol Metab.* 2011;96(1):225-E232.
28. Goldstone AP, Thomas EL, Brynes AE, et al. Elevated fasting plasma ghrelin in Prader-Willi syndrome adults is not solely explained by their reduced visceral adiposity and insulin resistance. *J Clin Endocrinol Metab.* 2004;89(4):1718-1726.

29. Miller J, Lynn C, Driscoll D, et al. Nutritional phases in Prader–Willi syndrome. *Am J Med Genet A.* 2011;155(5):1040-1049. doi:10.1002/ajmg.a.33951
30. Kim S, Cho S, Jin D. Prader-Willi syndrome: an update on obesity and endocrine problems. *Ann Pediatr Endocrinol Metab.* 2021;26(4):227-236. doi:10.6065/apem.2142164.082
31. Crinò A, Fintini D, Bocchini S, Grugni G. Obesity management in Prader–Willi syndrome: current perspectives. *Diabetes Metab Syndr Obes.* 2018;11:579.
32. Goldstone AP. Prader-Willi syndrome: advances in genetics, pathophysiology and treatment. *Trends Endocrinol Metab.* 2004;15(1):12-20.
33. Shapira NA, Lessig MC, Lewis MH, Goodman WK, Driscoll DJ. Effects of topiramate in adults with Prader-Willi syndrome. *Am J Ment Retard.* 2004;109(4):301-309.
34. Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr.* 2000;71(6):1455-1461. doi:10.1093/ajcn/71.6.1455
35. Irizarry KA, Mager DR, Triador L, et al. Hormonal and metabolic effects of carbohydrate restriction in children with Prader-Willi syndrome. *Clin Endocrinol (Oxf).* 2019;90(4):553-561. doi:10.1111/cen.13933
36. Cronin P, Joyce SA, O'Toole PW, O'Connor EM. Dietary fibre modulates the gut microbiota. *Nutrients.* 2021;13:1655.
37. Zhang C, Yin A, Li H, et al. Dietary modulation of gut microbiota contributes to alleviation of both genetic and simple obesity in children. *EBioMedicine.* 2015;2:968-984.
38. Alsaif M, Elliot S, MacKenzie M, et al. Energy metabolism profile in individuals with Prader-Willi syndrome and implications for clinical management: a systematic review. *Adv Nutr.* 2017;8(6):905-915. doi:10.3945/an.117.016253
39. Alsaif M, Triador L, Colin-Ramirez E, et al. Effect of high-protein diet on postprandial energy expenditure in children with Prader-Willi syndrome: a pilot and feasibility study. *Curr Dev Nutr.* 2021;5(3).
40. Calcaterra V, Magenes VC, Destro F, et al. Prader–Willi syndrome and weight gain control: from prevention to surgery—a narrative review. *Children.* 2023;10(3):564.
41. Teke Kısa P, Güzel O, Arslan N, Demir K. Positive effects of ketogenic diet on weight control in children with obesity due to Prader-Willi syndrome. *Clin Endocrinol (Oxf).* 2023;98(3):332-341.
42. Papavramidis ST, Kotidis EV, Gamvros O. Prader-Willi syndrome–associated obesity treated by biliopancreatic diversion with duodenal switch. *J Pediatr Surg.* 2006;41(6):1153-1158.
43. Scheimann A, Butler M, Gourash L, Cuffari C, Klish W. Critical analysis of bariatric procedures in Prader-Willi syndrome. *J Pediatr Gastroenterol Nutr.* 2008;46(1):80.
44. Elena G, Bruna C, Benedetta M, Stefania DC, Giuseppe C. Prader-Willi syndrome: clinical aspects. *J Obes.* 2012;473941:13.
45. Joharapurkar AA, Dhanesha NA, Lewis MH, Goodman WK, Driscoll DJ. Inhibition of the methionine aminopeptidase 2 enzyme for the treatment of obesity. *Diabetes Metab Syndr Obes.* 2014:73-84.
46. Artuch R, Salviati L, Jackson S, Hirano M, Navas P. Coenzyme Q10 deficiencies in neuromuscular diseases. In: *Inherited Neuromuscular Diseases: Translation from Pathomechanisms to Therapies.* 2009:117-128.
47. Miller JL, Lynn CH, Shuster J, Driscoll DJ. Carnitine and coenzyme Q10 levels in individuals with Prader–Willi syndrome. *Am J Med Genet A.* 2011;155(3):569-573.

doi: 10.53446/actamednicomedia.1718546

Acta Medica Nicomedia

Cilt: 8 Sayı: 2 Haziran 2025 / Vol: 8 Issue: 2 June 2025
https://dergipark.org.tr/tr/pub/actamednicomedia

Review | Derleme

POTENTIAL USE OF QUANTUM IMAGING AND ARTIFICIAL INTELLIGENCE TECHNOLOGIES IN NEUROSURGERY

NÖROŞİRÜRJİDE KUANTUM GÖRÜNTÜLEME VE YAPAY ZEKÂ TEKNOLOJİLERİNİN POTANSİYEL KULLANIMI

Yahya Turan[1], Ayfer Turan[2]

[1]Primary VM Medicalpark Kocaeli Hospital, Department of Neurosurgery, Kocaeli, Turkiye. [2]Kocaeli Healty and Technology University, Faculty of Pharmacy, Kocaeli, Turkiye.

ABSTRACT

This study examines the current status and potential future areas of use of artificial intelligence and quantum technologies in neurosurgery within a conceptual framework. Artificial intelligence is widely used, especially in the analysis of imaging data, surgical planning and intraoperative decision support systems, and is rapidly being integrated into clinical practices. Quantum imaging technologies, on the other hand, attract attention with their capacity to provide higher resolution and lower radiation dose, but are currently limited to experimental and pilot studies.

The study reveals that artificial intelligence and quantum technologies have complementary properties in neurosurgery; it shows that hybrid approaches such as quantum artificial intelligence can increase clinical success by increasing accuracy, speed and predictive capacity in diagnosis and treatment processes. In addition, it is emphasized that elements such as ethical responsibilities, user education and interdisciplinary collaboration are of critical importance in the clinical integration of these technologies. As a result, artificial intelligence and quantum technologies are seen as important tools that will shape the future surgical practices in neurosurgery.

Keywords: Neurosurgery, artificial intelligence, quantum imaging.

ÖZ

Bu çalışma, nöroşirürjide yapay zekâ ve kuantum teknolojilerinin mevcut durumunu ve gelecekteki potansiyel kullanım alanlarını kavramsal bir çerçevede incelemektedir. Yapay zekâ, özellikle görüntüleme verilerinin analizinde, cerrahi planlama ve intraoperatif karar destek sistemlerinde yaygın olarak kullanılmakta ve klinik uygulamalara hızla entegre olmaktadır. Kuantum görüntüleme teknolojileri ise, daha yüksek çözünürlük ve daha düşük radyasyon dozu sağlama kapasitesiyle dikkat çekmekle birlikte, henüz deneysel ve pilot düzeyde çalışmalarla sınırlandırılmıştır.

