Year: March 2024 Volume: 11 Issue: 1

E-ISSN: 2587-2478

# The Journal of Pediatric Research




galenos Publishing House

JPR
# The Journal of Pediatric Research

Official Journal of Ege University Children's Hospital

## EDITORIAL BOARD



**The Journal of Pediatric Research is the official publication of Ege University Faculty of Medicine, Department of Pediatrics and Ege Children's Foundation.**

**Publisher Contact**
**Address:** Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Turkey
**Phone:** +90 (530) 177 30 97 / +90 (539) 307 32 03
**E-mail:** info@galenos.com.tr/yayin@galenos.com.tr
**Web:** www.galenos.com.tr
**Publisher Certificate Number:** 14521

**Online Publication Date:** March 2024
**E-ISSN:** 2587-2478
International scientific journal published quarterly.

JPR
The
Journal of Pediatric Research
Official Journal of Ege University Children's Hospital

## EDITORIAL BOARD

JPR
# The Journal of Pediatric Research

Official Journal of Ege University Children's Hospital

## Scientific Advisory Board

JPR
# The Journal of Pediatric Research

Official Journal of Ege University Children's Hospital

Please refer to the journal's webpage **(https://jpedres.org/)** for "Aims and Scope", "Instructions to Authors" and "Ethical Policy".

The editorial and publication processes of the Journal of Pediatric Research are shaped in accordance with the guidelines of ICMJE, WAME, CSE, COPE, EASE, and NISO. The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing.

The Journal of Pediatric Research is indexed in **Emerging Sources Citation Index (ESCI), Embase, Directory of Open Access Journals (DOAJ), EBSCO, CINAHL Complete Database, ProQuest, CABI, Gale/Cengage Learning, Ulakbim TR Dizin, TurkMedline, J-GATE, IdealOnline, Hinari, GOALI, ARDI, OARE, AGORA** and **Türkiye Citation Index**.

The journal is published online.

**Owner:** Ege University and Ege Children's Foundation

**Responsible Manager:** Özgür ÇOĞULU

JPR
The
Journal of Pediatric Research
Official Journal of Ege University Children's Hospital

## Contents

### Original Articles

The
Journal of Pediatric Research

Official Journal of Ege University Children's Hospital

## Editorial

Dear Esteemed Colleagues and Readers of JPR,

We are pleased to share the latest issue of The Journal of Pediatric Research, a rich compilation of ten articles that span a spectrum of pediatric studies. In this edition, we are witnessing a synergy of artificial intelligence, genetic insight, and psychological assessment converge to further our understanding of pediatric health and disease.

Our cover article, "Exploring Predictive Role of Inflammatory Markers in Neuropathic Bladder-Related Kidney Damage with Machine Learning," heralds a pioneering use of machine learning to identify markers of kidney damage in neuropathic bladder conditions, offering a valuable tool for healthcare professionals operating in resource-constrained environments.

Moving from the digital to the practical, "Effects of a Mobile Application to Improve Oral Hygiene in Children" evaluates the efficacy of a mobile application in instilling better oral hygiene practices, reflecting a trend towards digital health interventions.

Our subsequent study, Why Infants with Some Inherited Metabolic Diseases do not Develop Neonatal Indirect Hyperbilirubinemia?: An Overlooked Detail probes a curiously underexplored area of pediatrics, challenging established understandings and opening the door to further inquisitive research.

The article "Epigenetic Mechanisms of Genes Influencing Immune Response in Patients with Celiac Disease" shines a light on the potential of a non-invasive biomarker in diagnosing Celiac Disease.

Highlighting the vulnerabilities of our youngest patients, "Newborns are Prone to More Hypothermic in the Low Temperature of Operating Rooms" raises a crucial aspect of perioperative care, calling for refined temperature regulation protocols in surgical environments.

Two unique studies, "Evaluation of Premature Ventricular Contractions in Children with Structurally Normal Hearts: A Single-Center Study" and "Evaluation of Heart Rate Variability in Children with Stutter" spotlight relatively uncharted medical issues.

"The Relationship Between Premature Adrenarche and Markers of Inflammation in Complete Blood Count" highlights the intriguing link between premature adrenarche and cardiovascular health.

The study "Comparison of Anxiety of the Children of Healthcare Workers and Non-Healthcare Workers during the COVID-19 Pandemic" highlights the significant impact of the pandemic on the mental health of healthcare workers' children, underscoring the urgent need for targeted support and interventions to help these children navigate the unique challenges posed by global health crises.

Finally, we close with an essential aspect of pediatric care "The Evaluation of Quality of Life in Children and Adolescents in an Inpatient Oncology Unit: A 6 Months Follow-up Study" delves into a deeply sensitive issue, offering crucial insights into the experiences and evolving needs of young patients during their cancer journey.

We extend our heartfelt gratitude to the authors, reviewers, the editorial team, and especially Galenos Publishing House for their indefatigable efforts. As we look towards the horizon of pediatric research and practice, your commitment and contributions shape the future of child health.

**Sincerely,**
**Assoc. Prof. Dr. Ali Tekin**

J Pediatr Res 2024;11(1):1-10
DOI: 10.4274/jpr.galenos.2024.08624

Original Article

# Exploring the Predictive Role of Inflammatory Markers in Neuropathic Bladder-Related Kidney Damage with Machine Learning

Su Özgür[1], Sevgin Taner[2], Gülnur Gülnaz Bozcuk[3], Günay Ekberli[4]

[1]Ege University, Translational Pulmonary Research Center-EgeSAM, İzmir, Turkey
[2]Adana City Training and Research Hospital, Clinic of Pediatric Nephrology, Adana, Turkey
[3]Adana City Training and Research Hospital, Clinic of Pediatrics, Adana, Turkey
[4]Adana City Training and Research Hospital, Clinic of Pediatric Urology, Adana, Turkey

**ABSTRACT**

**Aim:** The main objective of this study was to predict upper urinary tract damage utilizing novel approaches, such as machine learning models, by incorporating simple predictors alongside established radiological and clinical factors.

**Materials and Methods:** In this retrospective study, a total of 191 patients who underwent blood tests, urine analysis, imaging, and urodynamic studies (UDS) in order to assess their nephrological and urological status were included. Basic statistical analyses were conducted using IBM SPSS Version 25. A significance level of $p<0.05$ was employed to establish statistical significance. The machine learning analyses were performed on Ddsv4-series Azure Virtual Machines, equipped with 32 vCPUs with a memory capacity of 128 GiB.

**Results:** In the model where clinical and imaging data were jointly assessed, the k-nearest neighbor (KNN) model demonstrated the highest performance, achieving values of 0.813 area under the curve and 0.854 accuracy. For the KNN Model, the best predictors for kidney function loss were as follows: neutrophil/lymphocyte (1.0577), abnormal bladder in ultrasound (1.054), vesicoureteral reflux (0.901), ferritin (0.898), neutrophil/albumin (0.678), platelet/lymphocyte (0.619), increased detrusor leakage pressure (0.435), age (0.3505), decreased bladder capacity in urodynamics (0.3009), and white blood cell (0.266).

**Conclusion:** Based on our findings, initial patient evaluation through basic blood and urine tests, ultrasonography, UDS, and voiding cystourethrography is crucial for identifying risk factors and preventing renal damage. Complete blood count-derived inflammatory biomarkers offer cost-effective and accessible alternatives to other radiological tools in primary care settings. These machine learning models may hold clinical relevance in pre-clinical or resource-limited hospitals, by guiding clinicians in implementing preventative measures.

**Keywords:** Neuropathic bladder dysfunction, kidney damage, inflammatory markers, machine learning, k-nearest neighbor, random forest

## Introduction

Neuropathic bladder dysfunction (NBD) occurs as a result of a lesion at any level of the central nervous system (1). The most common pathology causing NBD is spinal dysraphism. Tethered cord, spinal cord tumors, spinal cord injuries, cerebral palsy, anorectal malformations, and posterior urethral valve are other pathologies of spinal cord causing NBD (2). Elevated bladder pressure leads to vesicoureteral reflux (VUR), upper urinary tract dilatation (UUTD), structural bladder changes and renal insufficiency

**Address for Correspondence**
Sevgin Taner, Adana City Training and Research Hospital, Clinic of Pediatric Nephrology, Adana, Turkey
**Phone:** +90 505 312 07 78 **E-mail:** sevgintaner@gmail.com **ORCID:** orcid.org/0000-0003-1578-789X
**Received:** 10.01.2024 **Accepted:** 07.02.2024

in patients with NBD (3). High intravesical pressure transmitted to the upper urinary tract causes decreased glomerular filtration and impaired urine flow from the renal collecting system to the bladder. As a common result of his pathology, progressive damage and kidney failure develop (4). An initial evaluation of patients with ultrasonography (US), voiding cystourethrography (VCUG), 99m Technetium Dimercaptosuccinic acid (DMSA), and blood and urine test can guide clinicians in terms of early diagnosis, treatment and the possible avoidance of negative consequences in the future. Identifying risk factors and indicators of upper urinary tract damage and progression to chronic kidney disease is an important cornerstone in the monitoring of these patients. Knowing the prognostic indicators in terms of upper system damage can guide medical and surgical treatment in order to prevent kidney damage and can also protect those patients who do not require medical or surgical treatment from undergoing unnecessary and troublesome advanced imaging.

Machine learning is increasingly being utilized in healthcare services. It demonstrates better accuracy in diagnosing diseases such as VUR and urinary tract infection (UTI), which are often challenging to differentiate in the clinic, by employing decision support algorithms. It provides significant support to clinicians in early diagnosis. While some biomarkers are utilized in clinical practice for the early diagnosis of NBD in the literature, there are no machine learning studies employing innovative approaches. This was the first study on this topic using machine learning techniques.

It is crucial to extract meaningful information from complex patterns in clinical data. Our objectives in this patient group were to make an early diagnosis of UUTD and, more importantly, to identify those patients at risk of UUTD. In this way, it may be possible to prevent kidney damage by using more aggressive diagnostic and treatment methods in this patient group.

## Materials and Methods

The electronic medical records of patients diagnosed with neuropathic bladder in the Pediatric Urology and Nephrology units at Adana City Training and Research Hospital were retrospectively reviewed. Ethical approval was obtained from the Adana City Training and Research Hospital Clinical Research Ethics Committee (approval no.: 1367, date: 08.04.2021).

A total of 191 patients who underwent blood tests, urine analysis, imaging, and urodynamic studies (UDS) for evaluating nephrological and urological status were included in this study. Those patients under the age of one year were excluded in terms of a time period for renal scar development. Demographic characteristics, medical history, and laboratory and imaging results were documented. Differentiated kidney functions and scarring were evaluated via Tc-99m DMSA scan. The presence of more than a 10% decrease in differentiated kidney functions in scintigraphic evaluation was considered as loss of kidney function (5).

Accordingly, those patients with loss of kidney function were categorized as Group 1, and those patients without loss of kidney function were categorized as Group 2.

### K-Nearest Neighbor Algorithm

K-nearest neighbors (KNN) is a standard machine learning method which has been extended to large-scale data mining efforts. Test samples are classified to the class most frequently occurring among the KNN in a multidimensional parameter space. Despite its simplicity, this method has a sound theoretical basis in non-parametric density estimation and can often outperform much more sophisticated methods. The method requires only the choice of k, the number of neighbors to be considered when making the classification. Small values of k will select the closest training points, which are best able to estimate the correct classification at the test point. Usually, k is chosen as the value which minimizes the classification error on some independent validation data or through cross-validation procedures (Figure 1) (6).

### Random Forest

Random forest (RF) is an ensemble learning algorithm widely used for both classification and regression tasks in machine learning. It operates by constructing a multitude of decision trees during training and outputs the mode of the classes (classification) or the mean prediction (regression) of the individual trees (Figure 2) (7).

### Variable Importance

Variable importance in machine learning models refers to the measure of the impact which individual input features (variables) have on the model's predictive performance or the outcome of interest. It quantifies the degree to which each variable contributes to the model's ability to make accurate predictions. Variable importance helps in understanding which features are the most influential in making decisions, allowing practitioners to focus on the most relevant factors and so potentially improves model interpretability and generalization.

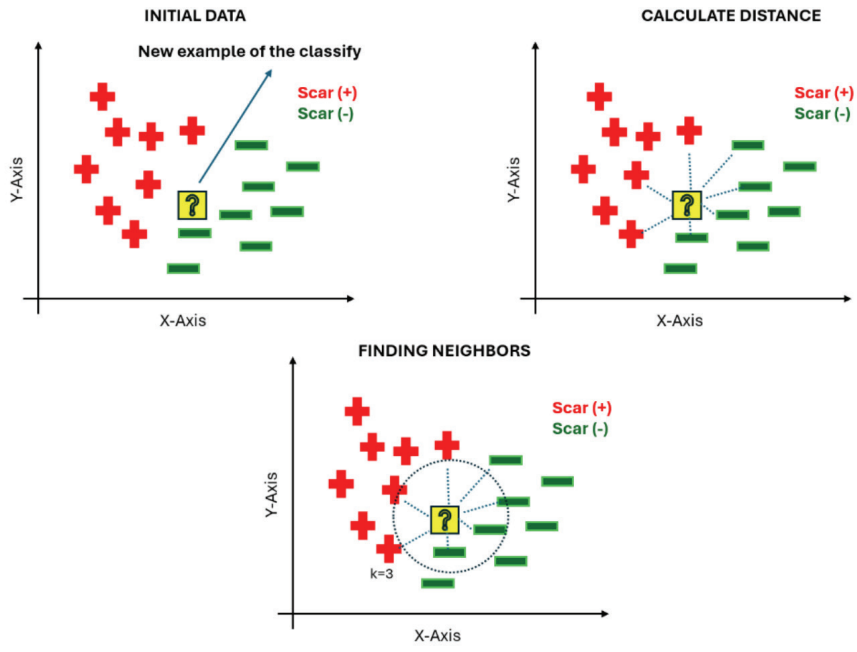


**Figure 1.** K-nearest neighbor (KNN) algorithm

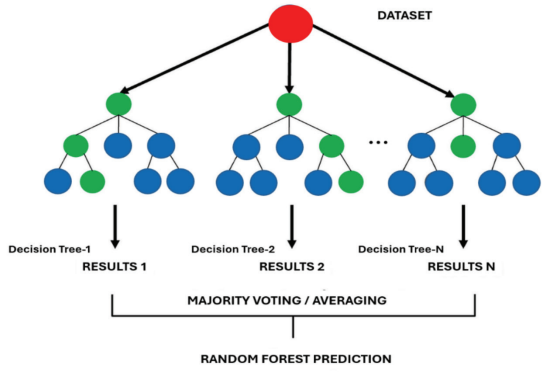


**Figure 2.** Random forest algorithm

Various techniques can be employed to gauge variable importance, including permutation importance, feature importance scores derived from algorithms like RF, or the analysis of coefficients in linear regression. These methodologies assign scores or rankings to each feature based on the degree to which the model's performance is compromised when a particular feature is altered or omitted (8).

### Performance Metrics

Performance metrics play a crucial role in evaluating the effectiveness of classification models, particularly those used in machine learning, statistics, and data analysis, with a focus on binary classification scenarios (Table I).

**Accuracy:** As a fundamental metric, accuracy calculates the ratio of correct predictions to the total number of predictions. Widely employed in medical machine learning applications, it should be interpreted cautiously, especially when handling imbalanced datasets.

**Area under the receiver operating characteristic curve (AUC-ROC):** Assessing a model's ability to distinguish between positive and negative classes, the AUC-ROC metric utilizes a visual representation through the ROC curve, where a higher value signifies enhanced class discrimination.

**Recall:** Also known as sensitivity or the true positive rate, recall quantifies the proportion of true positives relative to all actual positive instances. It is crucial in scenarios where the identification of all positive instances is of the utmost importance.

**Precision:** Precision gauges the ratio of true positive predictions among all positive predictions, emphasizing the minimization of false positive errors. This metric is particularly valuable when the cost associated with false positives is significant.

**F1 Score:** Representing the harmonic mean of precision and recall, the F1 Score provides a balanced single metric which accounts for both false positives and false negatives. It proves especially beneficial in addressing class imbalance.

**Matthews Correlation Coefficient (MCC):** Serving as a metric to evaluate the quality of binary classifications, MCC considers true positives, true negatives, false positives,

**Table I.** Performance measurements of the models

$$\text{Accuracy} = \frac{(TP + TN)}{(TP + TN + FP + FN)}$$

$$\text{Precision} = \frac{TP}{(TP+FP)} \qquad \text{Recall} = \frac{TP}{(TP+FN)}$$

$$\text{F1 score} = 2\,x\frac{\text{Precision x Recall}}{\text{Precision} + \text{Recall}}$$

$$\text{MCC} = \frac{TN\,x\;TP - FN\,x\,FP}{\sqrt{(TP + FP)\;(TP + FN)\;(TN + FP)\;(TN + FN)}}$$

| Diagnostic test | Gold standard | |
|---|---|---|
| | **Positive** | **Negative** |
| **Positive** | TP | FP |
| **Negative** | FN | TN |

TP: True Positive; TN: True Negative;
FN: False Negative; FP: False Positive

and false negatives. It offers a balanced measure, ensuring reliability even in the face of imbalanced datasets (9).

### Statistical Analysis

The sociodemographic and disease-related characteristics of the patients are presented using percentages (%), numbers (n), mean, and standard deviation (SD), as well as median, minimum, and maximum values. To compare the sociodemographic and disease-related characteristics between scar-positive and scar-negative cases, chi-square, Student's t-tests, and Mann-Whitney U tests were employed. IBM SPSS Version 25 was utilized for basic statistical analyses. A significance level of $p<0.05$ was considered when determining statistical significance.

The machine learning analyses were conducted using Ddsv4-series Azure Virtual Machines, featuring a vCPU count of 32 and a memory capacity of 128 GiB. The results and parameters of the best model obtained from the analyses conducted in Azure Automated ML (KNN and RF) are presented.

In the analyses of this study, both classical algorithms (RF, KNN, logistic regression) and innovative algorithms (XGBoost, LightGBM, Gradient Boosting) were employed. The findings section focuses on those models which achieved high performance in line with the research purpose.

## Results

A total of 191 patients who underwent blood tests, urine analysis, imaging, and UDS to assess nephrological and urological status were evaluated. The cases included in the study consisted of 62.3% (119) girls and 37.7% (72) boys ($p>0.05$).

The median age of children in Group 1 was 95 (3-207) months, whereas in Group 2 it was 63.5 (1-225) months, with a statistically significant difference ($p=0.005$). In Group 1, the median leak point pressure was 40 (7-100) cm $H_2O$, compared to 30 (5-120) cm $H_2O$ in Group 2 ($p>0.05$). The estimated glomerular filtration rate (eGFR) level in Group 1 [94.4 (8.02-215.48) mL/dk/1.73 $m^2$] was significantly lower than that in Group 2 [193.28 (22.72-476.54) mL/dk/1.73 $m^2$] ($p<0.001$). The median hemoglobin level in Group 1 [10.95 (8-15.8) g/dL] was notably lower ($p=0.002$). In Group 1, the median urea level was 30 (5-275) mg/dL, while in Group 2, it was 22 (5-323) mg/dL ($p<0.001$). The creatinine level in Group 1 [0.47 (0.11-5.2) mg/dL] was higher than that in Group 2 [0.23 (0.04-3)] ($p<0.001$). The neutrophile/lymphocyte ratio ($p>0.05$), platelet/lymphocyte ratio ($p>0.05$), neutrophile/albumin ratio ($p>0.05$), and systemic immune index ($p>0.05$) were similar between the groups. However, the frequencies of MSUG ($p<0.001$) and VUR ($p<0.001$) were higher in Group 1 (Tables II and III).

Two different approaches were employed in the prediction models. In Model 1, imaging findings were considered, while in Model 2, only demographic and clinical variables were utilized in cases where imaging information was unavailable. The variables that most significantly contributed to the differential diagnosis are presented in both Model 1 and Model 2 (Table IV, Figures 3 and 4).

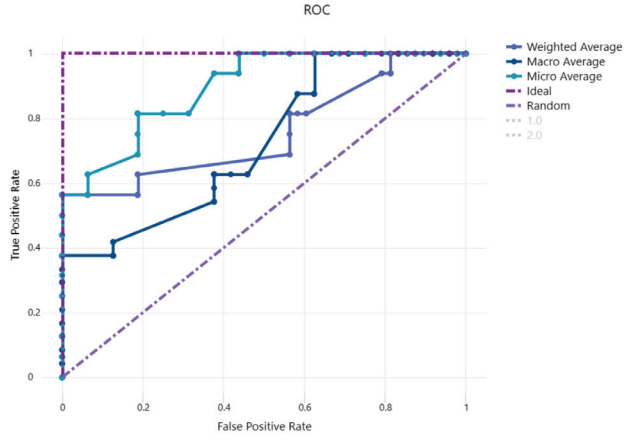


**Figure 3.** Model 2-performance for k-nearest neighbor

**Table II.** Laboratory characteristics of the patient with and without loss of kidney function

| Variables | Group 1: Loss of kidney function (+) n=51 (26.7%) | | Group 2: Loss of kidney function (-) n=140 (73.3%) | | Test statistics; p value |
|---|---|---|---|---|---|
| | Mean ± SD | Median (Min.-Max.) | Mean ± SD | Median (Min.-Max.) | |
| **Leak point pressure (cm $H_2O$)** | 42.43±27.75 | 40 (7-100) | 39.21±27.81 | 30 (5-120) | U=635.5 p=0.442 |
| **eGFR (mL/dk/1.73 $m^2$)** | 97.72±66.76 | 94.4 (8.02-215.48) | 204.66±85.27 | 193.28 (22.72-476.54) | U=372 p<0.001 |
| **Hemoglobin (g/dL)** | 11.2±1.55 | 10.95 (8-15.8) | 11.9±1.66 | 12.2 (6.5-15) | U=2431 p=0.002 |
| **MCV (fL)** | 77.17±9.15 | 78.65 (29.5-90.7) | 77.03±7.56 | 78.05 (49.4-98) | U=3199 p=0.537 |
| **WBC (x$10^3$/$mm^3$)** | 9.1±3.6 | 8.6 (4.5-20.0) | 8.6±2.7 | 8.3 (3.9-19.5) | U=1838.5 p=0.704 |
| **Neutrophil (x$10^3$/$mm^3$)** | 4.6±2.5 | 4.2 (1.8-15.2) | 3.9±1.7 | 3.7 (1.2-10.4) | U=1638.5 p=0.185 |
| **Lymphocyte (x$10^3$/$mm^3$)** | 3.3±15.2 | 3.2 (0.8-7.2) | 3.6±1.8 | 3.1 (1.2-12.2) | U=1809 p=0.603 |
| **Platelet (x$10^3$/$mm^3$)** | 325±102 | 338 (164-633) | 410±621 | 338 (153-650) | U=1668.5 p=0.236 |
| **Ferritin (mg/L)** | 61.63±72.08 | 35.85 (4-389) | 51.66±88.6 | 22.25 (2.5-49) | U=2270 p=0.063 |
| **Total protein (mg/dL)** | 40.23±33.36 | 47 (4.5-84) | 31.86±31.23 | 7.4 (3.7-81) | U=2593 p=0.013 |
| **Albumin (mg/dL)** | 20.19±19.03 | 4.4 (2.4-50.8) | 17.3±18.22 | 4.4 (2.6-47.8) | U=3089.5 p=0.663 |
| **Urea (mg/dL)** | 47.92±49.51 | 30 (5-275) | 25.16±27.96 | 22 (5-323) | U=2095.5 p<0.001 |
| **Creatinine (mg/dL)** | 0.86±1.01 | 0.47 (0.11-5.2) | 0.29±0.29 | 0.23 (0.04-3) | U=1775.5 p<0.001 |
| **Uric acid (mg/dL)** | 5.02±2.15 | 4.61 (2.04-10.08) | 3.55±1.18 | 3.44 (1.5-8.65) | U=1835.5 p<0.001 |
| **Cystatin C (ng/mL)** | 1.5±1.12 | 1 (0.41-6) | 0.91±0.53 | 0.8 (0.34-3.9) | U=1309 p<0.001 |
| **Parathormone level (pg/mL)** | 177.5±336.54 | 79.1 (12.8-2190) | 44.29±34.03 | 35 (7.1-271) | U=1437.0 p<0.001 |
| **Neutrophil lymphocyte ratio** | 1.67±1.32 | 1.28 (0.42-6.88) | 1.39±0.86 | 1.25 (0.15-3.9) | U=1688.5 p=0.276 |
| **Platelet lymphocyte ratio** | 0.12±0.07 | 0.1 (0.03-0.45) | 0.16±0.45 | 0.11 (0.03-4.64) | U=1849.5 p=0.743 |
| **Neutrophil albumin ratio** | 531.52±511.13 | 253.46 (41.3-1750) | 541.66±489.49 | 444.44 (26.09-1722.22) | U=1715.5 p=0.979 |
| **Systemic immune index** | 556.18±509.07 | 416.2 (143.58-2447.5) | 686.67±2067.07 | 363.35 (65.18-20892.86) | U=1806 p=0.593 |

U: Mann-Whitney U test statistics, p<0.05 Significance level
Min.-Max.: Minimum-maximum, SD: Standard deviation, MCV: Mean corpuscular volume, WBC: White blood cells, eGFR: Estimated glomerular filtration rate