Çalışma, yapay zekâ ve kuantum teknolojilerinin nöroşirürjide birbirini tamamlayıcı özelliklere sahip olduğunu ortaya koymakta; kuantum yapay zekâ gibi hibrit yaklaşımların, tanı ve tedavi süreçlerinde doğruluk, hız ve öngörü kapasitesini artırarak klinik başarıyı yükseltebileceğini göstermektedir. Ayrıca, bu teknolojilerin klinik entegrasyonunda etik sorumluluklar, kullanıcı eğitimi ve disiplinler arası iş birliği gibi unsurların kritik önemde olduğu vurgulanmaktadır. Sonuç olarak, yapay zekâ ve kuantum teknolojileri nöroşirürjide geleceğin cerrahi uygulamalarını şekillendirecek önemli araçlar olarak görülmektedir.

Anahtar Kelimeler: Nörosirürji, yapay zekâ, kuantum görüntüleme.

***Corresponding author/İletişim kurulacak yazar:** Yahya Turan; Primary VM Medicalpark Kocaeli Hospital, Department of Neurosurgery, Kocaeli, Türkiye.
Phone/Telefon: +90 (505) 588 38 76, **e-mail/e-posta:** dryahyaturan@hotmail.com
Submitted/Başvuru: 12.06.2024 • **Accepted/Kabul:** 16.06.2025 • **Published Online/Online Yayın:** 30.06.2025

Introduction

Neurosurgery is a medical specialty involving complex surgical interventions targeting the central nervous system, characterized by high risk and requiring precision. One of the most critical determinants of surgical success is the quality of imaging before and during the procedure. Conventional imaging techniques, such as magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET), have long been widely used in neurosurgery; however, limitations in resolution and temporal accuracy in these systems have increased the need for more advanced technologies.[1]

In response to this need, quantum imaging techniques, which have emerged in recent years, hold revolutionary potential in neurosurgery. Based on principles such as quantum entanglement, squeezing, and superposition, these techniques utilize photons to provide higher contrast and resolution, while also enabling more accurate and lower-dose imaging of biological tissues.[2] Especially when dealing with complex structures like neural tissue, the impact of quantum imaging systems on early diagnosis and surgical precision is expected to become clearer in the near future.[3]

Moreover, the increasing role of artificial intelligence (AI) systems alongside quantum technologies in neurosurgery is noteworthy. Deep learning and machine learning algorithms enhance clinical decision support systems in areas such as tumor segmentation, aneurysm detection, and surgical risk assessment, thereby reducing error rates.[4,5] The combination of quantum computing and AI has led to the emergence of a new discipline called "quantum artificial intelligence" (Quantum AI), which provides a theoretical framework that may enable faster, more accurate, and more predictable interventions in neurosurgical procedures.[6]

This study aims to systematically review the current literature on the potential applications of quantum imaging techniques and artificial intelligence in the field of neurosurgery. Within this context, both the fundamental principles of technological advancements and their clinical applicability will be discussed. Furthermore, innovative approaches that may arise from the integration of these two fields in the future will be highlighted.

The Transformation of Imaging Technologies in Neurosurgery

Neurosurgery is a specialized field encompassing high-risk surgical interventions performed on the central nervous system, one of the most complex and delicate structures of the human body. In procedures involving the brain, spinal cord, and peripheral nervous system, imaging technologies undoubtedly constitute a fundamental determinant of surgical success. High-resolution, low-risk, and reliable visualization methods are required at every stage—from diagnosis to treatment, from surgical planning to intraoperative guidance.[7] In line with this need, the primary imaging techniques traditionally employed in neurosurgical applications have been computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). These methods have long served as essential tools for surgeons in evaluating anatomical structures and guiding operations.[8]

However, conventional imaging systems may prove insufficient, particularly for detailed analysis and three-dimensional modeling of highly complex neural network structures. While methods such as MRI offer advantages in soft tissue resolution, they are limited by spatial resolution, imaging time, susceptibility to motion artifacts, and contrast accuracy.[9] Techniques like PET and CT, on the other hand, carry disadvantages such as ionizing radiation exposure and low tissue contrast. These fundamental limitations increase the risk of errors in surgical planning and intraoperative decision-making and complicate early-stage diagnosis of neurological diseases.[10, 11]

Increasing technological demands in neurosurgical practice have revealed the need for systems capable of real-time, low-dose, non-invasive imaging of biological tissues at micrometer resolution. At this point, innovative approaches that go beyond conventional methods are expected not only to improve imaging quality but also to be integrable into surgery, supported by analytical and predictive systems.

In this context, the transformation of imaging systems encompasses not only a hardware-based evolution but also the integration of imaging data with digital processing and decision support mechanisms. In particular, the development of artificial intelligence (AI) and quantum-based imaging systems stand out as two primary drivers of this transformation.[12] Deep learning algorithms increase clinical value in image data analysis, tumor segmentation, anatomical structure classification, and surgical targeting, while quantum imaging systems promise higher resolution and accuracy through next-generation photonic technologies that operate beyond classical physical principles.[2, 5]

Overall, the transformation of imaging technologies in neurosurgery holds the potential not only for improved visualization but also for creating a more holistic surgical ecosystem. This transformation aims to integrate next-generation systems that complement rather than replace conventional technologies, offering significant gains in accuracy, speed, and patient safety in neurosurgical practice.[4]

Fundamental Principles and Clinical Potential of Quantum Imaging Systems

Quantum imaging is a next-generation technology that transcends classical physical principles by utilizing the quantum properties of photons. This approach, based particularly on quantum mechanical phenomena such as entanglement, squeezing, and superposition, enables imaging of biological tissues with higher resolution and lower energy exposure.[2] While conventional imaging systems must balance image quality with radiation dose, quantum imaging enhances contrast without disrupting

this balance and simultaneously reduces the risk of biological damage.[13] One of the most common applications in quantum imaging involves the use of correlated photon pairs obtained through quantum entanglement. In this method, changes experienced by one photon as it passes through biological tissue are measured via its entangled partner, allowing for less invasive and more sensitive imaging.[14] Consequently, high-resolution images can be obtained even at low light levels, offering significant advantages in complex and light-sensitive areas such as neural tissues.

When considered specifically in the context of neurosurgery, the potential of quantum imaging technologies is particularly striking. Quantum imaging surpasses the resolution limits of conventional methods by providing a new paradigm for structural and functional mapping of brain tissue, tracking signal transmission pathways between neurons, and identifying microvascular structures.[3] Especially in real-time intraoperative applications, these systems could provide surgeons with greater control and precision, playing a critical role in delineating cancerous tissue margins or detecting delicate structures such as aneurysms.15 Although the use of quantum technologies in biomedicine is still at an early stage, research in this area has accelerated. Initiatives such as the European Union's Quantum Flagship and US-based quantum bioimaging programs are accelerating the integration of these technologies into healthcare applications. Moreover, quantum sensors enable the detection of electromagnetic fields at the nanosecond scale, allowing for more detailed modeling of brain activity.[16]

Despite these advances, the clinical integration of quantum imaging systems still involves various technical and logistical challenges. The sensitivity of quantum hardware, costs, and the requirement to operate under specific conditions pose barriers to the routine use of these systems in hospital environments. Nevertheless, despite these challenges, quantum imaging holds transformative potential for diagnostic and therapeutic approaches in neurosurgery and many other fields of biomedicine.[17]

The Role of Artificial Intelligence Applications in Neurosurgery

Artificial intelligence (AI) has recently become a major driver of transformation in medicine and health sciences, becoming an integral part of decision support processes, particularly in specialties requiring high precision. In fields such as neurosurgery, which demand microscopic-level detail, the algorithmic analysis capacity offered by AI technologies enhances diagnostic accuracy, optimizes surgical planning, and minimizes human errors.[18] Deep learning, machine learning, and image processing techniques are actively used in numerous areas ranging from image analysis and anatomical segmentation to risk classification and prognosis prediction.

AI algorithms contribute significantly to the analysis of neuroimaging data, especially magnetic resonance imaging (MRI) and computed tomography (CT). Tasks that traditionally require long processing times using conventional methods—such as tumor boundary detection, identification of vascular anomalies like aneurysms, or early-stage prediction of neurodegenerative diseases—can be performed faster and more accurately with AI-assisted systems. These systems not only perform data-driven analysis but also learn from past data to model future probabilities.[19]

Another advantage of AI in neurosurgical planning is the integration of multidimensional clinical data to personalize surgical strategies. In this context, patient-specific anatomical data, physiological indicators, and pathological findings are combined to create decision support systems that strengthen the surgeon's clinical decision-making process. For example, in aggressive tumors such as glioblastoma, AI systems model disease progression, enabling restructuring of both treatment plans and operative routes.[20]

The use of AI systems during surgery is also increasingly widespread. Algorithms integrated with intraoperative imaging systems provide real-time guidance to the surgeon, facilitating visualization of critical elements such as nerve pathways, tumor margins, or vascular structures. Particularly in robot-assisted surgical systems, AI enhances movement precision and reduces tremors at a microscopic level.[21]

All these developments demonstrate that AI in neurosurgery has become not merely an auxiliary technology but an active component of the decision-making process. However, unresolved issues remain concerning ethics, safety, and regulation. Topics such as the reliability, explainability, and legal accountability of AI systems in clinical applications play a decisive role in the widespread adoption of these Technologies.[22]

Comparative Analysis of Artificial Intelligence and Quantum-Based Systems in Neurosurgery

In the field of neurosurgery, both artificial intelligence (AI) and quantum technologies have emerged as innovative tools developed to surpass the limitations of traditional methods. Although these two technological approaches are based on different principles, their shared goal is to enhance accuracy, speed, and personalization in diagnostic, therapeutic, and surgical processes. However, AI and quantum systems differ significantly in their areas of application, operational mechanisms, clinical integration challenges, and technical infrastructure requirements.[23,24]

AI technologies demonstrate strong performance particularly in image analysis and the processing of large datasets. Deep learning algorithms operate with high accuracy in tasks such as tumor delineation, lesion classification, and risk assessment in neuroimaging data, occasionally surpassing human performance.[25] AI algorithms integrated into clinical decision support systems are currently actively used and subject to specific regulations. Their greatest advantage lies in their ability to quickly adapt to existing infrastructures and continuously improve through learning from extensive data pools.[18]

In contrast, quantum technologies have the potential to provide microscopic-level precision in imaging biological tissues by transcending classical physical limits. Systems operating with photon-level components, leveraging features such as quantum entanglement and superposition, can offer higher resolution imaging at lower energy levels, particularly providing significant advantages in delicate structures such as neural tissue.[13] However, the clinical integration of quantum systems faces significant limitations regarding hardware complexity, cost, and physical operating conditions.[2]

From a clinical perspective, AI technologies currently hold an advantage over quantum systems in terms of "applicability." Regulations, user interfaces, training programs, and IT infrastructure facilitate the integration of AI algorithms. Quantum systems remain largely in the research phase and are implemented only at limited pilot project levels.[17]

Nevertheless, these two approaches are not mutually exclusive but rather possess complementary potential. Research in the field of "quantum artificial intelligence" (QAI) aims to integrate the computational power of quantum computing with AI algorithms, creating new synergy in areas such as neurosurgery that require high resolution and high speed.[26]

Current Clinical Applications and Experimental Initiatives

Artificial intelligence (AI) and quantum technologies, in addition to offering significant theoretical potential, have recently begun to be tested in various pilot applications within clinical and semi-clinical settings. These initiatives in the field of neurosurgery are of great importance for demonstrating the integration of technologies into practice and their interaction with real patient data. In this context, AI-based decision support systems have entered routine use, while quantum-based technologies are still primarily in experimental and prototype stages.[27]

The most common clinical application of AI-assisted systems is observed in image analysis-based applications. For example, advanced imaging platforms developed by companies such as Brainlab and Surgical Theater operate integrated with AI algorithms, providing surgeons with three-dimensional virtual planning and intraoperative guidance during neurosurgical procedures. These systems are actively utilized, especially in delicate interventions such as tumor resection, assisting in tumor margin delineation, preservation of brain functional areas, and vascular structure analysis.[28]

Another example found in the clinical literature is the use of deep learning algorithms for preoperative risk stratification. A model developed at Johns Hopkins University predicts postoperative complication probabilities in glioma patients, providing personalized risk management. Such examples demonstrate that AI is effectively used not only in imaging but also in patient management and surgical decision-making processes.[29]

Regarding quantum technologies, although direct clinical use remains limited, prototype-level applications have been implemented in some research centers. For instance, a project conducted in collaboration between Massachusetts General Hospital (MGH) and Harvard University tested quantum entanglement-based biological imaging systems for low-dose, high-contrast imaging of neural tissues.[21] Similarly, quantum signal processing algorithms developed under the IBM Q project have been reported to offer more efficient results compared to classical algorithms in compressing and analyzing neuroimaging data.[30]

Moreover, experimental studies under the European Union–supported Human Brain Project aim to develop hybrid platforms combining quantum computing and AI for advanced applications such as neurological structure modeling, brain simulations, and virtual neurosurgical planning. Although these projects have not yet entered clinical practice, they provide preliminary evidence suggesting that quantum-assisted systems could be used across multiple domains, from preoperative simulations to intraoperative guidance.[31]

In summary, AI systems currently stand out as more mature, regulation-compliant, and field-integrated technologies, whereas quantum technologies remain in developmental stages with high future potential as experimental tools. However, the combined use of these two technologies, especially in hybrid systems, holds an exceptionally high capacity to transform neurosurgical applications.

Integration of Quantum Artificial Intelligence: Future Perspectives

The integration of quantum computing and artificial intelligence technologies has recently emerged as an interdisciplinary field extensively discussed in the literature under the term "Quantum Artificial Intelligence" (QAI). This new paradigm offers alternatives to problems that classical computers cannot solve, particularly in biomedical domains requiring the processing of high-dimensional and complex datasets.[5] In a field like neurosurgery, which demands simultaneous evaluation of numerous variables and real-time decision-making, the speed and predictive potential provided by quantum AI present promising opportunities.