**Table III.** Clinical and radiological characteristics of those patient with and those without loss of kidney function

| Variables | Loss of kidney function (+) (n=51) | Loss of kidney function (-) (n=140) | Total (n=191) | Test statistics; p value |
|---|---|---|---|---|
| **Clean intermittent catheterization** | 10 (25.0) | 35 (28.0) | 45 (27.3) | $X^2$=0.137 p=0.711 |
| **Oxybutynin use** | 4 (10.0) | 14 (11.2) | 18 (10.9) | $X^2$=0.045 p=0.832 |
| **Antibiotic prophylaxis use** | 10 (25.0) | 33 (26.4) | 43 (26.1) | $X^2$=0.031 p=0.861 |
| **Abnormal bladder in voiding cystourethrogram (VCUG)** | 38 (80.9) | 58 (43.3) | 96 (53.0) | $X^2$=19.72 p<0.001 |
| **Vesicoureteral reflux (VUR)** | 30 (63.8) | 31 (23.1) | 61 (33.7) | $X^2$=25.79 p<0.001 |
| **Abnormal bladder in US** | 34 (68.0) | 45 (32.6) | 79 (42.0) | $X^2$=18.869 p<0.001 |
| **Parenchymal thinning in US** | 24 (48.0) | 7 (5.1) | 31 (16.5) | $X^2$=49.11 p<0.001 |
| **Increased parenchymal echo in US** | 27 (54.0) | 134 (97.1) | 161 (85.6) | $X^2$=55.44 p<0.001 |
| **Hydronephrosis in US** | 36 (72.0) | 32 (23.2) | 68 (36.2) | $X^2$=37.88 p<0.001 |
| **Abnormal ureter in US** | 28 (56.0) | 14 (10.1) | 42 (22.3) | $X^2$=44.48 p<0.001 |
| **Decreased bladder capacity in urodynamics** | 19 (76.0) | 41 (56.9) | 60 (61.9) | $X^2$=2.997 p=0.319 |
| **Increased detrusor leakage pressure** | 18 (72.0) | 34 (52.3) | 52 (57.8) | $X^2$=2.87 p=0.090 |
| **Proteinuria** | 32 (69.6) | 42 (37.2) | 74 (46.5) | $X^2$=13.79 p<0.001 |
| **Recurrent urinary tract infections** | 18 (35.3) | 14 (10.1) | 32 (16.9) | $X^2$=16.972 p<0.001 |

$X^2$: Chi-square test statistics; p<0.05 Significance level, US: Ultrasonography

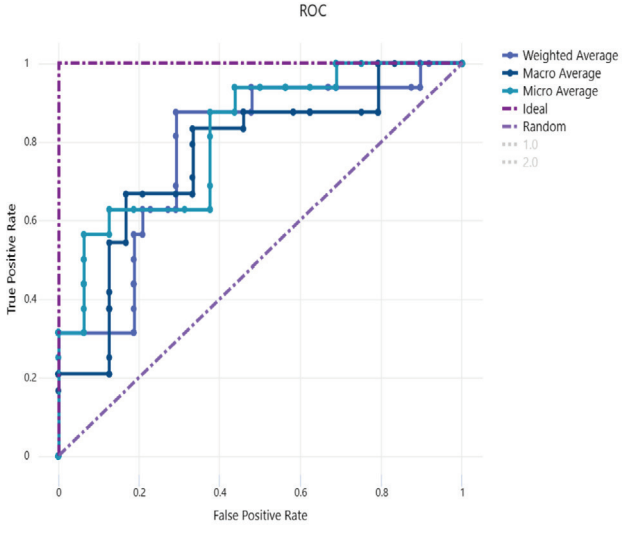


**Figure 4.** Model 3-performance for random forest

The variable importance obtained from KNN for Model 1 and RF for Model 2 are presented in Table IV. Accordingly, the ten variables which best predicted loss of kidney function for Model 2 are as follows: neutrophile/lymphocyte (1.0577) abnormal bladder in US (1.054), VUR (0.901), ferritin (0.898), neutrophile/albumin (0.678), platelet/lymphocyte (0.619), increased detrusor leakage pressure (0.435), age (0.3505), decreased bladder capacity in urodynamics (0.3009), and WBC (0.266). We used AUC (0.854), Accuracy (0.813), Precision (0.885), and Recall (0.625) as model performance criteria for the machine learning algorithm comparisons for Model 1 (KNN). Similarly, for Model 2 (RF), the variables determined to predict loss of kidney function were Ferritin (0.490), Age (0.172), neutrophile/albumin (0.093), neutrophile/lymphocyte (0.088), platelet/lymphocyte (0.082), sex (0.045), and WBC (0.018) (Figures 3 and 4). In order to assess the performance of machine learning algorithms, we employed criteria including AUC (0.833),

| **Table IV.** Results of the prediction models | | | |
|---|---|---|---|
| **Model 1: K-Nearest Neighbor** | | | |
| **Variables** | **Importance** | **Performances Metrics** | **Importance** |
| **Neutrophile/Lymphocyte** | 1.058 | **Accuracy** | 0.813 |
| **Abnormal bladder in US** | 1.054 | **AUC** | 0.854 |
| **Vesicoureteral reflux (VUR)** | 0.901 | **Precision** | 0.885 |
| **Ferritin** | 0.898 | **Recall** | 0.625 |
| **Neutrophile/Albumin** | 0.678 | **F1 score** | 0.767 |
| **Platelet/Lymphocyte** | 0.619 | **Mathews' Correlation** | 0.447 |
| **Detrusor leakage pressure** | 0.435 | | |
| **Age** | 0.350 | | |
| **Bladder Ccapacity in urodynamics** | 0.301 | | |
| **WBC (x10$^3$/mm$^3$)** | 0.266 | | |
| **Model 2: Random Forest** | | | |
| **Variables** | **Importance** | **Performances Metrics** | |
| **Ferritin** | 0.490 | **Accuracy** | 0.625 |
| **Age** | 0.172 | **AUC** | 0.833 |
| **Neutrophile/Albumin** | 0.093 | **Precision** | 0.823 |
| **Neutrophile/Lymphocyte** | 0.088 | **Recall** | 0.667 |
| **Platelet/Lymphocyte** | 0.082 | **F1 score** | 0.650 |
| **Sex** | 0.045 | **Mathews' Correlation** | 0.289 |
| **WBC (x10$^3$/mm$^3$)** | 0.018 | | |
| AUC: Area under the curve, WBC: White blood cells | | | |

accuracy (0.625), precision (0.823), and recall (0.667) for Model 3 (Table IV).

## Discussion

Studies have been conducted in order to assess the risk factors regarding UUTD (10-12) in patients with NBD. McGuire et al. (11) were the first to consider bladder changes as a risk factor for renal dilatation and VUR in children. Sixty-eight percent of patients with end-filling detrusor pressure more than 40 $H_2O$ had VUR and 81% had dilatation. Timberlake et al. (12) evaluated ultrasonographic findings and urodynamic parameters for the detection of risk factors in NBD. Higher detrusor pressure was reported to be related with renal scars. Additionally, trabeculation on US and the presence of VUR were also reported to be associated with renal scar (12).

Ekberli and Taner (13) conducted a retrospective review of patients diagnosed with neuropathic bladder. Logistic regression analysis was employed in order to identify significant predictors of scar formation in DMSA. Their results revealed a strong correlation between age, bladder changes observed in ultrasound and voiding cystogram, as well as high leak point pressure obtained during UDS, and upper urinary tract damage (13).

Li et al. (14) developed a predictive model for upper urinary tract damage in children with neurogenic bladder (NB). Their study revealed that recurrent UTI, bladder compliance, detrusor leak point pressure, overactive bladder, and clean intermittent catheterization are significant determinants of UUTD, and the efficacy of the model was validated. Univariate and multivariate logistic regression analyses were performed on the training cohort in order to identify predictors and create a nomogram. The nomogram exhibited strong discrimination, as indicated by the AUC-ROC in the training cohort [0.806, 95% confidence interval (CI): 0.737-0.874] and the validation cohort (0.831, 95% CI: 0.753-0.909). We achieved ROC values within the range of 83.3% to 85.4%, indicating a remarkably high level of predictive performance in our study (14).

In our study, leak point pressure was detected to be higher in the patient group with renal function loss. Also, abnormal ultrasonographic changes of the urinary tract,

the presence of VUR, recurrent UTI and bladder changes in VCUG can be considered as contributing factors for renal damage.

In addition to radiological evaluations, the accurate measurement of kidney function is essential in avoiding glomerular and tubular compromise. eGFR is defined as the GFR together with the serum creatinine value using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine-based formula and the updated Schwartz "bedside" formula for children (15,16). The GFR <90 mL/m/1.73 $m^2$≥ for 3 months with or without signs of renal damage (Shwartz) is defined as chronic renal disease. While evaluating our results, in line with the literature knowledge, eGFR values were found to be significantly lower in the group with loss of function than in the group without.

Another important parameter as renal lesions and chronic renal disease marker is protein excretion in urine. In children, protein excretion of <100 mg/$m^2$/day or <4 mg/$m^2$/hour in a 24 hr urine collection is considered normal. It is important to investigate proteinuria (up to 5 mg/kg/day in NB) as a marker of renal lesion (up to 5 mg/kg/day in NB) (17). In our study, there was a significant difference in proteinuria between the groups, consistent with the literature.

There is an increasing trend towards evaluating the potential value of hematological pro-inflammatory markers in the diagnosis and prognosis of various chronic diseases given the link between inflammation and changes in peripheral blood cells (18,19). Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune inflammation index are complete blood count (CBC)-derived inflammatory biomarkers which have been widely used in adult study populations (20,21).

Ohtaka et al. (22) noted considerable variations in the NLR at 1 and 3 months following renal transplantation. Patients with malignancies post-renal transplant exhibited a persistent increase in their NLR. Their research proposes that keeping track of the NLR in kidney transplant recipients may aid in the timely identification of malignancies. In our research, we identified neutrophil/lymphocyte, neutrophile/albumin, platelet/lymphocyte, and platelet/albumin ratios as significant predictors in anticipating kidney function loss (22).

Hobbs et al. (23) developed a machine learning algorithm which identified detrusor overactivity in UDS within the spina bifida population. Their time-based model exhibited the highest AUC at 91.9%, along with sensitivity values of 84.2%. Given the significant variability in individual interpretations of UDS data, there is a pressing need to standardize UDS interpretation. The successful development and implementation of machine learning algorithms in this population has the potential to be scaled to encompass all patients, both children and adults, experiencing lower urinary tract symptoms undergoing UDS. We introduced significant clinical models aimed at the early prediction of renal function loss, demonstrating high accuracy (81.3%) and AUC (85.4%) values in this study (23).

There are a limited number of studies recommending the use of the above-mentioned markers in the pediatric population (24,25). Nicoară et al. (24) in their study aimed to assess the relationship between CBC-derived inflammatory biomarkers and the presence of MetS in obese children. As a conclusion, they reported these markers which are inexpensive and universally available in primary care settings, to be an attractive alternative or addition to the frequently assessed inflammatory biomarkers (24). Another study in the pediatric obese patient population also revealed leukocyte, lymphocyte, erythrocyte, and platelet levels as being significantly higher in overweight/obese children but there was no significant difference between the groups regarding NLR and PLR (25).

In univariate analyses, statistical significance not identified between groups (loss of kidney function positive and negative cases) can gain significance through methods adept at extracting meaningful information from complex data structures, such as machine learning. Algorithms such as RF and KNN, which demonstrated the best performance in this research, can exhibit high efficacy in small datasets and scenarios where data relationships are less complex. Also, models generated using these approaches are often more straightforward to explain compared to boosting algorithms (26-28).

In this study, we achieved high-performance results by presenting models which both included and excluded imaging, when addressing a crucial clinical scenario such as the prevention of kidney loss. We believe that the increasing adoption of innovative approaches in clinical settings can enhance the precision of treatment plans for patients.

**Study Limitations**

This study was retrospective. Prospective studies are required to enhance the sensitivity of the models and enable real-time predictions.

The primary objective of this research was to identify crucial biomarkers for differential diagnosis in the NB and to promptly reveal predictive variables without directly impacting the patient. The results (XGBoost, LightGBM, Gradient Boosting, etc.) obtained from advanced machine learning methods employed in model development were not included.

## Conclusion

According to our results, it can be claimed that an initial evaluation of patients with basic blood and urine tests, US, UDS and VCUG is essential for the detection of risk factors and the prevention of renal damage. CBC-derived inflammatory biomarkers are inexpensive and more accessible compared to other radiological tools in primary care settings. These findings may have clinical relevance in pre-clinical settings or hospitals with limited resources and can guide clinician when taking preventative measures.

### Ethics

**Ethics Committee Approval:** Ethical approval was obtained from the Adana City Training and Research Hospital Clinical Research Ethics Committee (approval no.: 1367, date: 08.04.2021).

**Informed Consent:** Informed consent was obtained from all individual participants included in the study.

### Authorship Contributions

Surgical and Medical Practices: S.T., G.G.B., G.E., Concept: S.Ö., S.T., G.G.B., G.E., Design: S.Ö., S.T., Data Collection or Processing: S.Ö., S.T., G.G.B., G.E., Analysis or Interpretation: S.Ö., Literature Search: S.Ö., S.T., G.G.B., G.E., Writing: S.Ö., S.T., G.G.B., G.E.

**Conflict of Interest:** No potential conflict of interest was reported by the authors.

**Financial Disclosure:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

## References

1. Radmayr C, Dogan HS, Hoebeke P, et al. Management of undescended testes: European Association of Urology/ European Society for Paediatric Urology Guidelines. J Pediatr Urol 2016; 12:335-43.
2. Maerzheuser S, Jenetzky E, Zwink N, et al. German network for congenital uro-rectal malformations: first evaluation and interpretation of postoperative urological complications in anorectal malformations. Pediatr Surg Int 2011; 27:1085-9.
3. Liao L. Evaluation and Management of Neurogenic Bladder: What Is New in China? Int J Mol Sci 2015; 16:18580-600.
4. Lawrenson R, Wyndaele JJ, Vlachonikolis I, Farmer C, Glickman S. Renal failure in patients with neurogenic lower urinary tract dysfunction. Neuroepidemiology 2001; 20:138-43.
5. Ardela Díaz E, Miguel Martínez B, Gutiérrez Dueñas JM, Díez Pascual R, García Arcal D, Domínguez Vallejo FJ. Estudio comparativo de funcion renal diferencial mediante DMSA y MAG-3 en uropatías congénitas unilaterales [Comparative study of differential renal function by DMSA and MAG-3 in congenital unilateral uropathies]. Cir Pediatr 2002; 15:118-21.
6. Zhang Z. Introduction to machine learning: k-nearest neighbors. Ann Transl Med 2016; 4:218.
7. Rigatti SJ. Random Forest. J Insur Med 2017; 47:31-9.
8. Genuer R, Poggi JM, Tuleau-Malot C. Variable selection using random forests. Pattern recognition letters 2010; 31:2225-36.
9. Hicks SA, Strümke I, Thambawita V, et al. On evaluation metrics for medical applications of artificial intelligence. Scientific Reports 2022; 12:1-9.
10. DeLair SM, Eandi J, White MJ, Nguyen T, Stone AR, Kurzrock EA. Renal cortical deterioration in children with spinal dysraphism: analysis of risk factors. J Spinal Cord Med 2007; 30(Suppl 1):S30-4.
11. McGuire EJ, Woodside JR, Borden TA, Weiss RM. Prognostic value of urodynamic testing in myelodysplastic patients. J Urol 1981; 126:205-9.
12. Timberlake MD, Kern AJ, Adams R, Walker C, Schlomer BJ, Jacobs MA. Expectant use of CIC in newborns with spinal dysraphism: Report of clinical outcomes. J Pediatr Rehabil Med 2017; 10:319-25.
13. Ekberli G, Taner S. Risk determination for upper urinary tract damage in children with neuropathic bladder. J Paediatr Child Health 2023; 59:863-70.
14. Li Q, Cai M, Pu Q, et al. A nomogram for predicting upper urinary tract damage risk in children with neurogenic bladder. Front Pediatr 2022; 10:1050013.
15. Filler G, Gharib M, Casier S, Lödige P, Ehrich JH, Dave S. Prevention of chronic kidney disease in spina bifida. Int Urol Nephrol 2012; 44:817-27.
16. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. Clin J Am Soc Nephrol 2009; 4:1832-43.
17. Sager C, Barroso U Jr, Bastos JM Netto, Retamal G, Ormaechea E. Management of neurogenic bladder dysfunction in children update and recommendations on medical treatment. Int Braz J Urol 2022; 48:31-51.
18. Örgül G, Aydın Haklı D, Özten G, Fadiloğlu E, Tanacan A, Beksaç MS. First trimester complete blood cell indices in early and late onset preeclampsia. Turk J Obstet Gynecol 2019; 16:112-7.
19. Velioglu Y, Yuksel A. Complete blood count parameters in peripheral arterial disease. Aging Male 2019; 22:187-91.
20. Taha SI, Samaan SF, Ibrahim RA, Moustafa NM, El-Sehsah EM, Youssef MK. Can Complete Blood Count Picture Tell Us More About the Activity of Rheumatological Diseases? Clin Med Insights Arthritis Musculoskelet Disord 2022; 15:1-11.

21. Mercan R, Bitik B, Tufan A, et al. The Association Between Neutrophil/Lymphocyte Ratio and Disease Activity in Rheumatoid Arthritis and Ankylosing Spondylitis. J Clin Lab Anal 2016; 30:597-601.

22. Ohtaka M, Kawahara T, Takamoto D, et al. Neutrophil-to-Lymphocyte Ratio in Renal Transplant Patients. Exp Clin Transplant 2018; 16:546-9.

23. Hobbs KT, Choe N, Aksenov LI, et al. Machine Learning for Urodynamic Detection of Detrusor Overactivity. Urology 2022; 159:247-54.

24. Nicoară DM, Munteanu AI, Scutca AC, et al. Relationship between Systemic Immune-Inflammation Index and Metabolic Syndrome in Children with Obesity. Int J Mol Sci 2023; 24:1-15.

25. Mărginean CO, Meliț LE, Ghiga DV, Mărginean MO. Early Inflammatory Status Related to Pediatric Obesity. Front Pediatr 2019; 7:1-7.

26. Hastie T, Tibshirani R, Friedman J. The Elements of Statistical Learning. Springer New York, NY, 2009.

27. Breiman L. Random Forests. Machine Learning 2001; 45:5-32.

28. Ke G, Meng Q, Finley T, et al. LightGBM: A Highly Efficient Gradient Boosting Decision Tree. NIPS'17: Proceedings of the 31st International Conference on Neural Information Processing Systems 2017; 3149-57.

J Pediatr Res 2024;11(1):11-6
DOI: 10.4274/jpr.galenos.2024.82956

Original Article

# Effects of a Mobile Application to Improve Oral Hygiene in Children

Serap Sinan Özvarış, Dilşah Çoğulu

Ege University Faculty of Dentistry, Department of Pediatric Dentistry, İzmir, Turkey

**ABSTRACT**

**Aim:** Dental caries is one of the most common chronic diseases which affects oral health. Tooth brushing is considered the most effective method of preventing dental caries. Providing this motivation and ensuring this habit can be difficult, especially in children. Novel methods for improving tooth brushing habits are desired. This study aimed to investigate a mobile application's effectiveness in improving oral health in children.

**Materials and Methods:** Two hundred children between 5-12 years old who applied to the Department of Pedodontics, Ege University Faculty of Dentistry, for routine dental examination were included in this study. Children who had any systemic, physical, and/or mental disorders or those who had emergency dental complaints were not included in this study. A structured questionnaire including the child's oral hygiene habits was completed by the parents. In the clinical examination, the caries indices (DMFT/dmft and DMFS/dmfs), dental plaque, and gingival index scores of the children were recorded. Following this clinical examination, the "Brush DJ" mobile application was introduced to the children and their parents. After the first examination, the children were referred for their dental treatment and all were recalled after three months. At the recall examination, a second questionnaire was completed by the parents. The dental caries index (DMFT/dmft and DMFS/dmfs) scores, dental plaque, and gingival index scores were recorded again. A parental satisfaction questionnaire was administered to the parents. Statistical analysis was carried out using the SPSS 25.0 software program. The compliance of the parameters to the normal distribution was evaluated via the Shapiro-Wilk test. The Mann-Whitney U and Kruskal-Wallis tests were used for comparisons between the groups. For intra-group comparisons, the Wilcoxon test, chi-square test, Fisher's Exact test, and t-test were used. The results were evaluated at a 95% confidence interval and a statistical significance level of $p<0.05$.

**Results:** It was determined that 56.5% (n=113) of the children participating in this study were girls and 43.5% (n=87) were boys. One hundred and seventy-one of the 200 children were reported to be using the mobile application. It was observed that the tooth brushing frequency increased in 97 children due to their use of the mobile application. It was also observed that the tooth brushing duration increased in 143 of 171 children as a result of their use of the application. Initial plaque and gingival index scores (1.59±0.40; 1.18±0.40, respectively) were statistically higher than the recall examination scores (1.29±0.46; 1.09±0.49 respectively) ($p<0.05$). Based on the parental satisfaction survey data, it was observed that the use of the mobile app was effective in improving tooth brushing habits, and brushing became more regular and enjoyable in all children.

**Conclusion:** It was concluded that using the "Brush DJ" mobile tooth brushing app is effective in improving oral hygiene habits and making it a regular behavior in children. Long-term follow-up studies with a larger number of subjects comparing different oral hygiene education methods should be planned.

**Keywords:** Brush DJ, oral hygiene, pediatric dentistry

**Address for Correspondence**
Dilşah Çoğulu, Ege University Faculty of Dentistry, Department of Pediatric Dentistry, İzmir, Turkey
**Phone:** +90 532 430 33 31 **E-mail:** dilsah.cogulu@ege.edu.tr **ORCID:** orcid.org/0000-0002-3156-9801
**Received:** 19.02.2024 **Accepted:** 05.03.2024

## Introduction

Oral health plays an important role in protecting and maintaining general health. Oral and dental health problems are among the most common public health problems worldwide. Untreated oral and dental problems can often cause conditions which negatively affect general health, such as pain, chewing and feeding problems, loss of weight, and developmental deficiencies. Oral and dental health problems negatively affect quality of life (1-4).

Dental caries and periodontal diseases are among the most common oral and dental health problems. Dental caries affects 90% of the population (5-7). It has been observed that in developed countries with high socioeconomic status, these problems have started to decrease with proper oral hygiene habits, lower sugar consumption, widespread dental services, and regular dental visits (7-9). In developing countries, these problems continue to increase for many reasons; inadequate oral care and personal hygiene, inappropriate food and beverage consumption, irregular nutrition, inadequate preventive dentistry services, lack of regular dental visits, etc. (10-12).

Dental caries and periodontal diseases are caused by dental plaque, an organized form of biofilm. The most effective method to prevent dental plaque formation is brushing the teeth (13). Dentists need to encourage patients and their parents to practice these habits properly and regularly. These habits should be acquired from an early age through training which can be considered very easy and economical to implement. Training on oral and dental health has become an important part of preventive programs (14,15).

In recent years, mobile technology has revolutionized various aspects of our lives, including healthcare. One area where mobile apps have made a significant impact is oral hygiene. With the increasing accessibility of smartphones and the development of innovative dental care applications, individuals now have powerful tools at their fingertips to improve their oral health (16-18). Mobile apps serve as valuable educational tools, providing users with comprehensive information about proper oral hygiene practices, including brushing techniques, flossing methods, and the importance of regular dental visits. These apps often feature interactive tutorials, videos, and informative articles authored by dental professionals, empowering users to make informed decisions about their oral health (16,18).

Brush DJ, which was examined in the present study, was developed to motivate evidence-based oral hygiene routines and use the advantages offered by mobile applications. The British Pediatric Dentistry Association gave an award to Benjamin Underwood for creating the Brush DJ application (Outstanding Innovation Award 2018) (19).

The aim of developing this application was to encourage children to acquire improved brushing habits so as to contribute to their oral hygiene development. Within the scope of this application, reminders can be sent to the users on topics such as dental visits, how regularly they should replace their toothbrushes, the number of times they should brush their teeth per day, using mouthwash, etc. (19,20).

This study aimed to investigate the effectiveness of this mobile application, which can be downloaded freely to smartphones, in improving oral health in children.

## Materials and Methods

This study included 200 children between 5-12 years old who applied to the Department of Pedodontics, Ege University Faculty of Dentistry, for a routine dental examination, who did not have any systemic, physical, and/or mental illness, did not use any medication and who did not have any emergency dental complaints. Ethical approval was obtained from the Ege University Faculty of Medicine, Medical Research Ethics Committee (approval no.: 21-3.1T/49, date: March 18$^{th}$, 2021), and written informed consent was obtained from each parent.

An eleven-question survey was administered to the children and their parents before the clinical examination. Their initial oral and dental health status and oral hygiene habits were recorded. Dental caries (DMFT/dmft and DMFS/dmfs) (21) and periodontal indices [Silness & Löe plaque index (PI) and gingival index (GI)] scores were recorded (22,23).

Information was given about the "Brush DJ" application to the children and their parents and the application was installed on their smartphones. An alarm was set for the children to brush their teeth in the morning and in the evening. The application sends notifications such as "Good morning, time to brush your teeth" or "Good evening, time to brush your teeth" to remind the users. The application also reminds the users to brush their teeth for two minutes. During this period, it is aimed to make the act of brushing teeth more enjoyable for the children by playing music selected from the music lists in the application or the music lists from the user's smartphone. When the time is up, the music stops and the tooth-brushing action is terminated with a clapping effect.

To evaluate the effectiveness of this application, children were recalled three months later. In the third month follow-up, a nine-question survey was carried out in order to evaluate oral hygiene habits. Dental caries index (DMFT/dmft and DMFS/dmfs) scores, dental PI, and GI scores were recorded again. The Parental Satisfaction Survey consisting of five questions, scored out of 5 (1-5 points, from "strongly disagree" to "strongly agree") according to Likert scaling was performed on the parents.

### Statistical Analysis

Statistical analysis was carried out using the SPSS 25.0 (SPSS Inc., Chicago, Illinois, USA/Statistical Package for the Social Sciences) software program. The compliance of the parameters with normal distribution was evaluated with the Shapiro-Wilk test. The Mann-Whitney U and Kruskal-Wallis tests were used for comparisons between the groups. For intra-group comparisons the Wilcoxon test, chi-square test, Fisher's Exact test, and t-test were used. The results were evaluated at a 95% confidence interval and a statistical significance level of $p<0.05$.