Quantum algorithms can reveal deeper relationships with fewer data compared to classical machine learning methods and can significantly reduce processing time in tasks such as pattern recognition, classification, and optimization.[32] This advantage is particularly relevant for analyzing high-resolution images encountered in neurosurgery, providing real-time surgical guidance and enabling personalized treatment planning. For instance, quantum neural network architectures theoretically possess the potential to perform prediction and classification tasks in seconds that might take hours on classical systems.[26]

Quantum AI integration contributes not only to processing speed but also to explainability. The "black box" nature of most classical deep learning models raises safety and ethical concerns in healthcare. In contrast, quantum-supported models are expected to generate more interpretable mathematical solutions in nonlinear

multivariate systems, potentially rendering decision-making processes more transparent.[33]

From a clinical integration standpoint, quantum AI systems remain largely experimental, though some pilot applications have shown promising results. Quantum simulation platforms developed within projects such as IBM Q and Google Quantum AI are employed for modeling biological systems and testing clinical decision support systems. Nevertheless, their integration into high-risk clinical fields like neurosurgery is still limited due to hardware complexity, data security concerns, algorithm stability, and regulatory gaps.[24]

Nonetheless, the fusion of AI and quantum computing could pave the way for revolutionary transformations in neurosurgery in the future. Highly accurate predictive models may enable safer planning of minimally invasive surgeries and potentially facilitate fully automated surgical scenarios integrated with robotic systems. The success of these developments depends on effective multidisciplinary collaborations, ethical oversight mechanisms, and the advancement of open data–based system architectures.

Conclusion

Neurosurgery, as a medical discipline where high precision and rapid decision-making processes intersect, is among the fields most sensitive to technological advancements. In this context, recent progress particularly in artificial intelligence and quantum technologies has introduced a new dimension to imaging, diagnosis, and treatment applications in neurosurgery. Artificial intelligence has already begun to provide tangible contributions in image processing, decision support systems, and preoperative planning; especially through deep learning algorithms, enabling automatic recognition of anatomical structures and more precise evaluation of surgical risks. These developments enhance patient safety and improve surgical success rates in neurosurgery.

Although still in the experimental phase, quantum imaging emerges as an innovative approach with the potential to overcome the resolution and contrast limitations of conventional imaging technologies. Operating on fundamental principles such as quantum entanglement and superposition, these systems offer higher accuracy imaging with lower energy, potentially creating revolutionary impacts especially in delicate areas such as neural tissue. However, the clinical integration of quantum technologies involves multilayered challenges including cost, hardware complexity, infrastructure compatibility, and regulatory gaps.

As demonstrated in this study, artificial intelligence and quantum technologies should not be considered independent but rather complementary systems. Particularly, new-generation integrated structures known as quantum artificial intelligence enable faster and more meaningful analysis of multidimensional data, offering a potential new paradigm in both diagnostic and surgical processes in neurosurgery.

In conclusion, the integration of artificial intelligence and quantum-based technologies contributes to building a more predictable, personalized, and safer clinical future that goes beyond current neurosurgical applications. To facilitate the widespread adoption of these technologies, multidisciplinary research collaborations should be encouraged, ethical and legal frameworks clarified, and further studies conducted to establish clinical validity. The integration of scientific advancements into healthcare is not only a technological progression but also a direct investment in the quality of human life.

Conflicts of Interest

There is no conflict of interest between the authors.

Financial support

This study was not supported by any organization.

Author Contribution

All authors contributed equally to this work.

References

1. Panesar S, et al. Artificial intelligence and the future of surgical robotics. *Ann Surg.* 2019;270(2):223–226. doi:10.1097/SLA.0000000000003262
2. Moodley C, Forbes A. Advances in quantum imaging with machine intelligence. *Laser Photonics Rev.* 2024;18. doi:10.1002/lpor.202300939
3. Ortolano G, et al. Quantum enhanced non-interferometric quantitative phase imaging. *Light Sci Appl.* 2023; 12(171):1–30.
4. Lohmann P, et al. Radiomics in neuro-oncology: basics, workflow, and applications. *Methods.* 2021;[pages]112–121. doi:10.1016/j.ymeth.2020.06.003
5. Biamonte J, et al. Quantum machine learning. *arXiv.* 2017. doi:10.48550/arXiv.1611.09347
6. Efe A. Assessment of artificial intelligence and quantum computing in smart management information systems. *J Inf Technol.* 2023;16(3):177–188. doi:10.17671/gazibtd. 1190670
7. Tönge Ç, et al. Artificial intelligence and the future in neurosurgery. *Turk Neurosurg J.* 2022;32(2):136-141.
8. Abdallah A, Kitiş S. Imaging software and their contributions to neurosurgical practice. *Turk Neurosurg J.* 2018;28(3):284–288.
9. Yavaş G, Çalışkan KE. Intraoperative virtual and augmented reality in neurosurgery. *Turk Neurosurg J.* 2022;32(2):169–177.
10. Çeltikçi E. A systematic review on machine learning in neurosurgery: the future of decision-making in patient care. *Turk Neurosurg.* 2018;28(2):167–173.
11. Decupyer M, et al. Artificial intelligence with deep learning in nuclear medicine and radiology. *EJNMMI Phys.* 2021;8(1). doi:10.1186/s40658-021-00426-y
12. Şahin ÖS, et al. Artificial intelligence and human intelligence in neurosurgery. *Turk Neurosurg J.* 2018;28(3):277–283.
13. Brida G, Genovese M, Berchera IR. Experimental realization of sub-shot-noise quantum imaging. *Nat Photonics.* 2010;4:227–230.