## Results

The mean age of the 200 children was 8.13±2.10 years. It was determined that 56.5% (n=113) of the children were girls and 43.5% (n=87) were boys. One hundred and seventy-one of the 200 children were reported to be using the mobile application. It was observed that tooth brushing frequency increased in 97 children due to their use of this mobile application. It was also observed that the tooth brushing duration increased in 143 out of 171 children as a result of using this application.

A comparison of the baseline and follow-up caries index (DMFT/dmft and DMFS/dmfs) scores is given in Table I and II. No statistically significant differences were detected between these scores ($p>0.05$).

A comparison of the baseline and follow-up PI and GI scores is given in Table III. Baseline PI and GI scores were statistically significantly higher than the third-month follow-up scores ($p<0.05$).

**Table I.** Comparison of the baseline and check-up caries index scores for primary teeth

| | Baseline (T0) (Mean ± SD) | 3rd month follow-up (T1) (Mean ± SD) | p value |
|---|---|---|---|
| dmft | 6.10±3.72 | 6.16±3.74 | 0.236 |
| dmfs | 14.0±9.72 | 15.23±10.82 | 0.152 |

SD: Standard deviation

**Table II.** Comparison of the baseline and check-up caries index scores for permanent teeth

| | Baseline (T0) (Mean ± SD) | 3rd month follow-up (T1) (Mean ± SD) | p value |
|---|---|---|---|
| DMFT | 1.66±2.07 | 1.70±2.11 | 0.218 |
| DMFS | 2.78±3.85 | 2.81±3.88 | 0.391 |

SD: Standard deviation

**Table III.** Comparison of baseline and check-up periodontal index scores

| | Baseline (T0) (Mean ± SD) | 3rd month follow-up (T1) (Mean ± SD) | p value |
|---|---|---|---|
| PI | 1.59±0.40 | 1.29±0.46 | 0.00* |
| GI | 1.18±0.40 | 1.09±0.49 | 0.00* |

*p<0.05 Statistically significant
SD: Standard deviation, PI: Plaque index, GI: Gingival index

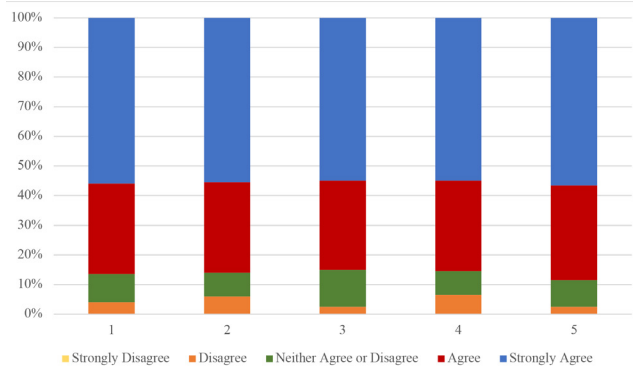


**Figure 1.** Responses for the parental satisfaction survey

The responses to the Parental Satisfaction Survey are given in Figure 1. Based on the parental satisfaction survey data, it was observed that the use of the mobile app was effective in improving tooth brushing habits, and brushing became more regular and enjoyable in all children.

## Discussion

It was aimed to effectively ensure oral hygiene in children by informing and motivating both children and their parents in the present study. Oral and dental health affects general health. Although the necessity and importance of oral hygiene habits in improving oral and dental health are known by many individuals, it has been observed that there are inadequacies in the implementation of these habits, especially in children. Oral and dental health depends on individuals practicing oral hygiene habits correctly and regularly (24,25). It is accepted that the most effective way to prevent dental caries and periodontal diseases is

through continuous individual oral hygiene practices and professional care (25).

Newly developed technologies and interactive applications create new environments and opportunities to provide health services. Using smartphones and the internet to communicate directly and quickly with healthcare providers can improve health management, especially for children and adolescents. Practices and studies on health are extremely important in terms of protecting and improving health. Mobile apps have emerged as valuable tools for promoting better oral hygiene practices and enhancing dental care outcomes. By providing education, reminders, personalized recommendations, and telehealth services, these apps empower individuals to take proactive control of their oral health. As technology continues to evolve, the integration of mobile apps into dental care workflows holds great promise for improving overall oral hygiene and reducing the prevalence of dental problems in the population (26). This study aimed to investigate a mobile application's effectiveness in improving oral health in children. For this reason, Brush DJ, a mobile application which can be downloaded freely on smartphones, was introduced to 200 children aged between 5-12 years old who were referred for routine dental examination.

At baseline and third-month follow-up visits, structured questionnaires were completed by the parents. The dental caries and periodontal index scores of the children were recorded. A parental satisfaction questionnaire was also administered to the parents.

Underwood et al. (19) also evaluated the user perception of the Brush DJ mobile tooth-brushing application. They reported that 77% of the participants stated that they brushed their teeth twice a day with the use of this application, and 88% stated that their duration of tooth brushing increased as a result of using this app (19). In the present study, 171 of the 200 children were reported to be using the mobile application. It was observed that the tooth brushing frequency increased in 97 children due to using this mobile application. It was also observed that the tooth brushing duration increased in 143 out of 171 children as a result of using the application.

When comparing the caries index (DMFT/DMFS, dmft/dmfs) scores, no statistically significant differences were detected between the baseline and follow-up scores in either the primary or the permanent teeth ($p>0.05$). It is thought that this result may change with long-term use of this mobile application.

In the study by Farhadifard et al. (27), in which the conventional oral hygiene education method and Brush DJ application were compared in patients receiving orthodontic treatment, it was reported that there was a significant decrease in PI and GI scores in both the control and test groups compared to the baseline scores and this decrease was higher in the group using the mobile application (27). Similarly, Alkadhi et al. (28) reported a decrease in PI and GI scores in the control and test groups, and these values were significantly lower in the mobile application group. Similar to the previous studies, the baseline PI and GI scores were statistically significantly higher than the third-month check-up scores in the present study ($p<0.05$).

It was also reported that 70% of the children started to brush their teeth more carefully and felt cleaner after using the Brush DJ mobile application (19). Similarly, in the present study, the rate of children who stated that they were motivated to brush their teeth at the end of the third month was 86%. Based on the parental satisfaction survey data, it was observed that the use of the mobile app was effective in improving tooth brushing habits, and brushing became more regular and enjoyable in all children. According to the responses to the parental satisfaction survey, it was observed that the parents were satisfied with their children using the Brush DJ mobile application.

## Conclusion

The Brush DJ mobile application, which can be downloaded freely to smartphones was developed to motivate oral hygiene routines. Given the increasing demand for health-related mobile applications and the societal trend toward using technology to support healthy behaviors, it is extremely important to investigate the effectiveness, efficiency, and acceptability of oral and dental health-related applications. The Brush DJ mobile application evaluated in the present study was found to be effective in motivating oral and dental health in children. It is thought that the results of this study will be a guide for future studies on oral and dental health education, which has an important place within the scope of preventive dentistry.

While mobile apps have the potential to improve oral hygiene practices significantly, there are certain challenges and considerations to address. These include ensuring the accuracy and reliability of the information provided within these apps, safeguarding user privacy and data security, and addressing disparities in access to technology among different demographics. Furthermore, it is essential to

promote evidence-based practices and collaborate with dental professionals in order to ensure that mobile apps complement, rather than replace, traditional dental care services.

Long-term follow-up studies with larger numbers of subjects comparing different oral hygiene education methods should be planned.

### Ethics

**Ethics Committee Approval:** Ethical approval was obtained from the Ege University Faculty of Medicine, Medical Research Ethics Committee (approval no.: 21-3.1T/49, date: March 18th, 2021).

**Informed Consent:** Written informed consent was obtained from each parent.

### Authorship Contributions

Surgical and Medical Practices: S.S.Ö., D.Ç., Concept: S.S.Ö., D.Ç., Design: S.S.Ö., D.Ç., Data Collection and/or Processing: S.S.Ö., D.Ç., Analysis and/or Interpretation: S.S.Ö., D.Ç., Literature Search: S.S.Ö., D.Ç., Writing: S.S.Ö., D.Ç.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Financial Disclosure:** The authors disclose that this research study was not funded by any specific grants from public, commercial, or not-for-profit sectors. The study was conducted independently without any financial support or sponsorship that could potentially introduce bias or influence the research outcomes.

## References

1. Haque SE, Rahman M, Itsuko K, et al. Effect of a school-based oral health education in preventing untreated dental caries and increasing knowledge, attitude, and practices among adolescents in Bangladesh. BMC Oral Health 2016; 16:44.
2. Heilmann A, Tsakos G, Watt RG. Oral Health Over the Life Course. In: Burton-Jeangros C, Cullati S, Sacker A, Blane D, eds. A Life Course Perspective on Health Trajectories and Transitions. Cham (CH): Springer 2015; 39-59.
3. Bernabé E, Masood M, Vujicic M. The impact of out-of-pocket payments for dental care on household finances in low and middle income countries. BMC Public Health 2017; 17:109.
4. Jung SH, Ryu JI, Jung DB. Association of total tooth loss with socio-behavioural health indicators in Korean elderly. J Oral Rehabil 2011; 38:517-24.
5. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005; 83:661-9.
6. Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis – a comprehensive review. J Clin Periodontol 2017; 44(Suppl 18):94-105.
7. Öztunç H, Haytaç MC, Özmeriç N, Uzel İ. Adana ilinde 6-11 yaş grubu çocukların ağız-diş sağlığı durumlarının değerlendirilmesi. GÜ Diş Hek Fak Derg 2000; 17:1-6.
8. Suni J, Helenius H, Alanen P. Tooth and tooth surface survival rates in birth cohorts from 1965, 1970, 1975, and 1980 in Lahti, Finland. Community Dent Oral Epidemiol 1998; 26:101-6.
9. Milen A, Hausen H, Heinonen OP, Paunio I. Caries in primary dentition related to age, sex social status, and county of residence in Finland. Community Dent Oral Epidemiol 1981; 9:83-6.
10. Şişko E, Dağhan Ş. Türkiye'de okul çağı çocuklarında ağız ve diş sağlığı araştırmalarının sonuçları bize ne söylüyor? STED 2022; 31:67-80.
11. Çelik ÖM, Ermumcu MŞK, Duran S, Şahin E. Evaluation of the relationship between oral care practices, food consumption and DMFT index in young adults. J BAUN Health Sci 2023; 12:516-24.
12. Pack AR. Dental services and needs in developing countries. Int Dent J 1998; 48:239-47.
13. Caries WI. Dental caries: etiology, clinical characteristics, risk assessment, and management. Sturdevant's Art Sci Oper Dent-E-Book 2017; 40.
14. Kandemir Ş, Atilla G. Islahevi çocuklarında farklı kişilerce uygulanan motivasyonun etkinliğinin karşılaştırmalı tetkiki. EÜ Diş Hek Fak Derg 1991; 12:56-60.
15. Tai B, Du M, Peng B, Fan M, Bian Z. Experiences from a school-based oral health promotion programme in Wuhan City, PR China. Int J Paediatr Dent 2001; 11:286-91.
16. Bhuyan SS, Lu N, Chandak A, Kim H, Wyant D, Bhatt J. Use of mobile health applications for health-seeking behavior among US adults. J Med Syst 2016; 40:153.
17. Tezcan C. Sağlığa yenilikçi bir bakış açısı: Mobil Sağlık. TÜSİAD Mobil Sağlık Raporu 2016.
18. Ventola CL. Mobile devices and apps for health care professionals: uses and benefits. PT 2014; 39:356-64.
19. Underwood B, Birdsall J, Kay E. The use of a mobile app to motivate evidence-based oral hygiene behaviour. Br Dent J 2015; 219:E2.
20. Chen R, Santo K, Wong G, et al. Mobile apps for dental caries prevention: Systematic search and quality evaluation. JMIR Mhealth Uhealth 2021; 9:e19958.
21. WHO. Oral Health Surveys Basic Methods. 5th Edition, 2013.
22. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22:121-35.
23. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967; 38:610-6.
24. Akkaya M, Boyraz M, Şevik N, Konak E. Bir grup öğrencide farklı motivasyon yöntemlerinin etkinliklerinin araştırılması. Atatürk Üniv Diş Hek Fak Derg 1994; 21:237-41.
25. Choo A, Delac DM, Messer LB. Oral hygiene measures and promotion: Review and Considerations. J Aust Dent 2001; 46:166-73.

26. Singh A, Wilkinson S, Braganza S. Smartphones and pediatric apps to mobilize the medical home. J Pediatr 2014; 165:606-10.

27. Farhadifard H, Soheilifar S, Farhadian M, Kokabi H, Bakhshaei A. Orthodontic patients' oral hygiene compliance by utilizing a smartphone application (Brush DJ): a randomized clinical trial. BDJ Open 2020; 6:24.

28. Alkadhi OH, Zahid MN, Almanea RS, Althaqeb HK, Alharbi TH, Ajwa NM. The effect of using mobile applications for improving oral hygiene in patients with orthodontic fixed appliances: a randomised controlled trial. J Orthod 2017; 44:157-63.

J Pediatr Res 2024;11(1):17-24
DOI: 10.4274/jpr.galenos.2024.80090

Original Article

# Why Infants with Some Inherited Metabolic Diseases do not Develop Neonatal Indirect Hyperbilirubinemia ? An Overlooked Detail

Gökçen Kartal Öztürk[1], Ayşe Korkmaz[2], Hasan Tolga Çelik[3], Şule Yiğit[3], Murat Yurdakök[3], Turgay Coşkun[4]

[1]Ege University School of Medicine, Department of Pediatrics, Division of Pediatric Pulmonology, İzmir, Turkey
[2]Acıbadem University School of Medicine, Department of Pediatrics, Division of Neonatology, İstanbul, Turkey
[3]Hacettepe University School of Medicine, Department of Pediatrics, Division of Neonatology, Ankara, Turkey
[4]Hacettepe University School of Medicine, Department of Pediatrics, Division of Pediatric Metabolism and Nutrition, Ankara, Turkey

**ABSTRACT**

**Aim:** Although indirect hyperbilirubinemia is the most common neonatal problem in term newborns, it is rarely observed in newborns with some inherited metabolic diseases. Therefore, we aimed to compare the frequency of indirect hyperbilirubinemia in newborns with these diagnoses and compare them with healthy newborns.

**Materials and Methods:** In the study group, term newborns with inherited metabolic diseases characterized by metabolic acidosis and/or hyperammonemia were included retrospectively and prospectively between January 1st, 2001, and December 31st, 2014. Healthy-term newborn infants were prospectively included in the control group.

**Results:** In the study group (n=106), 63.2% of the patients had organic acidemia, 20.8% urea cycle disorders, 4.7% mitochondrial diseases, 5.7% fatty acid oxidation disorders, and 5.7% other diseases, while the control group included 126 healthy term newborns. Mean serum indirect bilirubin levels were significantly lower in the study group compared to the control group (5.8±5.4 mg/dL vs 13.9±4.1 mg/dL, p<0.00, respectively). The frequency of phototherapy was 11.3% in the study group and 23.8% in the control group (p<0.05). While the incidence of jaundice was significantly lower in organic acidemia, urea cycle disorder, and fatty acid oxidation disorders (p<0.05), there was no difference in mitochondrial disease compared to the control group (p>0.05).

**Conclusion:** This was the first epidemiological study aiming to determine a very low incidence of neonatal jaundice in newborns with inherited metabolic diseases characterized by metabolic acidosis and/or hyperammonemia. The exact pathophysiological mechanism of this strikingly low incidence of indirect hyperbilirubinaemia in these newborns should be investigated with prospective biochemical, enzymatic, molecular, and genetic studies.

**Keywords:** Indirect hyperbilirubinemia, inherited metabolic diseases, metabolic acidosis, hyperammonemia, organic acidemia

## Introduction

Physiologic jaundice is defined as indirect hyperbilirubinemia. It occurs due to neonatal bilirubin metabolism in term infants, appearing after 24 hours of age and it resolves by approximately 2 to 3 weeks of age. Neonatal indirect hyperbilirubinemia is the most common neonatal problem and it is observed in nearly 20-50% of term newborns in the first weeks of life (1,2). In Turkey,

**Address for Correspondence**
Gökçen Kartal Öztürk, Ege University School of Medicine, Department of Pediatrics Pulmonology, İzmir, Turkey
**Phone:** +90 544 729 13 76 **E-mail:** gokcen_kartal@hotmail.com **ORCID:** orcid.org/0000-0002-0793-9710
**Received:** 18.12.2023 **Accepted:** 29.02.2024

the frequency of jaundice requiring phototherapy in term newborn infants has been reported to range between 10-50% depending on the source (3-6).

Inherited metabolic diseases are a group of diseases which occur as a result of a deficiency of an enzyme or carrier protein in the synthesis or degradation pathways of protein, carbohydrate, and fat. Although their frequencies increase in certain racial and ethnic groups, especially in societies where consanguineous marriages are common, their general prevalence is 1:4,000-1:5,000 (7-9). Our unit is a reference center for pediatric inherited metabolic diseases in Turkey. For many years, we have noticed that indirect hyperbilirubinemia was very rare among newborns who had severe metabolic acidosis and/or hyperammonemia.

We thought that determining the accuracy of this very interesting observation could provide a different perspective on bilirubin metabolism and metabolic diseases. Therefore, we aimed to define the frequency of physiologic jaundice in newborns who had inherited metabolic diseases characterized by metabolic acidosis and/or hyperammonemia and compare this with the frequency of physiologic jaundice in healthy newborns.

## Materials and Methods

This study was conducted in the divisions of Neonatology and Pediatric Metabolic Diseases and Nutrition covering the period between January 1st, 2001, and December 31st, 2014. The Hacettepe University Non-invasive Clinical Research Ethics Committee approved this study (approval no.: GO-14/410, date: 23.07.2014) and informed consent forms were obtained from the parents of each patient.

### Study Group

The study group comprised term newborn infants who were hospitalized in the neonatal intensive care unit with a diagnosis of inherited metabolic disease which was characterized by metabolic acidosis and/or hyperammonemia within the first 28 days of life. Preterm infants and infants with congenital anomalies or chromosomal abnormalities were excluded.

In our unit, the diagnosis of metabolic disease is made by biochemical, metabolic, and molecular tests such as blood and urine amino acid levels, plasma lactic acid, and pyruvic acid levels, urine organic acid profile, plasma ammonium levels, carnitine profile and amino acid analysis, and genetic tests. The diagnosis of hyperammonemia was defined as a plasma ammonia level >100 µmol/L (10). The type of acid-base imbalance was determined according to the acid-base nomogram (11).

In the study group, demographic and clinical data [gender, gestational age, birth weight, intrauterine growth status (12)], delivery type, Apgar score at the 5th minute, the need for resuscitation at birth, the presence of perinatal hypoxia (13), clinical symptoms and signs, nutritional status (type of enteral feedings such as breast milk or formula), the development of indirect hyperbilirubinemia and the need for phototherapy or exchange transfusion, and laboratory data [complete blood count and peripheral blood smear, infant and maternal blood groups, serum total/direct and indirect bilirubin levels, glucose-6-P-dehydrogenase (G6PD) enzyme activity, serum thyroid-stimulating hormone (TSH) level, hepatic and renal function tests, blood gas analysis, plasma lactic acid, pyruvic acid, ammonia levels, urine and blood amino acids, tandem mass spectrometry and special metabolic examination results] were obtained from the medical records. The need for phototherapy or exchange transfusion for indirect hyperbilirubinemia and the prognosis (survival/mortality) of the patients were noted retrospectively (14).

### Control Group

As the control group, healthy-term newborn infants who were born at the department of obstetrics on single days of the month between September 1st and December 31st 2014 were prospectively included. Newborns diagnosed with any diseases during the study period were excluded.

These newborn infants were followed for at least 2 days at the hospital. After discharge, they were followed by physical examination or by telephone call once every week in order to monitor for the development of neonatal hyperbilirubinemia until the end of the 28th day of life. Demographic data and nutritional characteristics (breast milk, breast milk + formula, or formula feeding) were noted. Laboratory examinations for hyperbilirubineamia were performed in cases with suspected physiologic jaundice during their follow-up.

In our unit, for those newborn infants with physiologic jaundice, complete blood count and peripheral blood smear, serum total bilirubin, direct bilirubin, and indirect bilirubin levels, infant and maternal blood groups, and direct Coombs test are routinely performed. In newborns requiring phototherapy, G6PD enzyme activity, serum TSH, and urine and blood amino acids are examined. The need for phototherapy or exchange transfusion is determined

according to the nomogram of the American Academy of Pediatrics, Clinical Practice Guideline, Subcommittee on Hyperbilirubinemia (14).

### Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows 15.0, Chicago, USA) program. The Kolmogorov-Smirnov test was used to test the normality of the data distribution. Continuous parameters are presented as mean ± SD and median ($25^{th}$ and $75^{th}$ percentile). Categorical parameters are presented as percentages. The Mann-Whitney U test was used to compare the two groups. The relationship between categorical variables was analyzed using $X^2$ test (Pearson chi-square, Fisher's exact chi-square). Statistical significance was accepted as $p<0.05$.

## Results

The study group comprised 106 newborns with inherited metabolic diseases characterized by metabolic acidosis and/or hyperammonemia. A total of 156 babies were born during the study period. However, 30 babies with various diseases (congenital heart disease, genetic disease, etc.) were excluded from this study. The control group comprised 126 healthy-term newborns.

The demographic and clinical characteristics of the study and control groups are shown in Table I. Although the mean gestational ages of the two groups were similar, the mean birth weight in the study group was significantly lower than the control group as the frequency of small for gestational age infants was higher in the study group ($p<0.01$). The rate of cesarean delivery in the control group was higher ($p<0.05$).

The inherited metabolic diseases of the study group are given in Table II. The most frequent inherited metabolic disease group was organic acidemias and amino acid disorders (n=67, 63.2%). In the study group, 20 (18.9%) cases just had metabolic acidosis, 24 (22.6%) cases just had hyperammonemia and 30 (28.3%) cases had a combination of both metabolic acidosis and hyperammonemia.

**Table I.** Demographic and clinical characteristics of study and control groups

| | **Study group (n=106)** | **Control group (n=126)** | **p value** |
|---|---|---|---|
| **Gender (M/F), n (%)** | 60/46 (56.6/43.4) | 69/57 (54.8/45.2) | 0.779 |
| **Gestational age (weeks)*** | 38.6±1.2 (37.0-42.0) | 38.5±0.9 (37.0-41.1) | 0.242 |
| **Birth weight (grams)*** | 3.138±488 (2.015-4.230) | 3.307±417 (2.450-4.630) | **0.005** |
| **Small for gestational age, n (%)** | 12 (11.3) | 2 (1.6) | **0.002** |
| **Parental consanguinity, n (%)** | 68 (64.2) | 16 (12.7) | **0.002** |
| **Type of delivery (V/CS), n (%)** | 53/53 (50.0/50.0) | 42/84 (33.3/66.7) | **0.010** |
| **Apgar score ($5^{th}$ min)*** | 9.8±0.7 (6-10) | 9.8±0.6 (7-10) | 0.459 |
| **Resuscitation at birth, n (%)** | 10 (9.4) | 12 (9.5) | 0.981 |
| **Perinatal hypoxia n (%)** | 1 (0.9) | - | 0.457 |
| **Age at hospitalization (days)*** | 9.2±7.2 (0-28) | - | - |
| **Hospitalization duration (days)*** | 13.5±9.8 (1-50) | 2.2±1.1 (1-5) | - |
| **Accompanying disorders, n (%)**<br>Blood culture (+) sepsis<br>DIC<br>Multiple organ failure | <br>28 (26.4)<br>32 (30.2)<br>17 (16.0) | <br>-<br>-<br>- | - |
| **Blood pH*** | 7.29±0.16 (6.87-7.58) | - | - |
| **Plasma $HCO_3$ level (mmol/L)*** | 17.8±7.6 (3.7-40.9) | - | - |
| **Metabolic acidosis n (%)**** | 50 (47.2) | - | - |
| **Plasma ammonia level (µg/dL)*** | 271 (34-2.554) | - | - |
| **Hyperammonemia (>100 µmol/L), n (%)**** | 54 (50.9) | - | - |
| **Mortality, n (%)** | 14 (13.2) | - | - |

*Mean ± standard deviation,
**More than one laboratory abnormality can be found in each patient
V: Vaginal birth, CS: Caesarean section, DIC: Disseminated intravascular coagulation

| **Table II.** Inherited metabolic diseases in the study group (n=106) | |
|---|---|
| **Inherited metabolic diseases** | **n (%)** |
| **Organic acidemia and amino acid disorders**<br>Maple syrup urine disease (MSUD)<br>Methylmalonic acidemia<br>Propionic acidemia<br>Isovaleric acidemia | 67 (63.2)<br>29 (27.4)<br>18 (17.0)<br>14 (13.2)<br>6 (5.7) |
| **Urea cycle disorders**<br>Carbamoyl phosphate synthetase deficiency (CPS 1)<br>Ornithine transcarbamylase deficiency (OTC)<br>Argininosuccinate synthase deficiency (ASS)<br>Argininosuccinate lyase deficiency (ASL)<br>Others | 22 (20.8)<br>2 (1.9)<br>1 (0.9)<br>12 (11.3)<br>4 (3.8)<br>3 (2.8) |
| **Fatty acid oxidation disorders** | 6 (5.7) |
| **Mitochondrial disease** | 5 (4.7) |
| **Others**<br>Congenital lactic acidosis<br>Inherited disorders of gluconeogenesis<br>Pyruvate carboxylase deficiency<br>Glutathione synthetase deficiency | 6 (5.7)<br>2 (1.9)<br>1 (0.9)<br>1 (0.9)<br>2 (1.9) |

The comparison of the clinical and laboratory findings of the study and control groups in terms of neonatal hyperbilirubinemia is given in Table III. Laboratory examination was performed with the suspicion of physiologic jaundice in 78 of the term healthy newborns. The mean hemoglobin level was significantly lower and the frequency of hemolytic findings on peripheral blood smear was higher in the study group when compared to the control group (13.8±2.7 gr/dL vs 16.8±2.6 gr/dL, p<0.001, and 17.9% vs 3.2% p<0.01, respectively). However, serum mean total and indirect bilirubin levels and the frequency of phototherapy were found to be significantly lower in the study group (6.6±5.6 mg/dL vs 14.6±4.2 mg/dL, and 5.8±5.4 mg/dL vs 13.9±4.1 mg/dL, and 11.5% vs 23.8%, p<0.05, respectively).

The comparison of each inherited metabolic disease group in the study group and the control group in terms of neonatal hyperbilirubinaemia is shown in Table IV.

| **Table III.** Clinical and laboratory findings of the study and control groups in terms of neonatal hyperbilirubinemia | | | |
|---|---|---|---|
| **Clinical and laboratory findings** | **Study group (n=106)** | **Control group (n=126)** | **p value** |
| **Color of the skin, n (%)**<br>Normal (pink)<br>Light (pale)<br>Icteric (yellow) | <br>39 (36.8)<br>35 (33.0)<br>32 (30.2) | <br>42 (33.3)<br>9 (7.1)<br>75 (59.5) | <br>0.582<br>**0.000**<br>**0.000** |
| **Nutritional status**<br>Breast milk<br>Breast milk + Formula<br>Formula | <br>76 (71.7)<br>18 (16.9)<br>12 (11.4) | <br>69 (54.8)<br>37 (29.4)<br>20 (15.8) | <br>0.089<br>0.068<br>0.432 |
| **Onset (diagnosis) of jaundice (day)*** | 2.9±0.9 (2-5) | 2.8±0.9 (1-5) | 0.431 |
| | **n=106** | **n=78** | |
| **Hematocrit (%)*** | 40.5±7.9 (23.2-65.4) | 50.3±8.0 (33.8-66.4) | **0.000** |
| **Hemolytic findings on peripheral blood smear, n (%)** | 19 (17.9) | 4 (3.2) | **0.001** |
| **Maternal-fetal blood group incompatibility, n (%)**<br>Rh<br>ABO<br>Rh+ABO | 10 (9.4)<br>3 (2.8)<br>6 (5.7)<br>1 (0.9) | 31 (24.6)<br>13 (10.3)<br>15 (11.9)<br>3 (2.4) | 0.696 |
| **Positive direct Coombs test, n (%)** | 1 (0.9) | 4 (3.2) | 0.068 |
| **G6PD deficiency, n (%)** | - | 1 (0.8) | 1.000 |
| **High serum TSH, n (%)** | 3 (2.8) | 3 (2.4) | 0.082 |
| **Time of bilirubin measurement (day)** | 9.9±6.5 (1-28) | 9.5±3.8 (1-28) | 0.516 |
| **Total bilirubin (mg/dL)*** | 6.6±5.6 (0.2-22.0) | 14.6±4.2 (7.1-27.6) | **0.000** |
| **Direct bilirubin (mg/dL)**** | 0.6 (0.01-4.90) | 0.6 (0.3-2.7) | 0.541 |
| **Indirect bilirubin (mg/dL)*** | 5.8±5.4 (0.1-20.6) | 13.9±4.1 (6.5-26.3) | **0.000** |
| **Phototherapy, n (%)** | 12 (11.3) | 30 (28.3) | **0.014** |
| **Duration of phototherapy (hours)** | 32.0±11.8 (24-48) | 34±16.9 (24-96) | 0.712 |
| **Exchange transfusion, n (%)** | - | - | - |

*Mean ± standard deviation, **Median
G6PD: Glucose-6-P-dehydrogenase

**Table IV.** Comparison of specific inherited metabolic disease groups of the study group and control group in terms of neonatal hyperbilirubinemia

| | **Organic acidemia and MSUD[a] (n=67)** | **Urea cycle disorders[b] (n=22)** | **Mitochondrial disease[c] (n=5)** | **Fatty acid oxidation disorders[d] (n=6)** | **Control group[e] (n=126)** | **p value** |
|---|---|---|---|---|---|---|
| Hemoglobin (gr/dL)* | 13.4±2.4 (9.1-20.3) | 15.4±3.2 (8.1-22.1) | 12.4±2.4 (9.8-15.7) | 12.7±1.9 (10.0-15.3) | 16.8±2.6 (11.6-22.5) | **0.000**[a-e]<br>0.050[b-e]<br>**0.001**[c-e]<br>**0.000**[d-e] |
| Hematocrit (%)* | 39.5±7.1 (27.9-60.4) | 45.0±9.6 (23.2-65.4) | 36.1±6.4 (28.1-44.0) | 38.3±5.8 (30.0-46.4) | 50.3±8.0 (33.8-66.4) | **0.000**[a-e]<br>**0.017**[b-e]<br>**0.000**[c-e]<br>**0.001**[d-e] |
| Total bilirubin (mg/dL)* | 6.3±6.0 (0.2-22.0) | 6.7±4.9 (0.4-19.6) | 6.9±5.9 (0.35-13.7) | 5.9±2.6 (0.9-7.6) | 14.6±4.2 (7.1-27.6) | **0.000**[a-e]<br>**0.000**[b-e]<br>**0.000**[c-e]<br>**0.000**[d-e] |
| Direct bilirubin (mg/dL)** | 0.4 (0.0-4.7) | 0.8 (0.0-4.9) | 0.8 (0.1-2.9) | 0.8 (0.3-1.0) | 0.6 (0.3-2.7) | 0.052[a-e]<br>**0.000**[b-e]<br>**0.019**[c-e]<br>0.688[d-e] |
| Indirect bilirubin (mg/dL)** | 4.1 (0.1-20.6) | 4.3 (0.3-18.1) | 6.9 (0.2-10.8) | 6.1 (0.7-7.3) | 13.8 (6.5-26.3) | **0.000**[a-e]<br>**0.000**[b-e]<br>**0.000**[c-e]<br>**0.000**[d-e] |
| Clinical jaundice, n (%) | 25 (37.3) | 4 (18.2) | 2 (40.0) | 1 (16.7) | 75 (59.5) | 0.154[a-b]<br>0.795[a-c]<br>0.381[a-d]<br>**0.001**[a-e]<br>0.299[b-c]<br>0.933[b-d]<br>**0.000**[b-e]<br>0.409[c-d]<br>0.386[c-e]<br>**0.039**[d-e] |
| Phototherapy, n (%) | 10 (14.9) | 2 (9.1) | - | - | 30 (23.8) | 0.571[a-b]<br>0.378[a-c]<br>0.335[a-d]<br>0.087[a-e]<br>0.492[b-c]<br>0.452[b-d]<br>0.123[b-e]<br>1.000[c-d]<br>0.216[c-e]<br>0.176[d-e] |

*Mean ± standard deviation, **Median
[a]: Organic acidemia and MSUD, [b]: Urea cycle disorders, [c]: Mitochondrial disease, [d]: Fatty acid oxidation disorders, [e]: Control group
MSUD: Maple syrup urine disease

In the study group, the mean hemoglobin and hematocrit levels were significantly lower in all disease groups except for urea cycle disorders ($p<0.001$). The mean total and median indirect bilirubin levels were found to be significantly lower in all disease groups than in the control group ($p<0.001$). While the frequency of jaundice was significantly lower in the organic acidemia, urea cycle disorder, and fatty acid oxidation disorder groups when compared to the control group ($p<0.05$), there was no difference for mitochondrial disease ($p>0.05$). The frequency of phototherapy was lower in the inherited metabolic disease groups compared to the control group, but no statistically significant difference was found ($p>0.05$).

## Discussion

This is the first epidemiological study in the literature which has demonstrated that the incidences of neonatal jaundice and the need for phototherapy are very low in newborns

with certain inherited metabolic diseases characterized by metabolic acidosis and/or hyperammonemia. In most of these infants, serum indirect bilirubin levels were found to be even lower than the physiological levels. In our unit, the incidence of neonatal hyperbilirubinemia requiring phototherapy or exchange transfusion has been reported to be nearly 25% (15). However, our observation about the low incidence of neonatal hyperbilirubinemia in newborn infants with some inherited metabolic diseases has been an overlooked detail for many years.

In the literature, we could not find any research investigating the incidence of neonatal jaundice in cases of inherited metabolic diseases. We find it interesting that this topic had not attracted the attention of researchers until this time. Essentially, our research was mainly an epidemiological study, and therefore our discussion about the possible mechanisms of 'low indirect bilirubin levels in newborn infants with some inherited metabolic diseases' would be theoretical and speculative, depending on the existing literature.

This situation might be explained by the following possible enzymatic and genetic changes in heme metabolism; 1) HO enzyme inhibition, 2) Biliverdin reductase inhibition, 3) UDP-glucuronyltransferase (UDPGT) activation, 4) Beta-glucuronidase inhibition and reduction of enterohepatic circulation, and 5) Genetic polymorphisms or mutations which lead to metabolic changes.

Bilirubin production begins with the conversion of the heme ring into biliverdin by the microsomal rate-limiting HO enzyme (16,17). It has been accepted that HO-1, one of the three isoforms of the enzyme, and its enzymatic products; biliverdin and carbon monoxide, have important biological roles in the interaction between cells during vascular endothelial structuring. HO-1 is present in all tissues and vascular smooth muscle cells and it is regulated by hypoxia, inflammation, oxidant mediators, nitric oxide, and hemodynamic forces. It has been shown that extracellular acidosis and hypoxia induce HO-1 expression (18-22). In one study, the half-life of HO-1 mRNA increased from 3.5 hours to 6.5 hours by lowering the extracellular pH from 7.4 to 6.8 (23). Based on studies like this, it can be thought that extracellular acidosis may cause an increase in bilirubin production. However, this finding is not compatible with our pathophysiological hypothesis (inhibition of HO) or the results of our study. The effects of metabolic acidosis on HO-1 enzyme activity in other cells are not known.

Another step in bilirubin production is the reduction of biliverdin to bilirubin by the cytosolic biliverdin reductase enzyme. In human biliverdin reductase studies, with the use of urea as a denaturing agent, the enzyme activity increases up to 3.5 M urea concentration and decreases enzyme activity at higher concentrations (24). This finding is also against our pathophysiological hypothesis. In this case, in urea cycle disorders characterized by low serum urea levels, an increase in serum bilirubin level should have been observed due to the increase in biliverdin reductase enzyme activity. However, in our study, the mean serum indirect bilirubin level in cases with urea cycle disorder was found to be quite low compared to the control group.

The function of UDPGT, an enzyme responsible for the conversion of indirect to direct bilirubin, drug metabolism, and detoxification in the liver, is genetically determined by single gene polymorphisms and these lead to functionally altered protein or expression levels (25). In rat astrocyte cell culture, with an increase in the synthesis of proinflammatory cytokines and free oxygen radicals in the inflammation induced by lipopolysaccharide, glucuronidation activity also increased. It has been determined that this occurs with an increase of the mRNA of the UGT1A6 isoform (26). In addition, UGT1A1 activity was increased in diabetic and fasting rats in animal studies (27). In our study, it can be thought that metabolic acidosis, hyperammonemia, and accompanying infections induced UGT1A1 activity in the liver by increasing the inflammatory response and this contributed to the decrease in the indirect bilirubin level. However, this pathophysiological mechanism cannot be considered to be responsible for the entire clinical status. Furthermore, this mechanism does not explain the increased incidence of indirect hyperbilirubinemia in otherwise healthy newborns with neonatal sepsis or urinary tract infections.

Phenobarbital is one of the most important anticonvulsant drugs used in newborn infants. In addition, it induces the activity of the UGT1A1 enzyme and is therefore an important drug in newborns with prolonged indirect hyperbilirubinemia of unknown cause (28). In our study, phenobarbital was given as an anticonvulsant drug in 24.5% of the newborns with inherited metabolic diseases. It is suspected that phenobarbital could be one of the factors contributing to the low levels of indirect bilirubin levels in the study group.

Studies have shown that hyperammonemia causes disturbances in bilirubin metabolism in hepatocytes, but its pathophysiological mechanism is not known exactly (29). It is thought that the increase in ammonia affects the expression of enzymes in bilirubin metabolism by reducing energy synthesis. In another study, hyperammonemia was found to inhibit cell growth, induce

apoptosis, damage the mitochondria, and lead to a reduction in energy synthesis, eventually affecting the expression of enzymes related to bilirubin metabolism (30). "Multidrug resistance protein 2 (MRP2)" is an adenosine triphosphate dependent pump which enables the excretion of bilirubin glucuronides from hepatocytes to the bile ducts. Ammonia affects MRP2 expression by decreasing energy synthesis and it causes an increase in conjugated bilirubin in the serum by decreasing bilirubin excretion (31-33). Consistent with this information, the mean conjugated bilirubin level in urea cycle disorders was significantly higher than the control group, but the mean indirect bilirubin level was significantly lower than the control group.

The β-glucuronidase enzyme, which is present in high levels in the small intestine in newborns, converts conjugated bilirubin in its mono- or diglucuronide form back into unconjugated bilirubin, allowing it to enter enterohepatic circulation. This physiological event contributes to the development of physiological neonatal jaundice by increasing the indirect bilirubin load in the plasma and must be conjugated in the liver (34). There is no research in the literature on the activity of the β-glucuronidase enzyme or its genetic polymorphisms which alter activity in newborns with inherited metabolic disease. Theoretically, in the study group, it was expected that there was insufficient enteral nutrition due to feeding difficulties and vomiting which started in the first days of life. This leads to an increase in enterohepatic circulation and leads to a tendency to indirect hyperbilirubinemia. However, in the vast majority of these newborns, the indirect bilirubin level remained well below the physiological levels. Consequently, the hypothesis of β-glucuronidase inhibition is unlikely to be valid in the pathophysiological process.

In our study, the frequency of phototherapy was found to be similar in those cases with metabolic acidosis or hyperammonemia and those without. This finding suggests that the lower frequencies of physiologic jaundice and phototherapy in newborns with inherited metabolic diseases could be due to the primary metabolic disease rather than metabolic acidosis or hyperammonemia. There is no study on genetic polymorphisms or mutations related to both metabolisms in these infants. Therefore, it is difficult to comment on this issue.

### Study Limitations

The most important limiting aspect of our study was its retrospective evaluation of a part of the study group. It is recommended to investigate the alteration of heme metabolism with prospective biochemical and molecular studies in large case series.

## Conclusion

Many studies have shown that many enzymes involved in heme metabolism and indirect bilirubin have antioxidant and anti-inflammatory effects (35-38). However, the neurotoxic effect of high indirect bilirubin levels in newborns is also well known. The levels of indirect bilirubin which are beneficial as an antioxidant and those which are harmful as a neurotoxic molecule for the newborn are unknown. We wonder if the low indirect bilirubin levels we have determined in those newborns with inherited metabolic diseases characterized by metabolic acidosis and/or hyperammonemia are beneficial or detrimental to these newborns. The answers to these questions should be investigated with prospective biochemical, enzymatic, molecular, or genetic studies in light of the findings of our study.

**Key message:** Although it is the most frequent neonatal disorder, the frequency of neonatal jaundice has been observed to be lower in newborns with inherited metabolic diseases characterized mainly by metabolic acidosis and/or hyperammonemia.

### Ethics

**Ethics Committee Approval:** EThe Hacettepe University Non-invasive Clinical Research Ethics Committee approved this study (approval no.: GO-14/410, date: 23.07.2014).

**Informed Consent:** Informed consent forms were obtained from the parents of each patient.

### Authorship Contributions

Concept: G.K.Ö., A.K., Design: G.K.Ö., A.K., Data Collection and/or Processing: G.K.Ö., A.K., Analysis and/or Interpretation: G.K.Ö., A.K., H.T.Ç., Ş.Y., M.Y., T.C., Literature Search: G.K.Ö., A.K., Writing: G.K.Ö., A.K.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Financial Disclosure:** The authors disclose that this research study was not funded by any specific grants from public, commercial, or not-for-profit sectors. The study was conducted independently without any financial support or sponsorship that could potentially introduce bias or influence the research outcomes.

## References

1. Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2022; 150:e2022058859.
2. Madam A, MacMahon JR, Stevenson DK. Neonatal hyperbilirubinemia. In: Taeusch HW, Ballard RA, Gleason CA

(eds). Avery's Diseases of Newborn (8th ed.), Philadelphia: Elsevier Saunders, 2005:1226-9.

3. Sarici SU, Serdar MA, Korkmaz A, et al. Incidence, course, and prediction of hyperbilirubinemia in near-term and term newborns. Pediatrics 2004; 113:775-80.
4. Çayönü N, Bülbül A, Uslu S, et al. Indirect hyperbilirubinemia in newborn infants change over the last decade. Med Bull Sisli Etfal Hosp 2011; 45:85-93.
5. Celik HT, Günbey C, Unal S, Gümrük F, Yurdakök M. Glucose-6-phosphate dehydrogenase deficiency in neonatal hyperbilirubinaemia: Hacettepe experience. J Paediatr Child Health 2013; 49:399-402.
6. Alpay F, Sarici SU, Tosuncuk HD, Serdar MA, Inanç N, Gökçay E. The value of first-day bilirubin measurement in predicting the development of significant hyperbilirubinemia in healthy term newborns. Pediatrics 2000; 106:E16.
7. Leonard JV, Morris AA. Inborn errors of metabolism around time of birth. Lancet 2000; 356:583-7.
8. Coşkun T. Inherited metabolic diseases. Beksaç S, Demir N, Koç A, Yüksel A (eds.) Obstetric Maternal-Fetal Medicine and Perinatology. Ankara: Medical Network, 2001; 409-28.
9. Coşkun T. Diagnostic approach principles. Coşkun T, Yurdakök M (ed.). Inherited Metabolic Diseases in Newborns. Ankara: Güneş Medical Bookstore, 2014; 29-57.
10. Coşkun T. Hyperammonemia. Coşkun T, Yurdakök M (eds.). Inherited Metabolic Diseases in Newborns Ankara: Güneş Medical Bookstore, 2014; 67-82.
11. Cogan MG, Rector FC Jr. Acid-base disorders. In: Kidney (3rd ed) Brenner BM, Rector FC Jr (eds). Saunders, 1986; 457-517.
12. Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. BMC Pediatr 2003; 3:13.
13. Sills J. Perinatal asphyxia. In: Gomella TL (ed). Lange Neonatology (6th ed) New York: Mc-Graw Hill 2009:624-636.
14. American Academy of Pediatrics, Clinical Practice Guideline, Subcommittee on Hyperbilirubinemia. Management of the newborn 35 or more weeks of gestation. Pediatrics 2004; 114:297-316.
15. Tekinalp G, Yurdakök M, Yiğit Ş, Korkmaz A. Hyperbilirubinaemia. In: Tekinalp G, Yurdakök M, Yiğit Ş, Korkmaz A (eds.). Hacettepe Practices in Neonatal Care. 2nd Edition. Ankara: Güneş Medical Bookstore 2009:104-10.
16. Hansen TWR, Wong RJ, Stevenson DK. Molecular Physiology and Pathophysiology of Bilirubin Handling by the Blood, Liver, Intestine, and Brain in the Newborn. Physiol Rev 2020; 100:1291-346.
17. Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 2006; 86:583-650.
18. Hartsfield CL, Alam J, Cook JL, et al. Regulation of heme oxygenase-1 gene expression in vascular smooth muscle cells by nitric oxide. Am J Physiol 1997; 273:980-8.
19. Wagner CT, Durante W, Christodoulides N, et al. Hemodynamic forces induce the expression of heme oxygenase in cultured vascular smooth muscle cells. J Clin Invest 1997; 100:589-96.
20. Yet SF, Pellacani A, Patterson C, et al. Induction of heme oxygenase-1 expression in vascular smooth muscle cells. A link to endotoxic shock. J Biol Chem 1997; 272:4295-301.
21. Morita T, Perrella MA, Lee M, et al. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proc Natl Acad Sci USA 1995; 92:1475-9.
22. Siow RC, Ishii T, Sato H, et al. Induction of the antioxidant stress proteins heme oxygenase-1 and MSP23 by stress agents and oxidized LDL in cultured vascular smooth muscle cells. FEBS Lett 1995; 368:239-42.
23. Christou H, Bailey N, Kluger MS, Mitsialis SA, Kourembanas S. Extracellular acidosis induces heme oxygenase-1 expression in vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 2005; 288:2647-52.
24. Franklin E, Mantle T, Dunne A. Activation of human biliverdin-IXalpha reductase by urea: generation of kinetically distinct forms during the unfolding pathway. Biochim Biophys Acta 2013; 1834:2573-8.
25. Erichsen TJ, Ehmer U, Kalthoff S, et al. Genetic variability of aryl hydrocarbon receptor (AhR)-mediated regulation of the human UDP glucuronosyltransferase (UGT) 1A4 gene. Toxicol App Pharmacol 2008; 230:252-60.
26. Heurtaux T, Benani A, Moulin D, et al. Induction of UGT1A6 isoform by inflammatory conditions in rat astrocytes. Neuropharmacology 2006; 50:317-28.
27. Braun L, Coffey MJ, Puskas F, et al. Molecular basis of bilirubin UDP-glucuronosyltransferase induction in spontaneously diabetic rats, acetone-treated rats and starved rats. Biochem J 1998; 336:587-92.
28. Dennery PA. Pharmacological interventions for the treatment of neonatal jaundice. Semin Neonatol 2002; 7:111-9.
29. Jia B, Yu ZJ, Duan ZF, et al. Hyperammonaemia induces hepatic injury with alteration of gene expression profiles. Liver Int 2014; 34:748-58.
30. Bhatia V, Singh R, Acharya SK. Predictive value of arterial ammonia for complications and outcome in acute liver failure. Gut 2006; 55:98-104.
31. Nies AT, Keppler D. The apical conjugate efflux pump ABCC2 (MRP2). Pflugers Arch 2007; 453:643-59.
32. Jedlitschky G, Leier I, Buchholz U, et al. ATP-dependent transport of bilirubin glucuronides by the multidrug resistance protein MRP1 and its hepatocyte canalicular isoform MRP2. Biochem J 1997; 327:305-10.
33. Wang Q, Wang Y, Yu Z, et al. Ammonia-induced energy disorders interfere with bilirubin metabolism in hepatocytes. Arch Biochem Biophys 2014; 555-556:16-22.
34. Stevenson DK, Dennery PA, Hintz SR. Understanding newborn jaundice. J Perinatol 2001; 21(Suppl 1):S21-4; discussion S35-9.
35. Dennery PA. Evaluating the beneficial and detrimental effects of bile pigments in early and later life. Front Pharmacol 2012; 3:1-5.
36. Liao S-L. The role of bilirubin and phototherapy in the oxidative/antioxidant balance. Pediatr Neonatol 2015; 56:77-8.
37. Garbagnati E. Could hypobilirubinemia be bad? Pediatrics 1990; 85:379-80.
38. Dennery PA, Weng YH, Stevenson DK, et al. The biology of bilirubin production. J Perinatol 2001; 21(Suppl 1):S17-20; discussion S35-9.

J Pediatr Res 2024;11(1):25-9
DOI: 10.4274/jpr.galenos.2024.08456

Original Article

# Epigenetic Mechanisms of Genes Influencing Immune Response in Patients with Celiac Disease

Maşallah Baran[1*], Tülay Kılıçaslan Ayna[2*], Melek Pehlivan[3], Betül Aksoy[4], Aslı Özkızılcık Koçyiğit[2], Yeliz Çağan Appak[1], Aslı Eldem[5], Bumin Nuri Dündar[6], Mustafa Soyöz[2], İbrahim Pirim[2]

[1]İzmir Katip Çelebi University Faculty of Medicine, Department of Pediatric Gastroenterology, Hepatology and Nutrition, İzmir, Turkey
[2]İzmir Katip Çelebi University Faculty of Medicine, Department of Medical Biology; University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Tissue Typing Laboratory, İzmir, Turkey
[3]İzmir Katip Çelebi University Vocational School of Health Services, Department of Medical Laboratory Techniques, İzmir, Turkey
[4]University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Clinic of Pediatric Gastroenterology, Hepatology and Nutrition, İzmir, Turkey
[5]İzmir Katip Çelebi University Faculty of Medicine, Department of Medical Biology, İzmir, Turkey
[6]İzmir Katip Çelebi University Faculty of Medicine, Department of Pediatric Endocrinology, İzmir, Turkey
*Maşallah Baran and Tülay Kılıçaslan Ayna are equivalent authors in this study.

**ABSTRACT**

**Aim:** The goal of this study was to investigate the expression levels of microRNAs (miRNAs) (miR-196b, miR-10a, miR-31-5p, and miR-338-3p) which regulate the genes involved in the proliferation and function of cells functioning in the inflammatory processes in Celiac patients' blood and tissue samples. Celiac disease (CD) is an inflammatory disease which affects people who are genetically predisposed to gluten consumption. The only treatment for this disease is a gluten-free diet.

**Materials and Methods:** The miRNA expressions were determined in blood and tissue samples from 12 pediatric patients with CD and from 8 healthy children using quantitative real-time PCR (qRT-PCR) and SybrGreen dye. The gene expression levels of miRNAs such as miR-196b, miR-10a, miR-338-3p, and miR-31-5p were compared between the two groups.

**Results:** There was a significant difference only in *miR-10a* gene expression levels between the control and patient blood samples. The greatest difference between the tissue and blood samples within the CD group were found in the expressions of miR-31-5p and miR-338-3p. It was seen that the patients' human leukocyte antigen tissue type was not associated with their miRNA expression profiles. In addition, there was no significant correlation between their Marsh classification and gene expression levels.

**Conclusion:** The significantly low level of miR-10a may be related to CD due to its effect on the immune response. Additionally, miR-10a may have potential as a non-invasive biomarker in the diagnosis of CD.