14. Dowling JP. Quantum optical metrology — the lowdown on high-N00N states. *Contemp Phys.* 2008;49(2):125–143.
15. Connor T, et al. Advances in deep brain imaging with quantum dots: structural, functional, and disease-specific roles. *Photonics.* 2025;12(1):3. doi:10.3390/photonics12010003
16. Taylor JM, et al. High-sensitivity diamond magnetometer with nanoscale resolution. *Mesoscale Nanoscale Phys.* 2008. doi:10.48550/arXiv.0805.1367
17. Giovannetti V, Lloyd S, Maccone L. Advances in quantum metrology. *Nature.* 2011;[pages]222–229.
18. Lundervold AS, Lundervold A. An overview of deep learning in medical imaging focusing on MRI. *Z Med Phys.* 2019;29(2):102–127. doi:10.1016/j.zemedi.2018.11.002
19. Ravi D, et al. Deep learning for health informatics. *IEEE J Biomed Health Inform.* 2017;21(1):4–21. doi:10.1109/JBHI.2016.2636665
20. Pinto dos Santos D, Baeßler B, Bigalke U. Diagnostic performance of artificial intelligence methods in medical imaging: a systematic review. *Eur Radiol.* 2019;29(9):4635–4645.
21. Zhou Z, Siddiquee MMR, Tajbakhsh N, Liang J. Unet++: a nested U-Net architecture for medical image segmentation. In: *Deep Learning in Medical Image Analysis and Multimodal Learning for Clinical Decision Support*. 2021:3–11. doi:10.1007/978-3-030-00889-5_1
22. Wiens J, et al. Do no harm: a roadmap for responsible machine learning for health care. *Nat Med.* 2019;25(9):1337–1340. doi:10.1038/s41591-019-0548-6
23. Cerezo M, et al. Variational quantum algorithms. *Nat Rev Phys.* 2021;3:625–644.
24. Schuld M, Petruccione F. *Supervised learning with quantum computers.* Springer; 2018.
25. Dunjko V, Briegel H. Machine learning & artificial intelligence in the quantum domain: a review of recent progress. *Rep Prog Phys.* 2018;81(7). doi:10.1088/1361-6633/aab406
26. Preskill J. Quantum computing in the NISQ era and beyond. *Quantum.* 2018. doi:10.22331/q-2018-08-06-79
27. Cabitza F, Rasoini R, Gensini GF. Unintended consequences of machine learning in medicine. *JAMA.* 2017;318(6):517–518. doi:10.1001/jama.2017.7797
28. Esteva A, et al. A guide to deep learning in healthcare. *Nat Med.* 2019;25(1):24–29. doi:10.1038/s41591-018-0316-z
29. Amunts K, et al. The human brain project: creating a European research infrastructure to decode the human brain. *Neuron.* 2016;92(3):574–581. doi:10.1016/j.neuron.2016.10.046
30. Cordier B, et al. Biology and medicine in the landscape of quantum advantages. *J R Soc Interface.* 2022;19(196). doi:10.1098/rsif.2022.0541
31. Chang K, et al. Residual convolutional neural network for determination of IDH status in low- and high-grade gliomas from MR imaging. *Clin Cancer Res.* 2019;24(5):1073–1081. doi:10.1158/1078-0432.CCR-17-2236
32. Douglas DB, et al. Virtual reality and augmented reality: advances in surgery. *Biol Eng Med.* 2017;3(1):1–8. doi:10.15761/BEM.1000131
33. Topol E. *Deep Medicine: How Artificial Intelligence Can Make Healthcare Human Again.* Basic Books; 2019.

doi: 10.53446/actamednicomedia.1232347

Acta Medica Nicomedia

Cilt: 6 Sayı: 1 Şubat 2023 / Vol: 6 Issue: 1 February 2023
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

THE EFFECTS OF VITAMIN D LEVELS ON PREGNANCY OUTCOMES IN PATIENTS RECEIVING FROZEN EMBRYO TRANSFER

DONMUŞ EMBRİYO TRANSFERİ YAPILAN HASTALARDA D VİTAMİNİ DÜZEYLERİNİN GEBELİK SONUÇLARINA ETKİSİ

Merve Cakır Kole[1], Emre Kole[2], Goksen Gorgulu[3], Barıs Candan[4], Ahmet Gulluoglu[2], Emek Doger[5], Lale Aksoy[6]

[1] Antalya Education and Research Hospital, Department of Obstetrics and Gynecology, Antalya, Türkiye. [2] Alanya Alaaddin Keykubat University School of Medicine, Department of Obstetrics and Gynecology, Antalya, Türkiye. [3] Tepecik Education and Research Hospital, Department of Obstetrics and Gynecology, Izmir, Türkiye. [4] Süreyyapaşa Education and Research Hospital, Department of Public Health, Istanbul, Türkiye. [5] Kocaeli University School of Medicine, Department of Obstetrics and Gynecology, Kocaeli, Türkiye. [6] Geyve State Hospital, Department of Obstetrics and Gynecology, Sakarya, Türkiye.

ABSTRACT

Objective: The aim of this study is to evaluate the effects of 25-OH vitamin D on pregnancy outcomes in infertile patients undergoing a frozen embryo transfer.

Methods: In this prospective, single-blind study conducted at Kocaeli University Medical Faculty Hospital, Center for Assisted Reproductive Techniques, baseline serum levels of 25-OH vitamin D were measured at the start of treatment in 276 infertile patients who were scheduled to undergo frozen embryo transfer (FET). Cases with 25-OH-D vitamin levels lower than the level of deficiency (<20 ng/ml, group A, n=48) and higher than the level of deficiency (≥20 ng/ml, group B, n=44) were compared in terms of the rates of pregnancy as an outcome of the FET cycle, clinical pregnancy, ongoing pregnancy, live birth, implantation, pregnancy loss, and multiple pregnancy.

Results: Cases in groups 1 and 2 had similar demographic characteristics, and the serum AMH levels, one of the cycle follow-up parameters, were statistically significantly higher in group 1 compared to group 2 (p=0.014). Pregnancy (41.6% vs. 31.8%), clinical pregnancy (35.4% vs. 25%), ongoing pregnancy (25% vs. 18.2%), live birth (20.8% vs. 18.2%), pregnancy loss (18.8% vs. 13.6%) and twin pregnancy (4.2% vs. 9.1%) were similar between the groups (p=0.328, p=0.278, p=0.428, p=0.749, p=0.507, p=0.421, respectively).

Conclusion: There was no correlation between pregnancy outcomes from frozen embryo transfer and baseline serum 25-OH vitamin D levels obtained at the start of treatment. There is no indirect evidence showing that vitamin D level exerts its effects on fertility through endometrial receptivity and the implantation process.

Keywords: 25-OH Vitamin D, Frozen embryo transfer, pregnancy outcome

ÖZ

Amaç: Donmuş embriyo transferi yapılan infertil hastalarda 25-OH vitamin D'nin gebelik sonuçlarına etkisini değerlendirmek.

Gereç ve Yöntem: Kocaeli Üniversitesi Tıp Fakültesi Hastanesi, Üremeye Yardımcı Teknikler Merkezinde prospektif -tek kör olarak yürütülen bu çalışmada donmuş embriyo transferi (DET) yapılması planlanan 276 infertil olgunun serum 25-OH vitamin D düzeyleri tedavi başlangıcında elde edildi. 25-OH-D vitamini seviyelerinin yetmezlik seviyesinde düşük olduğu olgular (<20 ng/ml, grup A, n=48) ve yetmezlik seviyesinin üstünde olduğu (≥20 ng/ml, grup B, n=44) olgular DET siklusu sonucundaki gebelik, klinik gebelik, devam eden gebelik, canlı doğum, implantasyon, gebelik kaybı ve çoğul gebelik oranları karşılaştırıldı.

Bulgular: Grup 1 ve grup 2 olguların demografik özellikleri benzerdi ve siklus takip parametrelerinden serum AMH düzeyleri grup 1 de grup 2 'ye göre istatiksel olarak anlamlı yüksek izlenmiştir (p=0,014). Gruplar arasında gebelik (% 41,6 vs %31,8), klinik gebelik (%35,4 vs %25), devam eden gebelik (%25 vs %18,2), canlı doğum (%20,8 vs %18,2), gebelik kaybı (%18,8 vs %13,6) ve ikiz gebelik (% 4,2 vs % 9,1) benzerdi (sırasıyla; p= 0,328 p= 0.278, p= 0,428, p= 0,749, p= 0,507, p= 0,421).