**Keywords:** miRNA, Celiac disease, qRT-PCR, gene regulation

## Introduction

Celiac disease (CD) is an autoimmune enteropathy which develops in the intestine because of gluten digestion in genetically susceptible people. Human leukocyte antigen (HLA)-DQ2.5, DQ8, and DQ2.2 haplotypes are found in genetically susceptible individuals. When these people consume gluten-containing foods, their immune systems react. This response causes chronic inflammation, which

**Address for Correspondence**
Maşallah Baran, İzmir Katip Çelebi University Faculty of Medicine, Department of Pediatric Gastroenterology, Hepatology and Nutrition, İzmir, Turkey
**Phone:** +90 505 263 04 59 **E-mail:** mbaran2509@gmail.com **ORCID:** orcid.org/0000-0003-3827-2039
**Received:** 20.11.2023 **Accepted:** 30.01.2024

harms the duodenum mucosa. The molecular processes which may aid in diagnosis are not completely understood (1).

The histopathological determination of villous atrophy and an increase in intra-epithelial lymphocytes in duodenal biopsies was previously accepted as the gold standard method for CD diagnosis. It will be extremely valuable to discover a new biomarker which can be detected in a patient's blood and can provide information about CD, particularly before intestinal damage occurs in the early stages (2).

Mature microRNAs (miRNAs) are small non-coding single-stranded RNAs which are 18-25 bp in length. They primarily prevent translation from the target mRNAs. These small molecules could be used as diagnostic biomarkers, particularly in CD. Several studies have been conducted to determine the role of several proteins which are targets for miRNAs (miR-192, miR-195-5p, miR-449a, and miR-638) in the pathogenesis of CD (3).

The goal of this study was to compare the gene expression levels of miRNAs such as miR-196b, miR-10a, miR-338-3p, and miR-31-5p, which may play a role in the pathogenesis of CD, in pediatric patients with a healthy control group. Our experiment was unique in that there had not been a study linking miR-196b and miR-10a to the etiology of CD, and miR-338-3p and miR-31-5p had not been explored in pediatric celiac patients to date.

## Materials and Methods

### Sampling

This study was a prospective case-control study which included 12 duodenal biopsy and blood samples from pediatric celiac patients and 8 blood samples from healthy children between 2019 and 2021. CD was excluded with negative anti-tissue transglutaminase (tTG) IgA-IgG and negative endomysial antibody (EMA) IgA-IgG in the healthy children group. CD diagnosis was made by duodenal biopsy in those who had positive tTG IgA- IgG and EMA IgA-IgG (4). All patients were screened for IgA deficiency. The HLA types of all participants were determined by the molecular method. Tissue samples were collected in an RNA saver tube (A.B.T, Turkey) and then lysed with liquid nitrogen. For the control tissue, a tissue sample was taken from a patient who was biopsied with the suspicion of CD but was found not to have CD. The association between the miRNA expression profiles and the clinical features of the patients was investigated.

Signed informed consent forms were obtained from the participants before this study took place. This study was conducted according to the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital (approval no.: 01, date: 29.03.2019).

### miRNA Isolation from Sera and Tissue Samples

Sera samples were separated from whole blood samples by centrifugation at 4,000 rpm for 5 minutes. miRNAs were isolated from fresh sera samples according to the manufacturer's instructions using miRNeasy Serum/Plasma Advanced Kit (Qiagen, Germany).

Firstly, tissue samples were lysed with liquid nitrogen using a sterile mortar and pestle. Then, miRNA isolation from these lysed tissue samples was performed according to the manufacturer's instructions using NucleoSpin® miRNA Kit (Macherey-Nagel, Düren, Germany).

The purity and concentration of miRNAs were measured with the NanoDrop 2000 Instrument (ThermoScientific, Wilmington, Delmare USA). Those samples with a ratio of 1.9-2.2 were accepted as pure (5).

### cDNA Synthesis

cDNA synthesis was performed according to the manufacturer's instructions using the MiScript II RT Kit (Qiagen, Germany). Before usage, the cDNA samples were diluted 1/100 according to the kit's instructions (6).

### The Analysis of *miRNA* Gene Expressions by Quantitative Real-Time PCR (qRT-PCR)

The quality and concentration of the cDNA samples were tested by qRT-PCR using RNU6 reference primer pairs (Qiagen, ABD). Threshold cycle (Ct) values between 15 and 35 were accepted as positive. In cases of low expression of RNU-6 control in blood, miR-16 primer was designed as the second control (F: CCGGAGTAGCAGCACGTAAAT R: ATCCAGTGCAGGTCCGA) (7). miR-196b, *miR-10a, miR-31-5p, and miR-338-3p* gene expression levels were determined by using gene-specific primer assays (Qiagen, USA) and reference primers in all cDNA samples. qRT-PCR was carried out with SYBR Green in a Rotorgene system. qRT-PCR conditions were optimized as 15 minutes at 95 °C, 40 cycles of 15 seconds at 94 °C, 30 seconds at 55 °C, and 30 seconds at 70 °C. Each experiment was repeated three times (7).

### Statistical Analysis

Ct values above 35 were excluded from this study. The fold changes in gene expression values of all individuals

were calculated by the ΔΔCt method and RT² Profiler PCR sequence data analysis platform on the Qiagen website (https://dataanalysis2.qiagen.com/pcr). The relationships between the variables were determined by the chi-square test using the IBM SPSS Statistics 25 Software Program. The correlations between the variables were identified using the Pearson correlation test. All p-values <0.05 were accepted as statistically significant. The GraphPad Prism 9 Software Program was used to perform unpaired and paired Student's t-tests to compare mean Ct values between groups and within groups. The fold changes in the gene expression levels of the miRNAs in the patient group's biopsy and blood samples were compared with the control group of healthy children. Furthermore, the difference in the expression profiles in the miRNA values between the blood and tissue samples within the groups was determined.

## Results

The demographic and clinical features of the patient and control groups are given in Table I. One patient had type 1 diabetes mellitus (T1DM), and 11 patients were free from T1DM.

We found that 66.7% (n=8) of the patients and 37.5% (n=3) of the controls had the DRB1*03DQB1*02DQA1*05 haplotype. The DRB1*07DQB1*02DQA1*02 haplotype was found in 25% of the patients (n=3) and 12.5% of the controls (n=1), while the DRB1*04DQB1*03DQA1*03 haplotype was found in 41.7% of patients (n=5) and 25% (n=2) of controls. Figure 1 gives the frequencies of the HLA types which are clinically associated with CD. Accordingly, 83.3% (n=10) of the patients had DQ2.5 (DQB1*02 DQA1*05) and 8.3% (n=1) had DQ2.2 (DQB1*02 DQA1*02) alleles, while 41.7% had the DQ8 allele.

There were no significant differences in the *miR-196b* and *miR-338-3p* gene expression levels between the control and patient blood samples (p>0.05 and p>0.05, respectively). In the CD group, there was a significant decrease in the *miR 196b* gene expression levels in the blood in comparison to the tissue samples (p<0.05). Furthermore, there were significant differences in the *miR-338-3p* gene expression levels between the tissue and blood samples in the CD group (p<0.05). The *miR-31-5p* gene expression level was significantly different in the patient blood samples compared to their tissue samples (p<0.05). There was a significant difference between the control and patient blood samples for miR-10a (p<0.05). In addition, there was no significant difference between the tissue and blood samples in the CD group (p>0.05).

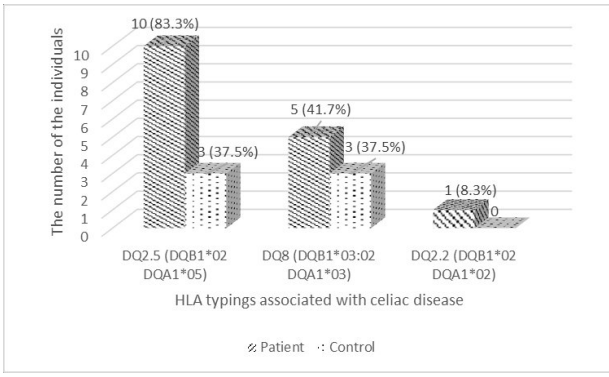


**Figure 1.** HLA-type frequencies in the patient and control groups
*HLA: Human leukocyte antigen*

The highest number of changes in gene expression in the patient blood and tissue samples were observed in the miR-338-3p expression. This expression decreased in seven patients (58.3%), while it increased in four patients (33.3%) in their blood samples. However, nine patients (75%) showed a decrease, while two (25%) showed an increase in their tissue samples.

Other modifications were not statistically significant. miR-10a had the greatest decrease in expression in patient blood (>2,000 fold) (Figure 2). The greatest decrease in patient tissues was observed in the expression of miR-31-5p (>500 fold). There was no statistically significant relationship between the patient's HLA tissue type and miRNA expression profiles (p>0.05). Additionally, there was no significant correlation between Marsh classification and gene expression levels (p>0.05). None of the patients had IgA deficiency and there was no significant correlation between the relative Ct values and the IgA levels of the patients (p>0.05).

**Table I.** Demographic and clinical information of the patient and control groups

| | | Patient group | Control group |
|---|---|---|---|
| **Age (years) mean ± SD** | | 7.67±3.52 | 6.62±3.58 |
| **Gender (% female)** | | 11 (91.7) | 5 (62.5) |
| **T1DM n (%)** | | 1 (8.3) | - |
| **Marsh classification n (%)** | **3a** | 1 (8.3) | - |
| | **3b** | 8 (66.7) | |
| | **3c** | 3 (25) | |

SD: Standard deviation, T1DM: Type 1 diabetes mellitus

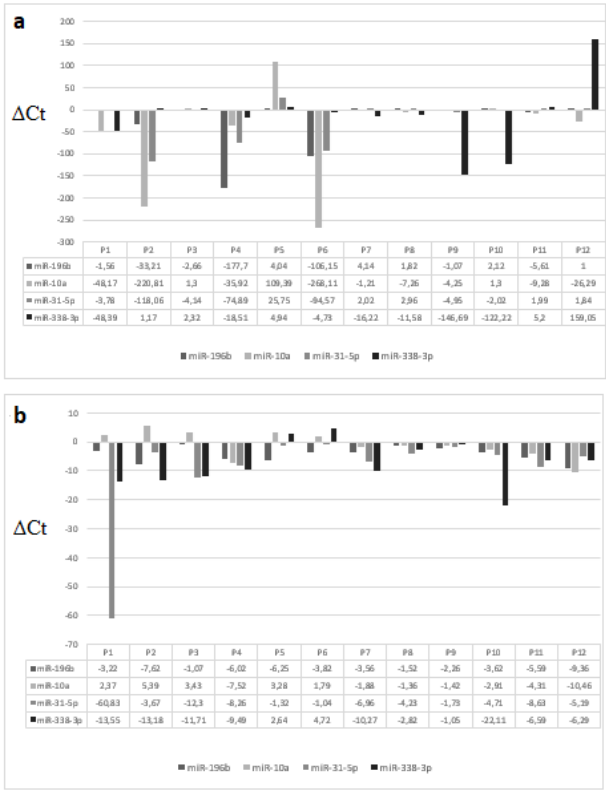


**Figure 2. a)** The gene expression changes of miRNAs in the blood samples of patients with celiac disease. **b)** The gene expression changes of miRNAs in the tissue samples of the patients with celiac disease
*miRNAs: microRNAs*

## Discussion

GATA-binding factor 6 (GATA6) is a zinc finger transcription factor which is involved in epithelial cell differentiation in the intestines (8). The GATA6 protein from the GATA transcription factor family is expressed in almost all gastrointestinal epithelial cells. It is important in intestinal epithelial cell proliferation and the development of colorectal cancer. GATA6 protein is suppressed by miR-196b (9). Although no studies have been conducted to directly link miR-196b to CD, it is thought that the GATA6 protein might play a role in the pathogenesis of this disease because it is involved in the transcription of some cytokine genes involved in the pro-inflammatory response. When we compared our patient group's blood samples to the control group, miR-196b expression was similar in the control and patient blood samples. In each group, the expression level was much lower in the tissue samples than in the blood samples. Due to its low expression, miR-196b may not have suppressed GATA6 protein synthesis in the patients, which may have resulted in the initiation of the inflammatory processes. In celiac patients, this miRNA has never been studied in blood samples. Felli et al. (10) found that miR-196a was downregulated in biopsy samples taken from celiac patients.

Bone morphogenetic protein 2 expression is suppressed by miR-10a. *In vitro*, this protein inhibited intestinal epithelial cell growth and induced apoptosis. It also promoted differentiation while suppressing proliferation (10). It has also been reported that this miRNA inhibits the release of the cytokine IL-12/IL-23p40, which is released by active macrophages and dendritic cells during innate and adaptive immune responses, and that it plays a role in the activation of NK cells and helper T-cell differentiation (11). In addition, it inhibits dendritic cell response, nucleotide-binding oligomerization domain 2 expression, and Th1/Th17 cell activations (12). Wu et al. (12) reported that miR-10a expression decreased in their patient group with inflamed bowel disease. Similarly, miR-10a expression was lower in the blood samples of the patients than in the control group in this study. These findings suggested that miR-10a could be used as a blood marker to predict CD.

It has been proposed that miR-31-5p is involved in the mitogen-activated protein kinase pathway, Wnt signaling, and cytoskeletal remodeling. It has been reported that miR-31-5p was downregulated in the serum of celiac patients compared to controls (13). Similarly, in our study, miR-31-5p expression was found to be lower in the blood samples of the patients when compared to the controls. Also, it was significantly downregulated in the patient's biopsy samples. By determining the levels of expression in biopsy samples, this miRNA may help in the diagnosis of this disease.

It has been demonstrated that in celiac patients, low expression of miR-338-3p increases the expression of innate and adaptive immune response proteins (14). According to Felli et al. (10), celiac patients have low expression, particularly in biopsy samples. The expression was similar in the control and patient blood samples. The expression levels in the tissue samples were lower than in the blood samples within the CD group.

The pathogenesis of CD is influenced by specific HLA alleles. The *HLA-DQA1* and *HLA-DQB1* genes are the main determinants which cause genetic susceptibility to this disease. While nearly all celiac patients have the HLA-DQ2 type, the remaining patients have the HLA-DQ8 heterodimer (15). HLA-DQ2 alleles were found in 91.7% (n=11) of our patients. However, the patients' HLA types were not associated with miRNA expression in our study.

### Study Limitations

Our study's limitations include that it was performed as a single-center analysis and involved a limited number of participants. In addition, the effects of epigenetic mechanisms on the clinical findings could not be evaluated due to the small number of patients.

## Conclusion

The expression levels of the miRNAs investigated in this study were found to be consistent with their molecular pathways and other published findings. When compared to the control group, all of the miRNAs had lower levels of expression in the patient blood samples than the control group. Among these, the significantly low level of miR-10a may be related to the autoimmune mechanism in CD due to its effect on the immune response. However, because of its similar expression levels in the blood and tissue samples from the CD group, particularly, miR-10a should be investigated further as a possible blood biomarker for the diagnosis of CD.

### Ethics

**Ethics Committee Approval:** This study was conducted according to the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital (approval no.: 01, date: 29.03.2019).

**Informed Consent:** Signed informed consent forms were obtained from the participants before this study took place

### Authorship Contributions

Surgical and Medical Practices: T.K.A., A.E., İ.P., Concept: M.B., M.S., Design: M.P., Y.Ç.A., B.N.D., İ.P., Data Collections or Processing: B.A., B.N.D., Analysis or Interpretation: M.P., A.E., M.S., Literature Search: A.Ö.K., Y.Ç.A., Writing: T.K.A., M.P., A.Ö.K.

**Conflict of Interest:** No potential conflict of interest was reported by the authors.

**Financial Disclosure:** This study was financially supported by Izmir Katip Celebi University Scientific Research Projects Coordination as a project of General Research Projects (Project no: 2020-GAP-TIPF-0008, 2020).

## References

1. Bascuñán KA, Pérez-Bravo F, Gaudioso G, et al. A miRNA-Based Blood and Mucosal Approach for Detecting and Monitoring Celiac Disease. Dig Dis and Sci 2020; 65:1982-91.
2. Tan IL, Coutinho de Almeida R, Modderman R, et al. Circulating miRNAs as Potential Biomarkers for Celiac Disease Development. Front Immunol 2021; 12:734763.
3. Domsa EM, Berindan-Neagoe I, Budisan L, et al. Expression of Selected Genes and Circulating microRNAs in Patients with Celiac Disease. Medicina 2022; 58:180.
4. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. JPGN 2012; 54:136-60.
5. Wan Y, Zhang Xi, Tang KD, et al. Overexpression of miRNA-9 enhances galectin-3 levels in oral cavity cancers. Mol Biol Rep 2021; 48:3979-89.
6. Wang G, Gu Y, Xu N, Zhang M, Yang T. Decreased expression of miR-150, miR146a, and miR424 in type 1 diabetic patients: Association with ongoing islet autoimmunity. BBRC 2018; 498:382-7.
7. Abolfathi H, Sheikhpour M, Mohammad Soltani B, Fahimi H. The comparison and evaluation of the miR-16, miR-155 and miR-146a expression pattern in the blood of TB and NSCLC patients: A Research paper. Gene Rep 2021; 22:100967.
8. Laudisi F, Stolfi C, Bevivino G, et al. GATA6 Deficiency Leads to Epithelial Barrier Dysfunction and Enhances Susceptibility to Gut Inflammation. JCC 2022; 16:301-11.
9. Fantini S, Salsi V, Reggiani L, et al. The miR-196b miRNA inhibits the GATA6 intestinal transcription factor and is upregulated in colon cancer patients. Oncotarget 2016; 8:4747-59.
10. Felli C, Baldassarre A, Masotti A. Intestinal and circulating microRNAs in coeliac disease. Int J Mol Sci 2017; 18:1-14.
11. Xue X, Feng T, Yao S, et al. Microbiota Downregulates Dendritic Cell Expression of miR-10a, Which Targets IL-12/IL-23p40. J Immunol 2011; 187:5879-86.
12. Wu W, He C, Liu C, et al. miR-10a inhibits dendritic cell activation and Th1/Th17 cell immune responses in IBD. Gut 2015; 64:1755-64.
13. Amr KS, Bayoumi FS, Eissa E, Abu-Zekry M. Circulating microRNAs as potential non-invasive biomarkers in pediatric patients with celiac disease. Eur J Allergy Clin 2019; 51:159-64.
14. Liu Q, Cui F, Wang M, et al. Increased expression of microRNA-338-3p contributes to production of Dsg3 antibody in pemphigus vulgaris patients. Mol Med Rep 2018; 18:550-6.
15. Sciurti M, Fornaroli F, Gaiani F, et al. Genetic susceptibility and celiac disease: what role do HLA haplotypes play? Acta Biomed Atenei Parmensis. 2018; 89:17-21.

J Pediatr Res 2024;11(1):30-7
DOI: 10.4274/jpr.galenos.2024.03710

Original Article

# Newborns are Prone to More Hypothermia in the Low Temperature of Operating Rooms

Eltun Guliyev, Cengiz Şahutoğlu, Canan Bor, Meltem Uyar

Ege University Faculty of Medicine, Department of Anesthesiology and Reanimation, İzmir, Turkey

**ABSTRACT**

**Aim:** Hypothermia (HT) is a common and serious problem during anesthesia. As the ratio of skin surface area to body volume is higher in neonates than in adults, heat loss and ultimately HT are more common in the intraoperative period. This study aimed to determine the incidence and independent risk factors of HT in the neonatal period.

**Materials and Methods:** This retrospective observational cohort study included 63 patients who underwent neonatal surgery within a one-year period. HT was defined as body temperature <36 °C and the patients were divided into two groups: Group I (body temperature <36 °C) and Group II (≥36 °C). Demographic data, ASA score, operative diagnosis, duration of surgery and anesthesia, amount of fluid, inotrope and vasopressor therapy, amount of bleeding, amount of blood transfusion, preoperative and postoperative temperatures, and the heating methods of the patients were recorded.

**Results:** The median age of the patients was 5 days (0-28 days) and their mean weight was 2,792±782 grams. The esophageal method was used for temperature monitoring in 88.9% (56 patients) and the rectal method in 10.1% (7 patients). HT developed in 54% of the patients. Caps and socks were used to prevent HT in 96.8% of the patients, forced-air warming blankets in 95.2%, warming gel mattresses in 27%, and fluid and blood warming devices in 17.5%. In the logistic regression model, the operating room temperature was identified as the only independent risk factor associated with neonatal HT.

**Conclusion:** Despite the use of active and passive warming methods, the incidence of HT in the neonatal period was found to be high. Cold operating rooms were found to be the only independent factor associated with neonatal HT.

**Keywords:** Neonate, hypothermia, pediatric anesthesia, body temperature, rewarmings

## Introduction

The neonatal (newborn) period refers to the first 28 days after the birth of a child. Neonates may undergo surgical procedures for a variety of reasons, many of which are emergency procedures. Maintaining a stable body temperature for patients in this age group during the perioperative period requires careful management of the balance between heat production and heat loss (1).

Hypothermia (HT) occurs in 70% of neonates during the intraoperative period, with several factors influencing body temperature, including vasodilating anesthetics, room temperature, systemic diseases, and/or intravenous cold fluids. Heat loss in neonates occurs by radiation, conduction, convection, evaporation, and respiration. Unlike adults, who can generate heat by shivering, neonates generate metabolic heat, relying primarily on brown adipose tissue. Premature infants have minimal brown fat, which affects

**Address for Correspondence**
Cengiz Şahutoğlu, Ege University Faculty of Medicine, Department of Anesthesiology and Reanimation, İzmir, Turkey
**Phone:** +90 232 390 21 42 **E-mail:** csahutoglu@yahoo.com **ORCID:** orcid.org/0000-0002-2664-4459
**Received:** 15.02.2024 **Accepted:** 25.02.2024

their heat production. In response to cold stress, newborns release norepinephrine from nerves in their brown adipose tissues in their back, neck, kidneys, and adrenal glands. This triggers the oxidation of fatty acids, which initiates lipolysis. Consequently, chemical thermogenesis occurs in the form of fat catabolism, as opposed to thermogenesis induced by shivering (1,2).

If no measures are taken to prevent heat loss, a drop in body temperature is inevitable in newborns. Basal metabolic rate and oxygen consumption are increased in order to maintain temperature and increase heat production. This can lead to hypoxia in patients or hypoglycemia as a result of the depletion of glycogen stores. Metabolic acidosis, pulmonary hypertension, and/or apnea may develop as a result of hypoxia and peripheral vasoconstriction (3,4). Complications such as disseminated intravascular coagulation, intraventricular hemorrhage, hypotension, severe sinus bradycardia, and/or increased mortality may occur in hypothermic infants. Despite the implementation of various precautions, HT may still occur in neonates due to a variety of factors (4,5).

The aim of this study was to investigate the incidence of HT in neonates and to identify any independent risk factors associated with its development.

## Materials and Methods

From November, 2019 to November, 2020, a total of 82 procedures were performed on 52 neonatal patients in the pediatric surgery operating room. This retrospective cohort study received approval from the Ege University Clinical Research Ethics Committee (approval no.: 20-12.1T/28, date: 17/12/2020), and informed consent was obtained from the parents of the patients. All the procedures were conducted in accordance with the Helsinki Declaration-2013. Of these, 63 procedures met the criteria for inclusion in this retrospective cohort study. Some patients underwent a single surgery, while others underwent multiple surgeries. Patients who underwent temperature monitoring by methods other than esophageal and rectal measurements were excluded from this study.

HT was defined as a decrease in body temperature below 36 °C (5,6). The patients were divided into two groups: Group I (patients with a core body temperature <36 °C, hypothermia group) and Group II (≥36 °C, normothermia group). Demographic data (age, weight, height, body surface area), American Society of Anesthesiologists Physical Status (ASA score), diagnosis, type of anesthesia, duration of anesthesia and operation, amount of fluid administered, inotrope and vasopressor treatment, amount of bleeding, amount of blood transfused, and the preoperative and postoperative temperatures of the patients were documented on the case report forms of the patients. This study's hypothesis was formulated as "Hypothermia is still prevalent at high rates in neonates". The primary objective of this study was to determine the incidence of HT, while its secondary objective was to identify any independent risk factors associated with neonatal HT.

### Statistical Analysis

This research utilized SPSS 21 (Statistical Package for the Social Sciences, IBM®) for statistical analysis. The Kolmogorov-Smirnov test was used to assess the normal distribution of the data. Descriptive statistics are presented as numbers (n) and percentages (%) for categorical variables, while mean and standard deviation are described by numerical variables.

Pairwise and multiple comparisons for categorical variables employed the chi-squared test and the Fisher's exact test. Quantitative variables underwent analysis through the independent t-test, one-way ANOVA test, and the Mann-Whitney U test. Binary logistic regression analysis was conducted in order to identify independent risk factors, ensuring the exclusion of multicollinearity. Statistical significance was set at $p<0.05$, with significance determined at the 95% confidence interval.

## Results

This study included 82 procedures performed on 52 neonates in the pediatric surgery operating room over a one-year period. Nineteen patients were excluded from this study due to axillary or tympanic temperature monitoring, leaving 63 procedures which met the criteria (patients monitored by rectal or esophageal methods) for inclusion in this study (Figure 1).

Of the patients included, 57.1% (36 patients) were male and 42.9% (27 patients) were female (p=0.344). The average age, weight, height, and body surface area (BSA) of all of the patients were 8.75±8.9 days (median: 5 days, range: 0-28 days), 2.79±0.78 kg (median: 2.8 kg, range: 0.88-4.5 kg), 49±3.5 cm (median: 50 cm, range: 40-60 cm), and 0.19±0.03 $m^2$ (median: 0.2 $m^2$, range: 0.1-0.27 $m^2$), respectively. Age, weight, height, BSA, and ASA were comparable between the two groups (Table I).

HT occurred in 54% of the patients (34 patients). While the preoperative body temperature was 35.95±0.78 °C in all patients, the postoperative temperature was 35.2±1.12 °C (p<0.001). The number of patients with a postoperative body temperature below 35 °C (32.3-35 °C) was 33.3%

| **Table I.** Demographic and preoperative data of the patients | | | | |
|---|---|---|---|---|
| | **All patients (n=63)** | **Hypothermia (n=34)** | **Normothermia (n=29)** | **p value** |
| Age (day)<br>Gender (male, %)<br>BSA ($m^2$)<br>ASA I/II/III<br>Preoperative temperature (°C) | 5 (0-28)<br>36 (57.1)<br>0.2 (0.1-0.27)<br>19/20/24<br>35.95±0.78 | 3 (0-26)<br>20 (58.8)<br>0.2 (0.14-0.26)<br>10/19/5<br>35.8±0.92 | 6 (0-28)<br>16 (55.2)<br>0.2 (0.1-0.27)<br>9/11/9<br>36.1±0.52 | 0.147<br>0.770<br>0.464<br>0.532<br>0.053 |
| Postoperative temperature (°C) | 35.2±1.12 | 34.42±0.88 | 36.1±0.52 | **<0.001** |
| Operating room temperature (°C) | 22.4±1.25 | 22.1±1.27 | 22.7±1.17 | 0.079 |
| Duration of operation (min) | 149±84 | 139±76 | 160±92 | 0.314 |
| Duration of anesthesia (min) | 169±85 | 156±70 | 183±99 | 0.212 |
| Amount of fluid (mL/kg/hr) | 15.5±7.2 | 16.9±6.4 | 13.9±7.7 | 0.108 |
| Urine output (mL/kg/hr) | 2.1±2.7 | 1.7±1.7 | 2.7±3.5 | 0.243 |
| Preoperative hematocrit (%) | 40.6±9 | 39.5±7.3 | 42.2±11.5 | 0.475 |
| Blood sugar level (mg/dL) | 79±44 | 75±42 | 85±46 | 0.500 |
| Continuous data are given as mean ± standard deviation, median (minimum-maximum). Dichotomous data are given as numbers (n) and percentages (%)<br>$m^2$: Square meter, ASA: The American Society of Anesthesiologists physical status classification system, °C: Degree celsius, min: Minute, mL: Milliliter, kg: Kilogram, hr: Hour, dL: Deciliter | | | | |

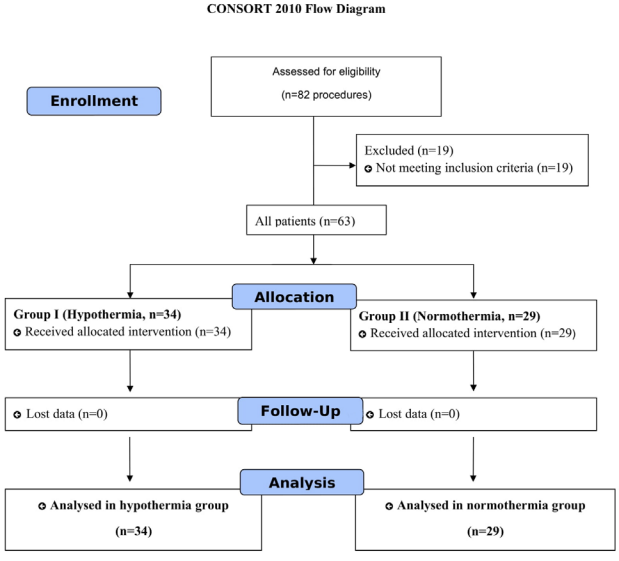


**Figure 1.** CONSORT flow chart of the data collection process

(21 patients), and there were no cases of hyperthermia (37.5 °C). Although basal (preoperative) body temperature was slightly lower in the HT group (35.8±0.92 °C vs. 36.1±0.52 °C), no statistical significance was observed (p=0.053). Temperature decreased in 68.3% of the patients, increased in 25.4%, and remained unchanged in 6.3%. Anesthesia and operating times were longer in the normothermic group, but these differences were not statistically significant (p>0.05). Although not statistically significant, the HT group showed a higher amount of fluid used (p=0.108). The perioperative characteristics of the patients are detailed in Table I.

| **Table II.** Preoperative diagnoses and percentage rates of the patients | | |
|---|---|---|
| | **n** | **%** |
| Tracheoesophageal fistula | 15 | 23.8 |
| Intestinal atresia | 10 | 15.9 |
| Omphalocele | 6 | 9.5 |
| Diaphragmatic hernia | 4 | 6.3 |
| Sacrococcygeal teratoma | 4 | 6.3 |
| Hirschsprung's disease | 3 | 4.8 |
| Necrotizing enterocolitis | 3 | 4.8 |
| Anorectal malformation | 2 | 3.2 |
| Exotropia vesica | 2 | 3.2 |
| Congenital lung malformation | 2 | 3.2 |
| Posterior ureteral valve | 2 | 3.2 |
| Others | 10 | 15.9 |
| Total | 63 | 100 |
| Others: Ambiguous genitalia, biliary atresia, inguinal hernia + undescended testicle, June syndrome, colon perforation, meconium ileus, gastric malrotation, midgut volvulus, ovarian torsion and umbilical hernia.<br>Data are given as numbers (n) and percentages (%) | | |

Some patients underwent intratracheal general anesthesia, most commonly for tracheoesophageal fistula (23.8%, 15 patients) and intestinal atresia (15.9%, 10 patients) (Table II). While open surgery was performed on 65.1% (41 patients), laparoscopic or thoracoscopic methods were used in 34.9% (22 patients). The choice of surgical method, such as open surgery, did not contribute to HT

(p=0.550). Temperature monitoring was performed using the esophageal method in 88.9% (56 patients) and the rectal method in 11.1% (7 patients). To prevent HT, cap and socks were used in 96.8% of the patients, forced air warming blankets in 95.2%, table-top warming gel mattresses in 27%, and fluid and blood warming devices in 17.5%. No significant relationship was observed between the heating methods and HT occurrence (p>0.05) (Table III).

Erythrocyte suspension (16.3±12 mL/kg) was administered to 7 patients (11.1%) because of intraoperative bleeding, with the amount of bleeding varying by 6.2±9 mL/kg (range: 5-120 mL). Twenty-seven patients (42.9%) were intubated and received mechanical ventilation support in the intensive care unit. Hypotension occurred in 22 patients (34.9%) during the intraoperative period, and vasoconstrictors were required in 17.5% of these cases due to a lack of response to fluid therapy. A significant association was found between HT and the development of complications (p=0.048) (Table IV).

To identify the risk factors associated with HT, a logistic regression model was constructed including age, sex, BSA, preoperative temperature, duration of anesthesia, active or passive warming method, open or laparoscopic surgery, operating room temperature, patient fluid administration rate (mL/kg/hour), bleeding volume, and preoperative hematocrit. Among these variables, only operating room

**Table III.** Heating methods used to protect patients from hypothermia

| Heating methods | All patients (n=63) | Hypothermia (n=34) | Normothermia (n=29) | p value |
|---|---|---|---|---|
| Cap | 61 (96.8) | 34 (100) | 27 (93.2) | 0.120 |
| Socks | 61 (96.8) | 34 (100) | 27 (93.1) | 0.120 |
| Forced air warming blanket | 60 (95.2) | 32 (94.1) | 28 (96.6) | 0.651 |
| Warming gel mattresses | 17 (27) | 8 (23.5) | 9 (31) | 0.504 |
| Fluid and blood warming device | 11 (17.5) | 6 (17.6) | 5 (17.2) | 0.966 |

Data are given as numbers (n) and percentages (%)

**Table IV.** Complications developing in the patients in the intraoperative and the postoperative periods

| Complications (yes) | All patients, n (%) | Hypothermia, n (%) | Normothermia, n (%) | p value |
|---|---|---|---|---|
| At least one complication | 28 (44.4) | 19 (55.9) | 9 (31) | 0.048 |
| Hypotension | 22 (34.9) | 14 (41.2) | 8 (27.6) | 0.259 |
| Use of vasoconstrictors | 11 (17.5) | 6 (17.6) | 5 (17.2) | 0.966 |
| Arrhythmia | 1 (1.6) | 0 (0) | 1 (3.4) | 0.460 |
| Hypercapnia | 6 (9.5) | 3 (8.8) | 3 (10.3) | 1.000 |
| Postoperative apnea | 6 (9.5) | 4 (11.8) | 2 (6.9) | 0.678 |
| Delayed recovery | 6 (9.5) | 5 (14.7) | 1 (3.4) | 0.205 |
| Hypoxia | 3 (4.8) | 2 (5.9) | 1 (3.4) | 0.651 |
| Hypoglycemia | 1 (1.6) | 1 (2.9) | 0 (0) | 1.000 |

Data are given as numbers (n) and percentages (%)

**Table V.** Analysis of independent risk factors associated with hypothermia by logistic regression model

| | B | p value | OR | 95% CI |
|---|---|---|---|---|
| Age (day) | -0.038 | 0.248 | 0.963 | 0.903-1.027 |
| Weight (kg) | -0.384 | 0.308 | 0.681 | 0.325-1.425 |
| Preoperative temperature (°C) | -0.590 | 0.130 | 0.554 | 0.258-1.191 |
| Duration of anesthesia (min) | -0.005 | 0.152 | 0.995 | 0.998-1.002 |
| Operating room temperature (°C) | -0.563 | 0.028 | 0.569 | 0.345-0.941 |
| Constant | 36.314 | 0.022 | - | - |

Hosmer-Lemeshow test model fit chi-square=4.748, p=0.784; omnibus test model effectiveness chi-square=11.316, p=0.045
°C: Degree celsius, min: Minute, kg: Kilogram, B: Regression coefficients, CI: Confidence intervals, OR: Odds ratio

temperature showed a significant association with HT (p=0.028; odds ratio: 0.681; 95% confidence interval: 0.345-0.941) (Table V).

## Discussion

Despite early efforts by pediatric anesthesiologists to monitor, identify, and implement effective interventions for perioperative HT, neonates and young children still frequently experience HT during surgery. Deviation from normothermia is strongly associated with numerous complications and adverse outcomes in neonates and young children, particularly those at highest risk. A comprehensive perioperative warming strategy is essential, including maintenance of normothermia during transport, active warming before the induction of anesthesia, during anesthesia and surgery, and accurate measurements of the core temperature (7).

The aim of this study was to investigate the occurrence of intraoperative HT and its associated risk factors in 63 neonates meeting specific criteria. The results showed that intraoperative HT developed in 54% (34 patients) of the study participants, and a significant correlation was found between the operating room temperature and the incidence of HT. Comparing our results with similar studies, Görges et al. (8) reported a HT rate of 45% in 6,737 children, while Pearce et al. (5) found an incidence of 52% in 717 children, with core body temperature measured in only 74% of cases and active warming applied in only 50%. Sim et al. (9) observed an 85% incidence of perioperative HT in premature infants undergoing laparotomy for necrotizing enterocolitis, and Cui et al. (10) reported an 82% incidence in a retrospective study of neonates. Ongun et al. (11) found an 83% rate of HT in infants undergoing craniosynostosis repair. In contrast, Thompson et al. (12) found severe intraoperative HT in 22% and 26% of infants undergoing open and endoscopic craniectomy for craniosynostosis repair, respectively.

Some studies suggest that the use of an appropriate protocol can reduce the incidence of HT to less than 10%, even in preterm infants (13-17). Consequently, the prevalence of perioperative HT seems to depend more on the effectiveness of warming strategies, regardless of the age of the patient or the type of surgical procedure.

Tander et al. (18) found that maintaining an operating room temperature above 23 °C was the most important factor in preventing perioperative HT in neonates undergoing major bowel surgery. Correspondingly, Morehouse et al. (19) observed a higher incidence of HT during surgery in the operating room compared to the intensive care unit. Certain studies have reported that risk factors for the development of HT include the type and duration of surgery (such as major orthopedic surgery), low initial core temperature, significant blood loss, and transfusion requirements (5,15,20). Additionally, Sun et al. (21) suggested that adequate warming, older age, and longer durations of anesthesia were protective factors against postoperative HT.

In our study, the room temperature experienced by patients in the hypothermic group during the perioperative period was lower than that of the normothermic group. Although this difference did not reach statistical significance in individual analysis, it emerged as the only independent factor in regression analysis. In addition, the lower baseline (preoperative) body temperature in the HT group did not show statistical significance. Temperature values showed an increase with prolonged anesthesia and surgery. Although not statistically significant, the HT group had a higher volume of fluid intake. In addition, the type of surgical method, such as open surgery, did not contribute to the occurrence of HT.

HT in pediatric patients can lead to a spectrum of adverse events ranging from thermal discomfort to increased morbidity and mortality. Particularly in neonates, cold stress can activate several physiological pathways, including a catecholaminergic response, vasoconstriction, increased metabolism, and decreased surfactant synthesis (15,22,23). As a result, these pathways may lead to pulmonary hypertension, arterial hypotension, hypoperfusion of the vital organs, and tissue hypoxia resulting in metabolic acidosis and hypoglycemia. Potential consequences include arrhythmias, an increased risk of infection, impaired neurological outcomes, apnea, the need for mechanical ventilation, prolonged hospital stays, and mortality (15,18,24).

Morehouse et al. (19) observed a significantly higher incidence of respiratory adverse events associated with perioperative HT. They reported that cardiac complications were five times more common and respiratory complications were three times more common in infants who developed HT compared with the normothermic group. Pearce et al. (5) identified a correlation between HT and elevated blood loss, leading to an increased demand for blood transfusion. In a separate investigation conducted by Görges et al. (25), the implementation of prewarming was linked to elevated core temperatures and reduced blood transfusion requirements in children undergoing spinal surgery.

In our study, we found a significant association between HT and the occurrence of at least one complication. Among those patients who experienced HT, 55.9% developed at least one complication, with cardiac and respiratory problems being the most common. However, in contrast to the existing literature, our study did not yield statistically significant results. In particular, there was no significant difference in bleeding volumes between the groups, which contradicts the established findings.

As body temperature is one of the vital signs, anesthesiologists have the primary responsibility for perioperative monitoring (26). Monitoring neonatal body temperature is critical not only to prevent HT, but also to identify iatrogenic, drug-induced, or emergent hyperthermic conditions. Therefore, the use of an accurate method of measurement is essential for accurate temperature management. Peripheral measurements can be inaccurate in the perioperative setting because the operating room is 2-5 °C colder than the standard environment, especially for neonates who do not have adequate heat retention on their skin surfaces prior to surgery. To overcome this, esophageal or rectal temperature measurements provide the most accurate results (27). In our study, the esophageal method was used in 88.9% (56 patients), while the rectal method was used in 11.1% (7 patients). Patients monitored by axillary or skin measurements were excluded from this study due to these methods inability to accurately reflect core temperature.

Implementing active warming therapies in pediatric patients before and during surgery, coupled with measures to maintain body temperature, can prove effective in preventing HT (28,29). Specifically, dressing newborns in berets and socks, employing active blown warm heating, applying gel heating from below, warming liquid and blood products, and maintaining operating rooms at elevated temperatures are successful strategies in averting HT. Continuous active heating during surgery is crucial, and irrigation and infusion solutions should be kept at body temperature. Areas of the body which cannot be actively heated should be properly insulated in order to reduce heat loss. The use of humidifiers which retain heat and moisture is recommended in order to maintain a consistent temperature, which reduces the amount of heat lost through evaporation from the airways (30).

In our clinic, a comprehensive approach was adopted to safeguard patients from HT. Berets and socks were utilized in 96.8% of patients, active blown warm heating in 95.2%, tabletop-bottom gel heating in 27%, and warming of liquids and blood products in 17.5%. No correlation was identified between the choice of heating methods and the occurrence of HT. Preoperative heating was not feasible for those patients monitored in the ward, as is the case in the intensive care unit. The use of heat-humidifiers in the breathing circuit resulted in carbon dioxide retention, limiting their application. Nevertheless, a determined effort was made to apply passive heating methods, particularly berets and socks, to nearly all patients. In the pursuit of HT prevention, we endeavored to employ multiple heating methods on the same patient whenever possible, depending on the nature of the surgical procedures, rather than adhering to a singular approach.

### Study Limitations

This study had several limitations: Firstly, it was designed retrospectively and so lacked the control inherent in a prospective controlled trial. Secondly, the application of standard heat treatment was not consistent across all of the patients, as the focus was on presenting the results of specific applications. Thirdly, the patient groups were homogeneous and standardization was difficult due to the limited number of patients. Finally, this study did not include late postoperative complications, focusing instead on intraoperative and early postoperative complications.

## Conclusion

Neonates are at increased risk for intraoperative HT due to their fragile physiology, environmental exposures, and inadequate perioperative warming. The consequences of perioperative HT are significant, contributing in particular to an increased incidence of cardiac and respiratory complications. Despite the implementation of active and passive warming methods in our study, a high incidence of HT (54%) was observed during the neonatal period. Notably, ambient operating room temperature emerged as the only independent risk factor for neonatal HT. Our findings underscore the importance of initiating warming measures in the preoperative phase, heating the operating room, utilizing available facilities, and minimizing neonatal exposure to cold environments in order to reduce the risk of HT.

### Acknowledgments

This article was presented as a poster at The Asian Society of Paediatric Anaesthesiologists Conference (ASPA-2021) on 13-14 November 2021.

### Ethics

**Ethics Committee Approval:** This study received ethical approval from the Ege University Faculty of Medicine Clinical Research Ethics Committee (president: Prof. Dr. Guzide Aksu) (approval no.: 20-12.1T/28, date: 17/12/2020).

**Informed Consent:** Informed consent was obtained from the parents of the patients.

### Authorship Contributions

Surgical and Medical Practices: E.G., C.Ş., C.B., M.U., Concept: E.G., C.Ş., C.B., M.U., Design: C.Ş., C.B., Data Collection and/or Processing: E.G., C.Ş., C.B., Analysis and/or Interpretation: E.G., M.U., Literature Search: E.G., C.Ş., Writing: E.G., C.Ş., C.B.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Financial Disclosure:** The authors disclose that this research study was not funded by any specific grants from public, commercial, or not-for-profit sectors.

## References

1. Litman RS. Pediatric Anesthesia, The Requisites in Anesthesiology, 1 st ed. Philadelphia, Elsevier Mosby 2004;3-6.
2. Kliegman R, Stanton B, Geme JW, Schor NF, Behrman RE, Nelson WE. Nelson Textbook of Pediatrics. 20th ed. Philadelphia, Pennsylvania, Elsevier 2016;1445-61.
3. Lowdermilk DL, Perry SE, Cashion K, Alden KR, Olshansky EF. Physiologic and Behavioral Adaptations of the Newborn. Maternity and Women's Health Care, 11th ed, St. Louis, MO, Elsevier 2016.
4. Benli GK. Yeni Doğan Bebek ve Özellikleri. 2021.
5. Pearce B, Christensen R, Voepel-Lewis T. Perioperative Hypothermia in the Pediatric Population: Prevalence, Risk Factors and Outcomes. J Anesth Clin Res 2010; 1:102.
6. Sahutoglu C, Bor C, Dokumcu Z, Balcioglu T. Is Temperature Monitoring Necessary in Pediatric Circumcision? Eurasian J Med 2022; 54:41-4.
7. Nemeth M, Miller C, Bräuer A. Perioperative Hypothermia in Children. Int J Env Res Pub He 2021; 18.
8. Görges M, Afshar K, West N, Pi SS, Bedford J, Whyte SD. Integrating intraoperative physiology data into outcome analysis for the ACS Pediatric National Surgical Quality Improvement Program. Pediatr Anesth 2019; 29:27-37.
9. Sim R, Hall NJ, de Coppi P, Eaton S, Pierro A. Core Temperature Falls during Laparotomy in Infants with Necrotizing Enterocolitis. Eur J Pediatr Surg 22012; 22:45-9.
10. Cui Y, Wang Y, Cao R, Li G, Deng LM, Li J. The low fresh gas flow anesthesia and hypothermia in neonates undergoing digestive surgeries: a retrospective before-after study. BMC Anesthesiol 2020; 20:1-8.
11. Ongun EA, Dursun O, Kazan MS, Nur B, Mihçi E. Early postoperative follow-up after craniosynostosis surgery. Turk J Med Sci 2018; 14;48:584-91.
12. Thompson DR, Zurakowski D, Haberkern CM, Stricker PA, Meier PM, Grp PCC. Endoscopic Versus Open Repair for Craniosynostosis in Infants Using Propensity Score Matching to Compare Outcomes: A Multicenter Study from the Pediatric Craniofacial Collaborative Group. Anesth Analg 2018; 126:968-75.
13. Brozanski BS, Piazza AJ, Chuo J, et al. STEPP IN: Working Together to Keep Infants Warm in the Perioperative Period. Pediatrics 2020; 145:e20191121.
14. Schroeck H, Lyden AK, Benedict WL, Ramachandran SK. Time Trends and Predictors of Abnormal Postoperative Body Temperature in Infants Transported to the Intensive Care Unit. Anesthesiol Res Pract 2016; 2016:1-7.
15. Kim P, Taghon T, Fetzer M, Tobias JD. Perioperative Hypothermia in the Pediatric Population: A Quality Improvement Project. Am J Med Qual 2013; 28:400-6.
16. Nemeth M, Lovric M, Asendorf T, Bräuer A, Miller C. Intraoperative zero-heat-flux thermometry overestimates esophageal temperature by 0.26 °C: an observational study in 100 infants and young children. J Clin Monit Comput 2021; 35:1445-51.
17. Hubbard R, Edmonds K, Rydalch E, Pawelek O, Griffin E, Gautam N. Anesthetic management of catheter-based patent ductus arteriosus closure in neonates weighing <3 kg: A Retrospective Observational Study. Pediatr Anesth 2020; 30:506-10.
18. Tander B, Baris S, Karakaya D, Ariturk E, Rizalar R, Bernay F. Risk factors influencing inadvertent hypothermia in infants and neonates during anesthesia. Pediatr Anesth 2005; 15:574-9.
19. Morehouse D, Williams L, Lloyd C, et al. Perioperative Hypothermia in NICU Infants. Adv Neonat Care 2014; 14:154-64.
20. Lai LL, See MH, Rampal S, Ng KS, Chan L. Significant factors influencing inadvertent hypothermia in pediatric anesthesia. J Clin Monit Comput 2019; 33:1105-12.
21. Sun Z, Honar H, Sessler DI, et al. Intraoperative Core Temperature Patterns, Transfusion Requirement, and Hospital Duration in Patients Warmed with Forced Air. Anesthesiology 2015; 122:276-85.
22. Engorn BM, Kahntroff SL, Frank KM, et al. Perioperative hypothermia in neonatal intensive care unit patients: effectiveness of a thermoregulation intervention and associated risk factors. Pediatr Anesth 2017; 27:196-204.
23. Mullany LC, Katz J, Khatry SK, LeClerq SC, Darmstadt GL, Tielsch JM. Risk of Mortality Associated With Neonatal Hypothermia in Southern Nepal. Arch Pediat Adol Med 2010; 164:650-6.
24. Deshpande SA, Platt MPW. Association between blood lactate and acid-base status and mortality in ventilated babies. Arch Dis Child-Fetal 1997;76:F15-F20.
25. Görges M, West NC, Cheung W, Zhou GH, Miyanji F, Whyte SD. Preoperative warming and undesired surgical and anesthesia outcomes in pediatric spinal surgery-a retrospective cohort study. Pediatr Anesth 2016; 26:866-75.
26. Sessler DI. Perioperative Temperature Monitoring. Anesthesiology 2021; 134:111-8.

27. Pasquier M, Paal P, Kosinski S, Brown D, Podsiadlo P, Darocha T. Esophageal Temperature Measurement. N Engl J Med 2020; 383:e93.

28. Triffterer L, Marhofer P, Sulyok I, et al. Forced-Air Warming During Pediatric Surgery: A Randomized Comparison of a Compressible with a Noncompressible Warming System. Anesth Analg 2016; 122:219-25.

29. Witt L, Dennhardt N, Eich C, et al. Prevention of intraoperative hypothermia in neonates and infants: results of a prospective multicenter observational study with a new forced-air warming system with increased warm air flow. Pediatr Anesth 2013; 23:469-74.

30. Bissonnette B, Sessler DI, Laflamme P. Passive and Active Inspired Gas Humidification in Infants and Children. Anesthesiology 1989; 71:350-4.

J Pediatr Res 2024;11(1):38-44
DOI: 10.4274/jpr.galenos.2024.60094

Original Article

# Evaluation of Premature Ventricular Contractions in Children with Structurally Normal Hearts: A Single-Center Study

Merve Tosyalı[1], Ertürk Levent[2]

[1]Ege University Faculty of Medicine, Department of Child Health and Diseases, Division of Social Pediatrics, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Child Health and Diseases, Division of Pediatric Cardiology, İzmir, Turkey

**ABSTRACT**

**Aim:** Premature ventricular contractions are a prevalent arrhythmia in children, with the majority of cases exhibiting normal cardiac function and no anatomical abnormalities. The objective of this study was to assess the clinical course of premature ventricular contractions in children who do not have any structural heart abnormalities.

**Materials and Methods:** In this study, 60 patients younger than 18 years of age who were diagnosed with premature ventricular contractions in our clinic and who did not have any structural cardiac abnormalities on echocardiography were included. Demographic data, 12-channel standard resting electrocardiography, 24-hour Holter electrocardiography monitoring, and the exercise test records of the patients were retrospectively analyzed from their follow-up files.

**Results:** This study assessed 60 patients diagnosed with premature ventricular contraction, of whom 55% were male. Out of the total number of patients, 28 (46.7%) were asymptomatic, while the most often reported symptom was palpitations. Additionally, it was stated that five children had syncope. Medical treatment was given to 40 patients (66.7%). Beta-blockers (52.2%) were the most commonly prescribed drugs. Malignant arrhythmia or sudden cardiac death did not occur in any of the patients throughout the follow-up period. According to all patients' follow-up Holter electrocardiography results, a 61.6% decrease in premature ventricular contraction rates was observed. Complete recovery was observed in 16.7% of the patients.

**Conclusion:** Premature ventricular contractions in children generally have a good prognosis. Most cases are asymptomatic, and regardless of the origin, spontaneous regression rates over time are quite substantial. Determining the origin may help predict the prognosis.