Sonuç: Donmuş embriyo transferinden elde edilen gebelik sonuçları ile tedavi başlangıcında elde edilen serum 25-OH vitamin D seviyeleri arasında ilişki tespit edilmemiştir. Vitamin D seviyesinin fertilite üzerindeki etkilerini endometriyal reseptivite ve implantasyon süreci üzerinden gösterdiğine dair dolaylı kanıt elde edilmemiştir.

Anahtar Kelimeler: 25-OH Vitamin D, Donmuş Embriyo Transferi, Gebelik Sonucu.

*** Corresponding author/İletişim kurulacak yazar:** Lale Aksoy; Geyve State Hospital, Department of Obstetrics and Gynecology, Sakarya, Turkiye.
Phone/Telefon: +90532 422 70 02 **e-mail/e-posta:** laleaksoy@gmail.com
Submitted/Başvuru: 11.01.2023 • **Accepted/Kabul:** 25.02.2023 • **Published Online/ Online Yayın:** 28.02.2023

Introduction

The current data of the World Health Organization show that 10-15% of married couples are affected by infertility. Assisted reproductive techniques are the only way to achieve pregnancy for most infertile couples.

In-vitro fertilization (IVF) is the ideal treatment method for couples who are unable to conceive naturally or by in-utero insemination, or for infertile patients who are not suitable for these methods. Pregnancy success in freeze-thaw cycles is still low in cases when embryos cannot be transferred in a fresh state, and novel strategies are being tested to improve success. One of these evaluations is whether the serum 25-OH-D vitamin levels of a female patient have an effect on pregnancy outcome in frozen embryo transfer (FET) cases.[1]

Vitamin D, produced mainly in the skin in the body, is a steroid hormone that is fat-soluble. Ergocalciferol, which is contained in plants, and cholecalciferol, which is prevalent in animal foods, are the main sources of exogenous vitamin D intake.[2] The effects of vitamin D on many systems in the body have been shown in numerous studies.[3,4] Studies both with humans and animals are conducted to clarify the potential role of vitamin D in female fertility.[5]

The purpose of this study is to compare the success of pregnancy outcomes between the cases where the baseline serum 25-OH-vitamin D level obtained from female patients at the beginning of the frozen embryo cycle is above (≥20 ng/mL) and below (<20 ng/mL) the insufficiency. Accordingly, it is aimed to reveal whether the serum vitamin D level affects endometrial receptivity and implantation success.

Methods

This study was carried out with 270 infertile women who were scheduled to undergo frozen embryo transfer (FET) at Kocaeli University Medical Faculty Hospital, Center for Assisted Reproductive Techniques. All participants were included in the study after obtaining informed consent.

Patients aged between 24-42 years who were scheduled to undergo FET and had at least one good quality frozen embryo with the diagnosis of single or combined unexplained infertility, male factor infertility, anovulation, low ovarian reserve, bilateral tubal factor and endometriosis were included in the study. Cases who did not want to participate in the study, who had endometrial polyps, submucous myomas, uncorrected uterine anomalies, hydrosalpinx, or uncontrolled systemic diseases, were excluded from the study.

Following the confirmation of ovulation in the luteal phase of the previous cycle, a suppressed FET cycle was performed in all cases by starting leuprolide acetate (Lucrin 5mg/ml/2.8ml vial, 14 Syringe Kit SC /Abbot) with a daily dose of subcutaneous 10 IU for pituitary suppression. On the third day of the menstrual cycle, the patients were then called for an ultrasound and blood tests. The patients were called for the measurement of serum 25-OH vitamin D levels, TSH, AMH, estradiol, and progesterone by drawing 3-5 cc blood daily on the third day of the menstrual cycle. The study continued using the blinded method by preventing the researcher and patient from knowing the 25-OH vitamin D levels. Cases whose TSH levels were not in the 0.5-4.5 mIU/L range were excluded from the study. Patients with an estradiol level of > 50 and a progesterone level of > 1 ng/mL continued to take Lucrin at a dose of 10 IU/day until complete suppression was achieved. Estrogen therapy was not started in these cases until it was determined by drawing blood every three days that suppression had been achieved. Estrogen treatment was not initiated in cases who had endometrial thickness of >5 mm or had a follicle cyst larger than >14mm in the adnexal area in the ultrasound examinations which was performed concurrently. Estrogen therapy was started in these cases when the endometrial thickness was ≤ 5 mm and no cyst was detected in the adnexal area in the ultrasonographic follow-up performed every three days.

In cases who met the criteria (estradiol <50 ng/mL, progesterone < 1 ng/mL, endometrial thickness ≤ 5mm, no cysts in the adnexal area) in the blood tests and ultrasound examinations performed, 6 mg oestradiol valerate/mL (Estrofem tablet 2 mg 28 tablets /Novo Nordisk) was started orally divided into three equal doses daily while Leuprolid acetate was continued with a daily dose of 5 IU. The patients were called for the first ultrasonographic evaluation at the earliest on the 10th day of the menstrual cycle and on day 7 of the estrogen therapy. In this evaluation, while the estrogen therapy was continued with the same dose in cases with an endometrial thickness of ≥ 8 mm and a blood progesterone level of <1 ng/mL, leuprolide acetate therapy was discontinued, and twice-a-day vaginal progesterone therapy was initiated (Crinone 90 mg gel 8% /Merck). Cases with an endometrial thickness of less than 8 mm were examined every other day to monitor for an increase in the endometrial thickness. Estrogen therapy was administered at a dose of 8 mg/day to the cases whose endometrial thickness did not increase sufficiently in 2 consecutive follow-ups. If available, two thawed embryos were transferred, and if not, one thawed embryo was transferred on the 4th day of vaginal progesterone in cases with a third-day embryo, on the 6th day of vaginal progesterone in cases with a 5th-day embryo, and on the 7th day of vaginal progesterone in cases with a 6th-day embryo.

The embryo transfer was performed under the supervision of transabdominal ultrasonography with a full bladder. After the visualization of the cervix with a speculum, the cervix was purified from drug residues with saline and cleared of mucus by aspiration with a mucus-attracting catheter. First, a mock transfer was performed to determine the cervical canal and the uterine position. Then, with a full echo soft catheter (Prodimed) the cervix was passed by using a stylet only in patients that necessitated it, and the embryo transfer was completed by applying the lowest pressure possible on the Hamilton syringe without approaching the middle

portion of the uterine cavity by more than 15 mm and without a fundal contact. No teneculum was used or no cervical dilatation was performed in any patient. The position of the air balloon was clearly observed in all cases. The embryo transfer catheter was slowly removed, and no bed rest was recommended for the patients after the transfer. Daily doses of 6 mg oral estrogen and 180 mg vaginal progesterone were continued after the embryo transfer. No vitamin treatments were recommended. On the 12th day after the embryo transfer, blood hCG levels were measured to confirm pregnancy.