**Keywords:** Premature ventricular contractions, ventricular arrhythmia, children

## Introduction

One of the most prevalent rhythm problems in children is premature ventricular contractions (PVCs). PVCs are usually asymptomatic in children and are diagnosed incidentally due to electrocardiography (ECG) evaluations performed for different purposes. Nearly all these cases have normal cardiac functioning and lack any structural cardiac abnormalities (1-3). PVCs are detected in 15% of babies and children and 35% of adolescents who do not have any pre-existing cardiac conditions (4).

**Address for Correspondence**
Merve Tosyalı, Ege University Faculty of Medicine, Department of Child Health and Diseases, Division of Social Pediatrics, İzmir, Turkey
**Phone:** +90 506 381 38 96 **E-mail:** merve.tosyali@ege.edu.tr **ORCID:** orcid.org/0000-0002-7920-1491
**Received:** 10.01.2024 **Accepted:** 09.02.2024

PVCs with a structurally normal heart in children are thought to be benign, although some studies have suggested adverse outcomes (1,5,6). Evidence from studies on the long-term consequences of PVCs shows that this arrhythmia is usually benign, and spontaneous regression is observed in the majority of cases (7). Therefore, it is not recommended to pursue routine medical therapy to reduce the frequency of PVCs (6).

However, some studies have shown that PVCs, which are common in pediatric patients, may cause cardiomyopathy during follow-up, which may worsen the prognosis (5). Evidence indicates that the frequency of PVCs exceeding 10% in adult patients is linked to ventricular dysfunction (8).

PVCs are an abnormal heart rhythm frequently seen in children. However, patients with frequent PVCs, even if isolated, are more likely to develop ventricular tachycardia (VT). It has been shown that the prognosis of PVCs with accompanying VT is worse (9). Currently, there is no universally accepted and clearly defined method for diagnosing, treating, and managing PVCs in children. This study aimed to determine the clinical progression of PVCs in children without any underlying cardiac structural abnormalities and assess the necessity and effectiveness of medical treatment.

## Materials and Methods

### Study Population

This retrospective study was conducted at the pediatric cardiology outpatient clinics of a tertiary referral hospital in Turkey. Sixty children younger than 18 years of age who were diagnosed with PVCs after incidental detection in the Well-Child Care Outpatient Clinics or who were symptomatic and diagnosed with PVCs as a result of investigations performed in the General Pediatric Outpatient Clinics were included in this study.

### Exclusion Criteria

This study did not include pediatric patients with structural heart disease, a history of heart surgery, malignant ventricular arrhythmia, a history of stimulant drug use, any underlying chronic disease, or a family history of major arrhythmia. Moreover, newborns were also excluded from this study. Only the files of those patients diagnosed with PVCs and with complete data in their files were evaluated.

### Definitions

PVCs are the early appearance of an abnormal QRS complex (typically duration ≥120 ms, the corresponding T-wave is wide and in the opposite direction of the major QRS deviation, not preceded by a P-wave) (8). The diagnosis of PVCs is based on the recommendations of the European Paediatric and Congenital Cardiology Guidelines (8). The ventricle (right/left) where the PVCs originated was evaluated by looking at the heart rate, axis, and development of the right and left bundle branch block on 12-channel resting ECG. Only the first ECG recording of all patients was examined at the time of diagnosis.

A 24-hour Holter ECG was conducted utilizing a six-channel Holter ECG device (DMS 300-7 HolterReader; DMS, Stateline, NV, USA). The Holter ECG recordings were analyzed using the CardioScan 12.0 software developed by DM Software Inc. The percentage of total QRS complex and PVCs percentages, monomorphic, polyform, and VT percentages, and whether they have a single focal (unifocal/multifocal), were assessed according to the 24-hour Holter ECG monitoring data. Frequent PVCs were defined as the occurrence of PVCs in more than 10% (8). This study considered a reduction in the proportion of PVCs to <5% as indicative of partial recovery, and the absence of PVCs in the most recent Holter ECG was considered a complete recovery (10).

During the exercise test, the patient's rhythm was monitored at regular intervals with a 12-channel ECG while walking on a treadmill whose incline and speed increased for certain periods. The decrease or disappearance of PVCs in the ECG performed at rest or during exercise was considered positive.

### Study Design

The examination comprised patients who were diagnosed with isolated PVCs in their Holter ECG recordings and did not have any structural cardiac abnormalities as determined by echocardiography (ECHO). All patient demographic information, admission symptoms, ECG, exercise test, Holter ECG monitorings (first and final), ECHO findings, medical treatments applied, responses to treatment, and follow-up periods were obtained from the patient files. Two ECGs and two Holter ECG recordings were evaluated for each patient, one at diagnosis and one during follow-up.

### Ethical Consideration

Approval for this study was obtained from the Clinical Research Ethics Committee of Ege University Faculty of Medicine (approval no.: 16-4.1/9, date: 09.08.2016).

### Statistical Analysis

Statistical analyses were performed with SPSS 25.0 software (IBM Corp., Armonk, NY, USA). The data's descriptive statistics mean, and compliance with the normal distribution were determined by kurtosis and skewness coefficients, Shapiro-Wilk, and Kolmogorov-Smirnov tests in the data analysis. In descriptive analysis, continuous data with normal distributions were expressed as mean ± standard deviation (SD) (range) and categorical variables as frequencies (numbers with percentages). The chi-square test was performed to compare the nominal data. Student's t-test was utilized to compare two normally distributed independent groups. The Kruskal-Wallis test was used to compare non-normally distributed parameters and ordinal variables. At the same time, the Mann-Whitney U test with Bonferroni correction was employed for group comparisons. Pearson's correlation test was used to assess univariate correlations between the patients' clinical follow-up data and, if available, response to medical treatment, prognosis, and different potential influencing factors. Statistical significance was defined as $p<0.05$ for all tests.

## Results

A total of 60 patients (55% were male) with PVCs were evaluated in this study. The patients' median age was 10.75 [minimum (min.): 6 maximum (max.): 15] years. While 28 (46.7%) of the patients were asymptomatic, palpitations were the most prevalent symptom, and 5 of the children suffered syncope. Before the diagnosis of arrhythmia, no patients were using any drug therapy which could have a proarrhythmic effect. Table I shows the participants' demographic and clinical information at the time of diagnosis.

While 56.7% of the children in the research had a left bundle branch block pattern (right ventricle origin), 43.3% of the children had a right bundle branch block pattern (left ventricle origin) (Table I). The PVCs morphology of all of the patients were monomorphic. The presence of the inferior axis was observed in 90.2% of those patients exhibiting a left bundle branch block pattern, while a superior axis was observed in 8.6% of the patients.

The echocardiographic evaluations of all of the patients at diagnosis were normal, and no structural heart disease or cardiomyopathy was detected. There was a decrease in the number of PVCs in 33 out of 60 patients who underwent exercise testing, and the test result was positive. However, only 5 (15.6%) of the symptomatic children increased their PVCs with exercise.

Ninety-five percent of the patients experienced unifocal PVCs. When those patients with right and left-origin PVCs were compared, no significant difference was found between the PVCs percentages (p=0.21) (Table II). Only

**Table I.** Demographic characteristics and ECG, holter ECG, exercise test data and medical treatment conditions of the patients included in the study at the time of diagnosis

| **Characteristics** | |
|---|---|
| **Median age (min.-max.), (year)** | 10.75 (6-15) |
| **Gender, n (%)**<br>Male<br>Female | <br>33 (55)<br>27 (45) |
| **Median follow-up duration (min.-max.), (month)** | 18 (3-120) |
| **Symptomatic patients, n (%)** | 32 (53.3) |
| Palpitation | 13 (21.7) |
| Chest pain | 10 (16.7) |
| Syncope | 5 (8.3) |
| Fatigue | 3 (5) |
| Presyncope | 1 (1.7) |
| **Asymptomatic patients, n (%)** | 28 (46.7) |
| **Echocardiography findings at diagnosis, n (%)**<br>Normal<br>Pathologic | <br>100 (100)<br>0 (0) |
| **PVCs features at diagnosis** | |
| Mean PVCs percentage | 8.7±6.4 |
| **Patients with VT, n (%)** | 18 (10.8) |
| Mean VT percentage | 2.11±1.5 |
| **PVCs morphology**<br>Monomorphic | <br>60 (100) |
| **PVCs focal [n (%)]**<br>Unifocal<br>Multifocal | <br>57 (95)<br>3 (5) |
| **PVCs origin** | |
| **LBBB, n (%)**<br>Inferior axis<br>Superior axis | 34 (56.7)<br>31 (90.2)<br>3 (8.8) |
| **RBBB, n (%)** | 36 (43.3) |
| **Exercise test response at diagnosis, n (%)** | |
| Decreased PVCs | 33 (55) |
| Not changed/Increased PVCs | 27 (45) |
| **Medical treatment, n (%)** | |
| **Medically treated patients**<br>Beta-blockers<br>Propafenone | 40 (66.7)<br>21 (52.2)<br>19 (47.5) |
| **Untreated patients, n (%)** | 20 (33.3) |

LBBB: Left bundle branch block, RBBB: Right bundle branch block, PVCs: Premature ventricular contractions, VT: Ventricular tachycardia, min.-max.: Minimum-maximum

18 patients had VT. The mean percentage of PVCs was 8.7±6.4%, and the mean VT percentage was 2.11±1.5% (Table I). The incidence of VT was higher in those cases with PVCs of right origin at the time of diagnosis than in those cases of left origin (p=0.046) (Table II).

The median follow-up period was 18 (min.: 3, max.: 120) months. After follow-up, none of the patients observed a rise in the occurrence of VT, experienced malignant arrhythmia or sudden cardiac death.

At the end of the follow-up, frequent PVCs were observed in 21.6% of the patients. 31.7% of the patients had a partial reduction in the frequency of PVCs, while 16.7% had a complete recovery. It was observed that those patients with right-origin PVCs had higher rates of spontaneous complete recovery during follow-up than those patients with left-origin PVCs. However, this difference was not statistically significant (p=0.49) (Table II). Moreover, in the follow-up, it was observed that the PVCs percentages decreased more in those patients with positive exercise tests compared to the baseline (p=0.586).

Medical treatment was given to 40 patients (66.7%) with a mean duration of 19.45 (SD=13.6) months. Beta-blockers (52.2%) were the most commonly prescribed drugs, followed by propafenone (47.5%). While 35% of

**Table II.** Comparison of demographic data, clinical findings, treatment, and follow-up data of patients with PVCs originating from the right and left ventricles

| | **PVCs with right ventricle origin (n/%)** | **PVCs with left ventricle origin (n/%)** | **p value** |
|---|---|---|---|
| **Number of patients** | 34/56.7 | 26/43.3 | NS |
| **Gender**<br>Male<br>Female | <br>19/11.4<br>15/9 | <br>14/8.4<br>12/7.2 | NS |
| Percentage of symptomatic patients (%) | 19/58.8 | 13/50 | 0.36 |
| Exercise test positivity (%) | 14/41.17 | 19/73.07 | 0.014 |
| Mean percentage of PVCs at diagnosis (min.-max.) | 11.12 (1.26-36) | 6.77 (0.9-17.9) | 0.21 |
| Number of patients with VT at the time of diagnosis | 15/44.1 | 3/11.5 | 0.046 |
| Untreated patients (%) | 9/26.4 | 11/42.3 | 0.39 |
| Treated patients (%) | 24/73.85 | 16/57.9 | |
| Beta-blocker (%) | 10/44 | 11/66.6 | |
| Propafenone (%) | 14/56 | 5/33.3 | |
| Frequent PVCs (>10% PVCs) at follow-up | 9/26.4 | 4/15.3 | 0.568 |
| **Improvement of PVCs in follow-up** | | | |
| Complete (%) | 5/14.7 | 5/19.2 | 0.49 |
| Partial (%) | 9/26.4 | 10/38.46 | |
| No improvement (%) | 20/58.8 | 11/42.3 | |

PVCs: Premature ventricular contractions, VT: Ventricular tachycardia, min.-max.: Minimum-maximum, NS: Not significant

**Table III.** PVCs frequency, PVCs origin among treated and untreated patients with antiarrhythmic medication at follow-up period, exercise test positivity situation

| | **Treated group** | **Untreated group** | **p value** |
|---|---|---|---|
| **Mean percentage of PVCs at the time of diagnosis (%)** | 19.2 (8.9-23.8) | 17.7 (7.9-18.6) | 0.007 |
| **Mean percentage of PVCs at last follow-up (%)** | 11.1 (5.2-19.5) | 7.2 (3.9-12.5) | |
| PVCs origin<br>Left ventricle<br>Right ventricle | <br>6.77 (0.9-17.9)<br>11.12 (1.26-36) | <br>4.06 (0.9-10.9)<br>5.4 (1.06-15.6) | 0.49 |
| **Exercise test positivity** | 19.3 (6.9-22.4) | 11.6 (4.1-18.5) | 0.586 |

PVCs: Premature ventricular contractions

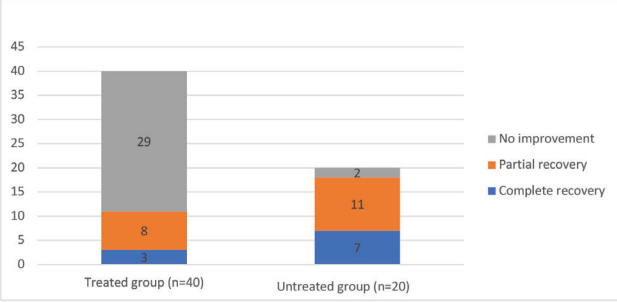


**Figure 1.** PVCs improvement rates between those who received medical treatment and those who did not in the follow-up of the patient (p=0.07)
*PVCs: Premature ventricular contractions*

patients who did not receive any medical treatment during follow-up had complete recovery, only 7.52% of those patients who received treatment had complete recovery. Thus, it was determined that antiarrhythmic treatment did not affect the course of the disease (p=0.007) (Figure 1, Table III). Among those patients receiving treatment, while no complete improvement in PVCs was observed in those patients using beta blockers, there was a complete clinical response in 15.7% of those using propafenone. In our study, propafenone was found to have a more successful clinical response than other medical treatments (p=0.033). When the origin of the PVCs was examined, it was seen that the spontaneous recovery rates of patients with PVCs originating from the right ventricle were higher than those of PVCs originating from the left ventricle. However, no significant difference was found (p=0.289).

No regression was observed in PVCs in any of the Holter ECG records of those patients with VT accompanying PVCs at the time of diagnosis, and these VT rates were found to be higher (p=0.0001). Four patients (6.7%) resistant to medical treatment were referred to a suitable center for electrophysiological studies.

Check-up ECHO could not be performed on any patient during the follow-up period. Therefore, the development of PVCs-induced cardiomyopathy could not be evaluated.

## Discussion

PVCs are prevalent cardiac arrhythmias in children, and their prevalence varies with age. Although asymptomatic PVCs are generally considered harmless in children, cardiac dysfunction can occur in certain patients, especially those with frequent PVCs (8,10,11). There is limited data on this topic in children, and it is unclear when and under what conditions cardiomyopathy will develop (10-15).

The clinical characteristics, ECG, ECHO, exercise test, first and last Holter ECG, and long-term follow-up results of 60 pediatric patients diagnosed with PVCs were investigated in this study.

Male dominance (55%) was found in our study, and the median age at diagnosis was 10.75 years, which is similar to previous investigations (5,7,16). In the literature, the majority of pediatric patients with PVCs are asymptomatic. One study stated that the symptom prevalence was less than 5% (16). Similar to existing studies, the majority of patients in our study were asymptomatic at the time of diagnosis.

It has been found in many studies that most children's PVCs resolved on their own over time (6,11,17,18). For this reason, it is not advised to undergo regular medical therapy to decrease the occurrence of PVCs (19). In our study, 31.7% of the children experienced a partial improvement in the incidence of PVCs, while 16.7% showed complete resolution. There was no critical link between the decrease in PVCs rates and where the PVCs originated. In our study, some of the patients received medical treatment. Contrary to the literature, although medical treatment was initiated at a high rate to treat PVCs, there was no significant difference in the healing rates of PVCs between the groups that were given and those that were not given medical treatment (17). Hence, it can be concluded that medical treatment might be unnecessary when trying to mitigate the occurrence of PVCs.

Beta-blockers are the most commonly used antiarrhythmic drugs to reduce PVCs according to the literature (19). While no complete improvement in PVCs was observed in any of the patients using beta blockers in our study, a complete clinical response was achieved in 15.7% of those using propafenone. In our study, unlike the information in the current literature, it was determined that propafenone had a more successful clinical response than other medical treatments.

PVCs often originate from the right ventricle (20). This may be related to the higher rate of spontaneous regression of PVCs originating from the left ventricle. However, current literature is not sufficient to explain this relationship. A recent study to support this emphasized that only PVCs originating from the left ventricle showed regression, while PVCs originating from the right ventricle remained unchanged throughout the follow-up period (13). In the only study in the literature investigating the relationship between the disappearance of PVCs during follow-up and the ventricle from which they originate, it was reported

that PVCs originating from the left ventricle showed a spontaneous decrease and disappearance in the follow-up (21). Similar to the literature, 56.7% of the cases in our study were in the right ventricle. When the source of the PVCs was examined, it was seen that the spontaneous recovery rates of those patients with PVCs originating from the right ventricle were higher than those with PVCs originating from the left ventricle. This was contrary to current literature, but no statistically significant difference was found.

In addition to the absence of structural heart disease, the absence of a family history of arrhythmia and the reduction of PVCs with exercise have been evaluated as good prognoses in many studies (6,18,22). One study showed that another good predictor of prognosis was a decrease in PVCs in children with exercise (18). In our study, the exercise test positivity rate was high (55%) at the time of diagnosis. Additionally, those patients with a positive exercise test at the time of diagnosis had higher rates of complete recovery when PVCs percentages were evaluated at follow-up. This result we obtained supports the idea that exercise test positivity is an indicator of good prognosis.

Additionally, it is known that the detection of VT during diagnosis negatively affects the prognosis of PVCs (22,23). Supporting this information, no regression was observed in PVCs in any of the check-up Holter ECGs of our patients with VT accompanying PVCs at the time of diagnosis, and their VT rates were found to be higher compared to those without VT at baseline (23).

PVCs are classified as frequent if they are ≥10% in 24 hours, and it is well known that this group of patients should be constantly monitored for the development of cardiomyopathy. There are well-defined risk factors (being male, being asymptomatic, and having frequent PVCs formation) for the development of PVC-induced cardiomyopathy in adults (24-26). However, there has been no large-scale study which looked into the factors which might make children more likely to develop PVC-induced cardiomyopathy.

Many studies in the literature argue that as the frequency of PVCs increases in adults, the rate of cardiomyopathy increases and vice versa (24-26). Despite this, some current studies argue that there is no relationship between the frequency of PVCs and the development of cardiomyopathy (27,28). In none of our patients during follow-up, malignant arrhythmia or sudden cardiac death were observed. However, we could not detect the prevalence of PVC-induced cardiomyopathy as check-up ECHO evaluation was not performed.

### Study Limitations

The primary constraint of this study was its retrospective design and small sample size. The patients' evaluation, testing, follow-up frequency, and treatment decisions were heterogeneous; thus, there is likely some selection bias in the data. Also, as left ventricular dysfunction was not assessed in our patients using follow-up echocardiography, we could not evaluate the link between the origin of PVCs and the risk of cardiomyopathy.

## Conclusion

There are few studies on the clinical course of PVCs in the pediatric age group. PVCs, frequently encountered in childhood clinical practice and diagnosed incidentally, show a good prognosis in those children without structural heart disease. The majority of individuals are asymptomatic, and the rate of spontaneous regression is exceedingly high, regardless of the underlying cause. There may be no need for medical treatment to reduce the frequency of PVCs. As a result, in light of this study's findings, since it is impossible to precisely determine which patient may develop cardiomyopathy, the heart functions of all patients should be monitored at regular intervals with Holter ECG and ECHO. The results of this study offer valuable data for planning comprehensive studies with a standardized protocol for the evaluation, risk stratification, and follow-up of PVCs in children, including a larger group of patients from different age groups, preferably with prospective long-term follow-up.

### Ethics

**Ethics Committee Approval:** Approval for this study was obtained from the Clinical Research Ethics Committee of Ege University Faculty of Medicine (approval no.: 16-4.1/9, date: 09.08.2016).

**Informed Consent:** Retrospective study.

### Authorship Contributions

Concept: E.L., Design: M.T., E.L., Data Collection or Processing: M.T., E.L., Analysis or Interpretation: M.T., E.L., Literature Search: M.T., E.L., Writing: M.T., E.L.

**Conflict of Interest:** No potential conflict of interest was reported by the authors.

**Financial Disclosure:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

## References

1. Crosson JE, Callans DJ, Bradley DJ, et al. PACES/HRS expert consensus statement on the evaluation and management of ventricular arrhythmias in the child with a structurally normal heart. Heart Rhythm 2014; 11:e55-78.
2. West L, Beerman L, Arora G. Ventricular ectopy in children without known heart disease. J Pediatr 2015; 166:338-42.
3. Drago F, Leoni L, Bronzetti G, Sarubbi B, Porcedda G. Premature ventricular complexes in children with structurally normal hearts: clinical review and recommendations for diagnosis and treatment. Minerva Pediatr 2017; 69:427-33.
4. Alexander ME, Berul CI. Ventricular arrhythmias: when to worry. Pediatr Cardiol 2000; 21:532-41.
5. Abadir S, Blanchet C, Fournier A, et al. Characteristics of premature ventricular contractions in healthy children and their impact on left ventricular function. Heart Rhythm 2016; 13:2144-8.
6. Spector ZZ, Seslar SP. Premature ventricular contraction-induced cardiomyopathy in children. Cardiol Young 2016; 26:711-7.
7. Mont L, Pelliccia A, Sharma S, et al. Pre-participation cardiovascular evaluation for athletic participants to prevent sudden death: position paper from the EHRA and the EACPR, branches of the ESC. Endorsed by APHRS, HRS, and SOLAECE. Eur J Prev Cardiol 2017; 24:41-69.
8. Baman TS, Lange DC, Ilg KJ, et al. Relationship between burden of premature ventricular complexes and left ventricular function. Heart Rhythm 2010; 7:865-9.
9. Bethge KP, Bethge D, Meiners G, Lichtlen PR. Incidence and prognostic significance of ventricular arrhythmias in individuals without detectable heart disease. Eur Heart J 1983; 4:338-46.
10. Pfammatter JP, Paul T. Idiopathic ventricular tachycardia in infancy and childhood: a multicenter study on clinical profile and outcome. Working Group on Dysrhythmias and Electrophysiology of the Association for European Pediatric Cardiology. J Am Coll Cardiol 1999; 33:2067-72.
11. Tsuji A, Nagashima M, Hasegawa S, et al. Long-term follow-up of idiopathic arrhythmias in otherwise normal children. Jpn Circ J 1995; 59:654-62.
12. Cağdaş D, Celiker A, Ozer S. Premature ventricular contractions in normal children. Turk J Pediatr 2008; 50:260-4.
13. Beaufort-Krol GC, Dijkstra SS, Bink-Boelkens MT. Natural history of ventricular premature contractions in children with a structurally normal heart: does origin matter? Europace 2008; 10:998-1003.
14. Grimm W, Menz V, Hoffmann J, Maisch B. Reversal of tachycardia induced cardiomyopathy following ablation of repetitive monomorphic right ventricular outflow tract tachycardia. Pacing Clin Electrophysiol 2001; 24:166-71.
15. Bogun F, Crawford T, Reich S, et al. Radiofrequency ablation of frequent, idiopathic premature ventricular complexes: comparison with a control group without intervention. Heart Rhythm 2007; 4:863-7.
16. Corrado D, Basso C, Thiene G. Essay sudden death in young athletes. Lancet 2005; 366:47-8.
17. Davis AM, Gow RM, McCrindle BW, Hamilton RM. Clinical spectrum, therapeutic management, and follow-up of ventricular tachycardia in infants and young children. Am Heart J 1996; 131:186-91.
18. Porcedda G, Brambilla A, Favilli S, et al. Frequent ventricular premature beats in children and adolescents: natural history and relationship with sport activity in a long-term follow-up. Pediatr Cardiol 2020; 41:123-8.
19. Wang S, Zhu W, Hamilton RM, et al. Diagnosis- specific characteristics of ventricular tachycardia in children with structurally normal hearts. Heart Rhythm 2010; 7:1725-31.
20. Collins KK, Schaffer MS, Liberman L, et al. Fascicular and nonfascicular left ventricular tachycardias in the young: an international multicenter study. J Cardiovasc Electrophysiol 2013; 24:640-8.
21. Harinder R. Arrhythmias in Children and Young Adults. Advances in Electrocardiograms - Clinical Applications 2012; 25:41-76.
22. Davis AM, Gow RM, McCrindle BW, Hamilton RM. Clinical spectrum, therapeutic management, and follow-up of ventricular tachycardia in infants and young children. Am Heart J 1996; 131:186-91.
23. Yabek SM. Ventricular arrhytmias in children with an apparently normal heart. J Pediatr 1991; 119:1-5.
24. Yokokawa M, Kim HM, Good E, et al. Relation of symptoms and symptom duration to premature ventricular complex-induced cardiomyopathy. Heart Rhythm 2012; 9:92-5.
25. Yokokawa M, Kim HM, Good E, et al. Impact of QRS duration of frequent premature ventricular complexes on the development of cardiomyopathy. Heart Rhythm 2012; 9:1460-4.
26. Song MK, Baek JS, Kwon BS, et al. Clinical spectrum and prognostic factors of pediatric ventricular tachycardia. Circ J 2010; 74:1951-8.
27. Niwano S, Wakisaka Y, Niwano H, et al. Prognostic significance of frequent premature ventricular contractions originating from the ventricular outflow tract in patients with normal left ventricular function. Heart 2009; 95:1230-7.
28. Hasdemir C, Ulucan C, Yavuzgil O, et al. Tachycardia-induced cardiomyopathy in patients with idiopathic ventricular arrhythmias: the incidence, clinical and electrophysiologic characteristics, and the predictors. J Cardiovasc Electrophysiol 2011; 22:663-8.