The main outcomes that were aimed to achieve in this study were the pregnancy rate revealed by hCG positivity, the clinical pregnancy rate obtained by ultrasonographic confirmation of the embryonic heartbeat, and the ongoing pregnancy rate confirmed by exceeding the 10th week of pregnancy. Additionally, the secondary aim of the study was to obtain the rates of multiple pregnancy and rates of abortion. After achieving the primary aims of the study, the study was unblinded, and the pregnancy success as a result of the FET cycle was compared between the cases with blood 25-OH-D vitamin levels above the deficiency level (≥20 ng/ml) and the cases at the insufficiency level (20 ng/ml).

The data analysis was performed with SPSS for Windows 20.0 package program. A Kolmogorov-Smirnov test was completed to check if the continuous variables were normally distributed. Descriptive statistics were presented as mean ± standard deviation or median (minimum-maximum) for continuous variables, while categorical variables were presented as number and percentage (%) of cases. The student's t-test was used to determine the significance of the difference between the groups in terms of means. The nonparametric Mann-Whitney U Test was used for the data whose means could be calculated as the group did not fit the normal distribution. The Pearson's Chi-Square Test was used for the data whose means could not be calculated. The results were considered statistically significant for a p value of <0.05.

Results

A comparison of the demographics of study groups are presented in Table 1. Both groups showed similar demographics and infertility diagnoses (Table 1).

While the comparison of the characteristics of the FET cycle between the groups is presented in Table 2, the comparison of the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancies is presented in Table 3.

The serum AMH value of the group with a serum 25-(OH) Vitamin D level higher than 20 was statistically significantly lower than the group with a low serum 25-OH vitamin D level. No significant difference was found between the groups in terms of both the characteristics of the FET cycle and the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, pregnancy loss, and twin pregnancy.

The comparison of the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancy between cases with 25-OH vitamin D levels of <20 ng/mL and cases with 25-OH vitamin D levels of ≥20 ng/mL is presented in Table 3. There was no significant difference in pregnancy achievement and pregnancy outcomes between the two groups (p>0.05).

The retrospective analysis of the means of 25-OH vitamin D levels of cases who got pregnant, achieved clinical pregnancy, had an ongoing pregnancy, and gave live birth revealed no significant differences in the means of vitamin D levels between the groups, and the results are presented in Table 4.

In the study, we did not find any relationship between the levels of serum vitamin D and achieving pregnancy in the FET cycle.

Discussion

There is no agreement on the ideal vitamin D levels for female reproductive health and fertility at the moment. Although the possible effect of vitamin D on the outcomes of assisted reproductive therapy (clinical pregnancy and live birth) has been evaluated in a limited number of studies, the data are inconsistent.[6-10]

Studies examining the relationship between serum vitamin D levels and the effectiveness of IVF cycles reported that the clinical pregnancy rate is associated with vitamin D deficiency. According to a study measuring the 25-OH vitamin D levels in follicular fluid instead of serum, high vitamin D levels are associated with significantly higher clinical pregnancy and implantation rates, and follicular fluid vitamin D levels are an independent predictor of IVF cycle success.[11] Similarly, research done over various IVF cycles has suggested a relationship between vitamin D levels and pregnancy outcome.[12-13]

In contrast to these studies, which discovered a significant correlation between vitamin D levels and the success of IVF cycles using fresh embryos, Anifandis et al. reported a negative correlation between follicular fluid 25-OH vitamin D level, embryo quality, and clinical pregnancy rate.[14]

We aimed to evaluate the data obtained from FET cycles of patients with good and very good quality embryos frozen in the previous cycle to rule out ovarian factors and reveal whether vitamin D has an effect on endometrial receptivity and the implantation process. As good quality embryos are already frozen, standardizing FET is easier. As a result, many concomitant variables associated with the patient and her partner in new cycles are eliminated, and the true effect on receptivity can be assessed. In this prospectively designed, single-blind study, no statistically significant relationship was found between the serum 25-OH vitamin D levels measured at the start of the FET cycle and pregnancy success. In this study, we evaluated case groups with vitamin D levels both below and above 20 ng/mL. Furthermore, unlike many other studies, all samples were examined

Table 1. Comparison of important characteristics of groups.

	Group 1 Vitamin D level <20 ng / mL. (n= 48)	**Group 2 Vitamin D level ≥ 20ng / mL (n= 44)**	**p value**
Age (year)*	30.48 ± 4.36	32.00 ± 4.30	0.144
Partner age (year)*	34.27 ± 4.16	35.10 ± 6.47	0.939
Marriage duration (year)*	7.62 ± 4.55 (n=24)	5.95 ± 4.39 (n=20)	0.210
Gravida (n)*	0.52 ± 1.24	0.55 ± 0.82	0.307
Parity (n)*	0.10 ± 0.31	0.14 ± 0.38	0.636
Abortion (n)*	0.35 ± 1.06	0.41 ± 0.76	0.243
BMI (kg/size^2) *	25.94 ± 4,94 (n=24)	24,99 ± 3,99 (n=33)	0.518
Smoking (n, %)	3 (6.3%)	5 (11.4%)	0.473 *
Chronic medical disease (n, %)	4 (8.3%)	9 (20.5%)	0.095
Previous uterine surgery (n, %)	9 (18.75%)	11 (2[illegible].0%)	0.468
Number of previous fresh IVF	1.08 ± 1.13	1.14 [illegible].98	0.443
Number of previous FET	0.75 ± 0.81	0.91 ± 0.[illegible]	0.728
Genetic (n, %)	1 (2.1%)	[illegible] (0.0%)	0.522 *
Advanced age (n, %)	0 (0%)	[illegible] (4.[illegible]%)	0.226 *
Endometriosis (n, %)	1 (2.1%)	2 (4.5%)	0.467 *
Bilateral Tubal factor (n, %)	2 (4.2%)	4 (9.1%)	0.298 *
Low ovarian reserve (n, %)	4 (8.3%)	7 (15.9%)	0.263
Azosperm (n, %)	5 (10.4%)	2 (4.5%)	0.255 *
Anovulation (n, %)	11 ([illegible]%)	6 (13.6%)	0.252
Unexplained infertility (n, %)	4 (8.[illegible]%)	5 (11.4%)	0.444 *

Values are given as mean ± standard deviation. *p value was calculated by Fischer Chi Square Test.**Abbreviations; BMI: Body mass index, ICSI: Intracytoplasmic sperm injection, FET: Frozen embryo transfer, PCOS: Polycystic ovary syndrome

Table 2. Comparison of FET cycle characteristics between groups

	Group 1 Vitamin D Level <20 Ng / Ml (n= 48)	**Group 2 Vitamin D Level ≥20 Ng / Ml (n= 44)**	**P Value**
AMH Level (Ng/Ml) *	9.73 ± 7.79 (n=26)	4.67 ± 4.21 (n=23)	**0.014**
TSH Level (Miu/L) *	1.96 ± 1.24 (n=41)	1.84 ± 0.77 (n=38)	0.702
AFC*	23.46 ± 16.09 (n=12)	14.38 ± 9.36 (n=16)	0.113
Estrogen Used Time (Day)	9.79 ± 2.93	9.98 ± 2.42	0.379
Endometrial Thickness (mm)*	9.94 ± 1.86 (n=47)	10.06 ± 1.69 (n=43)	0.487
Progesterone Level at the end of Proliferation Phase (ng/ml) *	0.53 ± 0.3 (n=20)	0.43 ± 0.27 (n=24)	0.094
Number Of Embryos Transferred (n)*			
Day 3 Embryos (n, %)	18 (37.5%)	13 (29.5%)	0.420
Day 5 Embryos (n, %)	21 (43.8%)	22 (50.0%)	0.548
Day 6 Embryos (n, %)	9 (18.8%)	9 (20.5%)	0.837

*Values are given as mean ± standard deviation. ** Abbreviations; AMH: Anti-mullerian Hormone, AFC: Antral Follicle Count, TSH: Thyroid Stimulant Hormone

Table 3. Comparison of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancy rates between groups

	Group 1 Vitamin D level <20 ng/ml (n= 48)	Group 2 Vitamin D level ≥20 ng / ml (n= 44)	p value
Pregnancy Rate	20 (41.6%)	14 (31.8%)	0.328
Clinical Pregnancy Rate	17 (35.4%)	11 (25.0%)	0.278
Ongoing Pregnancy Rate	12 (25.0%)	8 (18.2%)	0.428
Live Birth Rate	10 (20.8%)	8 (18.2%)	0.749
Abortion Rate	9 (18.8%)	6 (13.6%)	0.507
Twin Pregnancy Rate	2 (4.2%)	4 (9.1%)	0.421 *

*p value was calculated by Fischer Chi-Square Test.

immediately without being frozen, but neither the researchers nor the patients were aware of their vitamin D levels. This reflects the strength of our work in minimizing bias. Similar to our study, Van de Vijyer et al. in their prospective cohort studies, evaluated 280 infertile cases whose FET cycle was planned, in two separate groups as cases with 25-OH vitamin D levels below and above 20 ng/mL on the day of embryo transfer, the pregnancy rate in the vitamin D deficient group was found to be similar when compared to the vitamin D sufficient group (respectively; 40.9% vs. 48.3%, p=0.2).[15] Similarly, no difference was found between the clinical pregnancy rates (32.2% vs 37.9%, respectively, *p*=0.3). Clinical pregnancy rates in this study were similar in cases of insufficiency, deficiency, and normal levels of vitamin D, and the multivariate logistic regression analysis revealed that vitamin D status was not associated with pregnancy outcomes. Moreover, in their study, which involved randomizing 114 infertile cases with 25-OH vitamin D levels of <30 ng/L into two groups with and without vitamin D replacement, Aflatoonian et al. reported that the results of the two groups had similar results in terms of ongoing FET cycle pregnancy and clinical pregnancy.[1] When these findings are considered together with the findings of our study, we are of the opinion that there is no data showing that vitamin D exerts its effects on reproductive functions through endometrial receptivity and implantation.

The fact that the study was not designed to reveal the effects of vitamin D levels at the tissue level is a limitation. However, the study focused on the clinical outcomes needed in current practices and provided explanatory information on this subject. Nevertheless, when the limitations of our study are considered, it is clear that randomized controlled trials with high quality large samples are needed to determine the optimal 25(OH) vitamin D levels and the effects of vitamin D supplementation on fertility.

Compliance with Ethical Standards

This study was approved by Kocaeli University Non-interventional Clinical Research Ethics Committee (Decision number: 2017/176, Date: 07/06/2022)

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

Author Contribution

Authors have contributed equally to this work.

Financial Disclosure

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

References

1. Aflatoonian A, Arabjahvani F, Eftekhar M, Sayadi M. Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: A randomized clinical trial. Iran J Reprod Med. 2014;12(9):595–600.
2. Bringhurst FR, Demay MB, Krane SM, Kronenberg HM. Kemik ve mineral metabolizması bozuklukları. İçinde: Harrison. *İç Hastalıkları Prensipleri.* 17. Baskı. İstanbul: Nobel Matbaacılık; 2013. S.2365-77
3. Özsoylu S. Hormonal effect of cholecalciferol. *New Medical Journal.* 1986;2:3-6.
4. Yetgin S, Özsoylu Ş, Ruacan Ş, Tekinalp G, Sarialiolu F. Vitamin D deficiency rickets and myelofibrosis. *J Pediatr.* 1989;114(2):213-217. doi:10.1016/s0022-3476(89)80785-1
5. Anagnostis P, Karras S, Goulis DG. Vitamin D in human reproduction: a narrative review. *Int J Clin Pract.* 2013;67(3):225-235. doi:10.1111/ijcp.12031
6. Viganò P, Lattuada D, Mangioni S, et al. Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. *J Mol Endocrinol.* 2006;36(3):415-424. doi:10.1677/jme.1.01946
7. Miyashita M, Koga K, Izumi G, et al. Effects of 1,25-Dihydroxy Vitamin D3 on Endometriosis. *J Clin Endocrinol Metab.* 2016;101(6):2371-2379. doi:10.1210/jc.2016-1515
8. Chan SY, Susarla R, Canovas D, et al. Vitamin D promotes human extravillous trophoblast invasion in vitro. *Placenta.* 2015;36(4):403-409. doi:10.1016/j.placenta.2014.12.021
9. Du H, Daftary GS, Lalwani SI, Taylor HS. Direct regulation of HOXA10 by 1,25-(OH)2D3 in human myelomonocytic cells and human endometrial stromal cells. *Mol Endocrinol.* 2005;19(9):2222-2233. doi:10.1210/me.2004-0336
10. Rajaei S, Mirahmadian M, Jeddi-Tehrani M, et al. Effect of 1,25(OH)2 vitamin D3 on cytokine production by endometrial cells of women with repeated implantation failure. *Gynecol Endocrinol.* 2012;28(11):906-911. doi:10.3109/09513590.2012.683062

11. Ozkan S, Jindal S, Greenseid K, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertil Steril.* 2010;94(4):1314-1319. doi:10.1016/j.fertnstert.2009.05.019
12. Rudick BJ, Ingles SA, Chung K, Stanczyk FZ, Paulson RJ, Bendikson KA. Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles. *Fertil Steril.* 2014;101(2):447-452. doi:10.1016/j.fertnstert.2013.10.008
13. Garbedian K, Boggild M, Moody J, Liu KE. Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization. *CMAJ Open.* 2013;1(2):E77-E82. doi:10.9778/cmajo.20120032
14. Anifandis GM, Dafopoulos K, Messini CI, et al. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. *Reprod Biol Endocrinol.* 2010;8:91. doi:10.1186/1477-7827-8-91
15. van de Vijver A, Drakopoulos P, Van Landuyt L, et al. Vitamin D deficiency and pregnancy rates following frozen-thawed embryo transfer: a prospective cohort study. *Hum Reprod.* 2016;31(8):1749-1754. doi:10.1093/humrep/dew107