J Pediatr Res 2024;11(1):45-51
DOI: 10.4274/jpr.galenos.2024.50374

Original Article

# Evaluation of Heart Rate Variability in Children with Stutter

Pelin Özcan Ulubeli[1], Eser Doğan[2], Mustafa Şahin[3], Zülal Ülger Tutar[2], Mehmet Fatih Öğüt[4]

[1]Çukurova University Faculty of Health Sciences, Department of Speech and Language Therapy, Adana, Turkey
[2]Ege University Faculty of Medicine, Department of Pediatric Cardiology, İzmir, Turkey
[3]Aydın Adnan Menderes University Faculty of Medicine, Department of Otorhinolaryngology, Aydın, Turkey
[4]Ege University Faculty of Medicine, Department of Otorhinolaryngology, İzmir, Turkey

**ABSTRACT**

**Aim:** The autonomic nervous system has a direct or indirect effect on motor speech and its development. The results of studies evaluating autonomic functions in stuttering individuals show that further research is needed in different age groups. In this study, the aim was to evaluate autonomic function by analysing heart rate variability (HRV) in children with stutter.

**Materials and Methods:** In this study, a total of 41 individuals (11 females, 30 males) between the ages of 6-17 years (mean age: 10.17±2.75), diagnosed with stuttering were evaluated. The control (healthy/normal) group comprised 41 individuals (12 female, 29 male) between the ages of 6-17 years (mean age: 10.78±2.78), who did not have any speech disorder complaints and no family history. The level of stuttering was designated by applying the Turkish version of the Stuttering Severity Instrument Fourth Edition to the diagnosed group. All cases were tested for HRV and analysed using the 24-hour Holter electrocardiography recording method. Correlations between stuttering severity and the HRV parameters of the stuttering group, and correlations of HRV parameters in both groups were examined.

**Results:** A positive significant correlation was found between secondary behaviours in the stuttering group and the standard deviation of the mean NN intervals in 5-minute recordings (SDANN) of the HRV test. Additionally, when the correlation of HRV parameters between the groups was examined, the SDANN parameter in the stuttering group was statistically significantly higher ($p<0.05$). The other parameters were not statistically significantly different between the groups.

**Conclusion:** In this study, when the HRV parameters of the stuttering children were compared with the non-stuttering children, no significant differences were found to prove autonomic nervous system dysregulation.

**Keywords:** Stuttering, autonomic nervous system, heart rate variability

## Introduction

Stuttering is a speech disorder which affects the normal flow of speech through the repetition or prolongation of sounds, syllables, or words. It can also be accompanied by secondary behaviours.

The autonomic nervous system controls involuntary movements and organ functions in the body and it plays a role in motor speech and development. Furthermore, emotions can cause physiological changes which activate the sympathetic part of the autonomic nervous system,

**Address for Correspondence**
Pelin Özcan Ulubeli, Çukurova University Faculty of Health Sciences, Department of Speech and Language Therapy, Adana, Turkey
**Phone:** +90 322 338 64 84 **E-mail:** pelinozcan6@gmail.com **ORCID:** orcid.org/0000-0003-2392-5148
**Received:** 24.11.2023 **Accepted:** 19.01.2024

which can disrupt speech patterns and affect the speed, rhythm, and fluency of speech (1,2).

Several theories have been proposed to explain the aetiology of stuttering. However, studies examining autonomic responses in individuals with stuttering problems are insufficient. Some studies have shown that a decrease in heart rate (HR) during speech tasks in adults with stuttering indicates that sympathetic arousal is accompanied by impairments in motor processes characteristic of stuttering (3,4). In their study, Doruk et al. (2) investigated the presence of autonomic nervous system dysregulation in young adult stutterers by analysing the results of 24-hour Holter electrocardiogram HR variability (HRV). Their study concluded that stuttering patients tended to have parasympathetic system dominance (2). In 2010, a study was conducted with young adult stuttering patients which obtained results supporting autonomic nervous system dysregulation through the application of the tilt test (5). In 2009, Tarkowski and Paprzcki (6) conducted a study which was not entirely compatible with previous research. They stated that autonomic responses were not significantly related to the fluency of speech (6).

Conture et al. (7) investigated childhood stuttering in order to determine whether emotional intelligence interacts with emotional stress. They found that increased sympathetic activity had a negative impact on speech (7). In 2016, Choi et al. (8) studied the relationship between mood, emotional stress, and stuttering in preschool children. They concluded that the autonomic nervous system plays a role in stuttering (8). Contrary to these findings, Walsh et al. (9) reported that speech-related sympathetic arousal was not a significant factor in early childhood stuttering. They suggested that developmental profiles in different age groups should be explored in future studies involving children who continue to stutter.

This study aimed to evaluate the severity of stuttering in school-age children and compare HRV between stuttering and healthy individuals. The hypothesis was that there are differences in HRV values between stuttering and non-stuttering children.

## Materials and Methods

### Study Design

This study was conducted jointly by the otorhinolaryngology and pediatric cardiology departments in accordance with the Declaration of Helsinki. The study was conducted with 41 patients who presented with stuttering complaints and met the inclusion criteria, as well as 41 healthy individuals without stuttering complaints. All of the individuals were between the ages of 6-17 years. The Clinical Research Ethics Committee of Ege University Faculty of Medicine approved this study (approval no.: 16-9/1, date: 14.10.2016).

This study excluded those individuals with hearing impairments, those outside the 6-18 year age range, those with a history of cardiac and circulatory disease, head trauma, metabolic disease, convulsions or central nervous system infection, and those using psychiatric drugs.

The volunteers were divided into two groups: the control group, consisting of patients with normal hearing and no stuttering complaints, and the subject group, consisting of patients diagnosed with stuttering. All patients received an Informed Consent Form, Case Report Form, and a hearing assessment with pure tone threshold audiometry. The severity of stuttering was evaluated using the Turkish version of the Stuttering Severity Instrument (SSI-4TR), and HRV analysis was conducted with a 24-hour Holter application. The Holter was applied in the cardiology department on the first day, and the findings were recorded and extracted the following day (10).

### Stuttering Severity Instrument Fourth Edition

Stuttering Severity Instrument Fourth Edition (SSI-4) is a scale widely used to assess the severity of stuttering. SSI-4TR was adapted by Mutlu et al. (11) in 2014. This tool can be used for both clinical and screening purposes. The severity of stuttering in children and adults is measured by evaluating four areas of speech behaviours: frequency, duration, secondary behaviours, and the naturalness of speech. Frequency is expressed as a percentage and converted into scale scores ranging from 2 to 18, duration is scored on a scale of 2 to 18, and secondary behaviours are scored on a scale of 0 to 20. The total score determines the severity of stuttering, with higher scores indicating greater severity. Naturalness of speech is rated on a visual analogue scale from 1 (very natural speech) to 9 (very unnatural speech) (10,11).

### HRV

HRV reflects physiological changes in HR resulting from the interaction between the sympathetic and parasympathetic nervous systems. HRV records are obtained through 24-hour Holter electrocardiography. The records are first transferred to a computer environment and evaluated using the Holter program. They are then visually examined by an experienced professional, who excludes any parasitic areas from the evaluation.

On the first day of testing, a Holter device is installed in the cardiology department. The findings are recorded and extracted the following day and evaluated by experienced paediatric cardiology physicians.

### Statistical Analysis

This study analysed data using the SPSS 20.0 program (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp, USA). Gender and age distributions are presented through cross-tabs. Normal distribution was assessed using the Shapiro-Wilk test. For data without normal distribution, the Mann-Whitney U test was used for comparison. Continuous variables are presented as mean ± standard deviation and categorical variables as numbers and percentages. Pearson's correlation analysis was used to examine relationships between continuous variables, while chi-square analysis was used to analyse differences between categorical variables. A p value <0.05 was considered statistically significant.

## Results

This study analysed data from 41 patients diagnosed with stuttering and 41 healthy controls, a total of 82 individuals. Table I shows the demographic characteristics of the patients included in this study. The mean duration of stuttering at the time of diagnosis was 16±4.7 months. There was no statistically significant difference between the two groups in terms of age or gender distribution. Analysis of family history in the stuttering group revealed that 16 individuals (39%) had a family history of stuttering, whereas 25 (61%) did not.

The severity of stuttering in the patient group ranged from mild in 1 patient (2.4%), moderate in 19 (46.3%), severe in 14 (34.1%), and to very severe in 7 (17.1%). The results of the assessment of stuttering frequency, duration, secondary behaviours and the naturalness of speech are given in Table II.

Upon examining the average distribution of the HRV parameters between the groups, the standard deviation of the mean NN intervals in 5-minute recordings (SDANN) values for the stuttering group were significantly higher than the control group ($p<0.05$). No significant differences were observed between the groups in terms of the other variables ($p>0.05$) (Table III).

When examining the correlation between reading, speaking, frequency, seconder behaviours, and duration values which make up the total SSI-4TR scores in the stuttering group, along with HRV parameters, a weak positive correlation was found between the secondary behaviour scores and the SDANN values, and a weak negative correlation was found between maxHR values and beats values ($p<0.05$) (Table IV).

When the correlation between the total score of SSI-4TR, severity and naturalness of speech values of the stuttering group were analysed with the HRV parameters, a weak negative correlation was found between the total score of SS1-4TR and beats values (Table V).

**Table I.** The demographic characteristics of the cases

| | Stutter (n=41) | Control (n=41) | p value |
|---|---|---|---|
| **Age (Mean ± SD)** | 10.17±2.75 | 10.78±2.78 | 0.228 |
| **Gender n (%)** | | | |
| Male | 30 (73.2) | 29 (70.7) | 0.806 |
| Female | 11 (26.8) | 12 (29.3) | 0.317 |

SD: Standard deviation

**Table II.** The mean of the parameters SSI-4 score in stuttering children

| | Mean ± SD | Median (Min.-Max.) |
|---|---|---|
| **Frequency** | 15.24±1.9 | 15 (18-11) |
| Reading | 7.49±1.19 | 8 (9-5) |
| Speaking | 7.76±1.18 | 8 (9-4) |
| **Secondary behaviours** | 6.21±3.82 | 6 (17-0) |
| **Duration** | 7.29±2.4 | 8 (12-2) |
| **Naturalness of speech** | 5.78±2.13 | 7 (9-2) |

SD: Standard deviation, Max.: Maximum, Min.: Minimum, SSI-4: Stuttering Severity Instrument Fourth Edition

**Table III.** The average distribution of HRV parameters according to the groups

| | Control group | Stuttering group | Z | p value |
|---|---|---|---|---|
| | Median (Min.-Max.) | Median (Min.-Max.) | | |
| **MinHR** | 50 (40-70) | 51 (40-67) | -0.851 | 0.395 |
| **AVGHR** | 83 (56-126) | 85 (67-106) | -1.819 | 0.069 |
| **MaxHR** | 142 (106-256) | 147 (120-178) | -1.412 | 0.158 |
| **Beats** | 113,080 (31,389-151,742) | 116,727 (74,458-141,172) | -0.969 | 0.332 |
| **SDNN** | 152 (80-285) | 152 (114-275) | -0.060 | 0.952 |
| **SDANN** | 123 (49-233) | 132 (81-360) | -2.203 | **0.028** |
| **SDNNIndex** | 99 (41-210) | 85 (52-163) | -1.271 | 0.204 |
| **RMSSD** | 112 (36-289) | 93 (49-248) | -1.442 | 0.149 |
| **SDSD** | 112 (36-289) | 93 (49-248) | -1.517 | 0.129 |
| **NN50** | 27.64 (4.04-55.2) | 26.54 (7.65-65.95) | -0.107 | 0.915 |
| **pNN50** | 31.45 (11-58.71) | 26.98 (14.32-55.51) | -0.645 | 0.519 |
| **TR.INDEX** | 34.88 (15.76-55.15) | 38.6 (14.43-56.63) | -1.053 | 0.293 |
| **ULF** | 9,862.46 (2,558.84-30,595.06) | 12,069.96 (3,222.52-36,116.41) | -1.526 | 0.127 |
| **VLF** | 2,637.94 (673.58-10,982.59) | 2356.12 (894.44-5,194.71) | -0.858 | 0.391 |
| **LF** | 3,043.11 (400.29-21,651.75) | 2234.54 (808.62-6,089.95) | -1.748 | 0.080 |
| **HF** | 3,596.94 (417.88-30,371.53) | 2756.28 (1,240.19-9,719.89) | -1.118 | 0.264 |
| **TP** | 8,602.26 (1,254.81-58,280.7) | 6430.95 (3,115.13-18,862.72) | -1.247 | 0.212 |
| **LF/HF** | 0.88 (0.5-1) | 0.83 (0.34-1) | -1.925 | 0.054 |

Mann-Whitney U Analysis
Max.: Maximum, Min.: Minimum, MinHR: Minimum heart rate beats/min, MaxHR: Maximum heart rate beats/min, AVGHR: Mean heart rate beats/min, NN: Cycle length between two normal beats, SDNN: Standard deviation of all NN intervals during the examination, SDNN index: Mean of the standard deviations of all NN intervals in 5-min recordings, SDANN: Standard deviation of average NN intervals in 5-min recordings, NN50: Number of adjacent NN intervals with a difference of more than 50ms during the entire recording, pNN50: NN50 number divided by the total number of NNs, RMSSD: Square root of the sum of the differences of consecutive NN intervals in the 24-hour recording) TR.INDEX: Division of all NN interval number to the number of NN intervals in the mode length, HF: High-frequency band, LF: Low-frequency band, VLF: Very-low-frequency band, TP: Total power; variance of all NN intervals, ULF: Asleep, power in the low-frequency range

**Table V.** Correlation between stuttering children's SSI-4 Total score, severity and naturality of speech values with HRV parameters

| | SSI-4 Total score | | Severity | | Naturality of speech | |
|---|---|---|---|---|---|---|
| | r | p value | r | p value | r | p value |
| **MinHR** | 0.083 | 0.604 | 0.054 | 0.737 | 0.074 | 0.644 |
| **AVGHR** | -0.062 | 0.699 | -0.036 | 0.823 | 0.065 | 0.688 |
| **MaxHR** | -0.273 | 0.084 | -0.088 | 0.584 | 0.061 | 0.705 |
| **Beats** | -0.317 | **0.044** | -0.220 | 0.166 | -0.101 | 0.529 |
| **SDNN** | -0.013 | 0.935 | 0.006 | 0.970 | -0.122 | 0.447 |
| **SDANN** | 0.269 | 0.089 | 0.263 | 0.096 | 0.187 | 0.241 |
| **SDNNIndex** | 0.048 | 0.766 | 0.025 | 0.877 | -0.112 | 0.487 |
| **RMSSD** | 0.123 | 0.443 | 0.068 | 0.672 | -0.077 | 0.634 |
| **SDSD** | 0.132 | 0.410 | 0.074 | 0.644 | -0.071 | 0.658 |
| **NN50** | -0.046 | 0.775 | -0.020 | 0.901 | -0.170 | 0.288 |
| **pNN50** | -0.113 | 0.480 | -0.090 | 0.576 | -0.119 | 0.457 |

**Table V.** Continued

| | SSI-4 Total score | | Severity | | Naturality of speech | |
|---|---|---|---|---|---|---|
| | r | p value | r | p value | r | p value |
| **TR.INDEX** | -0.041 | 0.797 | -0.001 | 0.993 | -0.113 | 0.483 |
| **ULF** | -0.179 | 0.264 | -0.157 | 0.328 | -0.263 | 0.097 |
| **VLF** | 0.232 | 0.145 | 0.224 | 0.159 | 0.084 | 0.599 |
| **LF** | 0.219 | 0.169 | 0.175 | 0.273 | 0.099 | 0.539 |
| **HF** | 0.167 | 0.296 | 0.158 | 0.323 | 0.028 | 0.862 |
| **TP** | 0.209 | 0.189 | 0.188 | 0.240 | 0.062 | 0.701 |
| **LF/HF** | -0.160 | 0.316 | -0.207 | 0.194 | -0.068 | 0.672 |

Pearson correlation
MinHR: Minimum heart rate beats/min, MaxHR: Maximum heart rate beats/min, AVGHR: Mean heart rate beats/min, NN: Cycle length between two normal beats, SDNN: Standard deviation of all NN intervals during the examination, SDNN index: Mean of the standard deviations of all NN intervals in 5-min recordings, SDANN: Standard deviation of average NN intervals in 5-min recordings, NN50: Number of adjacent NN intervals with a difference of more than 50ms during the entire recording, pNN50: NN50 number divided by the total number of NNs, RMSSD: Square root of the sum of the differences of consecutive NN intervals in the 24-hour recording, TR.INDEX: Division of all NN interval number to the number of NN intervals in the mode length, HF: High-frequency band, LF: Low-frequency band, VLF: Very-low-frequency band, TP: Total power; variance of all NN intervals, ULF: Asleep, power in the low-frequency range

**Table IV.** Correlation of stuttering children's reading, speaking, frequency, secondary behaviour, and duration values with HRV parameters

| | Reading | | Speaking | | Frequency | | Seconder behaviour | | Duration | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | p value | r | p value | r | p value | r | p value | r | p value |
| **MinHR** | -0.307 | 0.051 | 0.221 | 0.165 | -0.054 | 0.735 | 0.126 | 0.432 | 0.019 | 0.905 |
| **AVGHR** | -0.104 | 0.519 | 0.304 | 0.053 | 0.124 | 0.439 | -0.207 | 0.194 | -0.032 | 0.842 |
| **MaxHR** | -0.001 | 0.996 | 0.139 | 0.386 | 0.086 | 0.593 | -0.393 | **0.011** | -0.158 | 0.325 |
| **Beats** | 0.008 | 0.960 | 0.132 | 0.410 | 0.087 | 0.587 | -0.455 | **0.003** | -0.228 | 0.151 |
| **SDNN** | 0.002 | 0.990 | -0.103 | 0.520 | -0.063 | 0.695 | -0.008 | 0.958 | 0.049 | 0.760 |
| **SDANN** | -0.190 | 0.233 | 0.064 | 0.690 | -0.079 | 0.623 | 0.334 | **0.033** | 0.256 | 0.106 |
| **SDNNIndex** | -0.003 | 0.987 | -0.088 | 0.584 | -0.056 | 0.726 | 0.073 | 0.651 | 0.069 | 0.670 |
| **RMSSD** | -0.108 | 0.502 | 0.019 | 0.907 | -0.056 | 0.729 | 0.135 | 0.401 | 0.120 | 0.456 |
| **SDSD** | -0.105 | 0.513 | 0.023 | 0.888 | -0.052 | 0.748 | 0.143 | 0.371 | 0.125 | 0.435 |
| **NN50** | 0.126 | 0.432 | -0.050 | 0.757 | 0.048 | 0.766 | -0.086 | 0.592 | -0.024 | 0.882 |
| **pNN50** | -0.192 | 0.230 | 0.042 | 0.794 | -0.094 | 0.560 | -0.051 | 0.750 | -0.100 | 0.535 |
| **TR.INDEX** | 0.062 | 0.698 | -0.023 | 0.885 | 0.025 | 0.879 | 0.098 | 0.540 | -0.218 | 0.171 |
| **ULF** | -0.139 | 0.388 | -0.154 | 0.337 | -0.182 | 0.254 | -0.162 | 0.312 | -0.064 | 0.693 |
| **VLF** | 0.113 | 0.481 | 0.078 | 0.627 | 0.119 | 0.457 | 0.220 | 0.167 | 0.168 | 0.294 |
| **LF** | -0.094 | 0.560 | 0.033 | 0.839 | -0.038 | 0.812 | 0.241 | 0.129 | 0.227 | 0.153 |
| **HF** | -0.077 | 0.631 | 0.058 | 0.719 | -0.012 | 0.939 | 0.130 | 0.419 | 0.229 | 0.150 |
| **TP** | -0.060 | 0.711 | 0.055 | 0.733 | -0.003 | 0.985 | 0.191 | 0.232 | 0.242 | 0.127 |
| **LF/HF** | -0.001 | 0.993 | -0.231 | 0.147 | -0.144 | 0.368 | -0.071 | 0.659 | -0.171 | 0.285 |

Pearson correlation
MinHR: Minimum heart rate beats/min, MaxHR: Maximum heart rate beats/min, AVGHR: Mean heart rate beats/min, NN: Cycle length between two normal beats, SDN: Standard deviation of all NN intervals during the examination, SDNN index: Mean of the standard deviations of all NN intervals in 5-min recordings, SDANN: Standard deviation of average NN intervals in 5-min recordings, NN50: Number of adjacent NN intervals with a difference of more than 50ms during the entire recording, pNN50: NN50 number divided by the total number of NNs, RMSSD: Square root of the sum of the differences of consecutive NN intervals in the 24-hour recording, TR.INDEX: Division of all NN interval number to the number of NN intervals in the mode length, HF: High-frequency band, LF: Low-frequency band, VLF: Very-low-frequency band, TP: Total power; variance of all NN intervals, ULF: Asleep, power in the low-frequency range

## Discussion

Although many theories have been proposed to explain the aetiology of stuttering, our study focused on investigating the background of speech initiation. HRV parameters were examined in school-age children who stutter to examine possible differences in autonomic nervous system and these were compared with the values for children of similar age without any speech problems.

Our study found that the mean age in the stuttering group was 10.17±2.75 years, which was not significantly different from the control group's mean age of 10.78±2.78 years. This provided an advantage when evaluating the parameters. Karabulut (12) reported significant positive correlations with age for SDNN, SDNN-index, SDANN-index, low-frequency band (LF), very-low-frequency band, and total power parameters in their study of 51 healthy children with a mean age of 10.78 years. The emergence of different correlations in adults and children was attributed to the incomplete development of the autonomic nervous system in children (12). Previous studies found a negative correlation between HRV parameters and age in adults, while a significant positive correlation was found in children (13). In our study, no significant difference was found between age and HRV parameters in the case group.

In a study of 106 healthy children aged 1-20 years, Silvetti et al. (14) found that SDNN and SDANN values were higher in boys, while SDNN-index, square root of the sum of the differences of consecutive NN intervals in the 24-hour recording (RMSSD), and pNN50 values did not differ between the genders. Similarly, Karabulut (12) reported no significant difference in HRV parameters between the genders in a study of 51 healthy children aged 4-17 years. Our study also found no significant difference in HRV parameters between genders.

Doruk et al. (2) investigated the presence of autonomic nervous system dysregulation in young adult stutterers by analysing their HRV using a 24-hour Holter monitor. Compared to the control group, the stuttering group exhibited high RMSSD and differences in LF, LF/high-frequency band (HF), normalised LF, pNN50, SDNN, HF, normalised HF, total power, and GSI values. Additionally, negative correlations were found between subjective and total anxiety scores and LF and total power. Their study concluded that stuttering patients tended to have parasympathetic system dominance (2). Based on an analysis of our study results, the SDANN values of those children who stutter were significantly higher than those of the control group ($p=0.028$). No statistically significant differences were observed between the groups in terms of the other variables ($p>0.05$). In our study, a weak positive correlation was identified between secondary behaviour values and SDANN values, a weak negative correlation with maxHR values, and a moderate negative correlation with Beats values ($p<0.05$).

SDANN is the standard deviation of the average NN intervals over short periods of 5 minutes. There are differing opinions regarding the SDANN parameter as a reflection of the autonomic nervous system. Fantoni et al. (15) interpreted an increase in SDANN as reflecting changes in autonomic tone, particularly changes in the interaction between the sympathetic and parasympathetic nervous systems in the heart. Adamson et al. (16) reported that a decrease in physical activity corresponds to a decrease in SDANN before clinical deterioration. Raj et al. (17) suggested that the change in SDANN value may not indicate a change in the autonomic nervous system, despite all these findings. The evidence supporting a positive correlation between the SDANN value and secondary behaviours in stuttering children suggests that physical activity may increase the SDANN value. Therefore, the significant SDANN value found in our study is interpreted as being related to the autonomic nervous system and secondary movements during speech in stuttering children. However, the lack of significant changes in the other HRV parameters does not support our hypothesis that the autonomic functions of stuttering children were different. In order to analyse the relationship between the autonomic nervous system and stuttering, it would be useful to use the autonomic nervous system in combination with other nervous dysfunction assessment tools.

To the best of our knowledge, there is no study in the literature investigating the relationship between stuttering and autonomic nervous system functions in school-age children using HRV measurements.

### Study Limitations

There is the possibility of the population not being fully represented by both groups in our study. It would be beneficial to increase the number of participants in both groups and to work with more similar groups in terms of gender and age.

## Conclusion

No significant correlation was found, except for the SDANN parameter, when examining the HRV parameters of school-age children with and without stuttering. The literature lacks sufficient and precise information regarding

the ability of SDANN to show autonomic function alone, which does not support our hypothesis that the autonomic functions of stuttering children are different. When examining the demographic characteristics (gender, age, and family history) of the group with stuttering, a positive and significant correlation was found between the SDANN parameter and secondary behaviours ($p>0.05$). However, there were no significant correlations between the other parameters, including stuttering severity, speech spontaneity, and the HRV parameters. The literature suggests that physical activity increases the SDANN value and supports a positive correlation with the SDANN value of secondary behaviours in stuttering children. This is the first study to examine autonomic dysfunctions of stuttering children using the 24-hour Holter measurement HRV. Our study may contribute to future research into the aetiology of stuttering.

### Ethics

**Ethics Committee Approval:** The Clinical Research Ethics Committee of Ege University Faculty of Medicine approved this study (approval no.: 16-9/1, date: 14.10.2016).

**Informed Consent:** All patients received an Informed Consent Form, Case Report Form, and a hearing assessment with pure tone threshold audiometry.

### Authorship Contributions

Surgical and Medical Practices: E.D., M.Ş., Z.Ü.T., M.F.Ö., Concept: P.Ö.U., M.Ş., M.F.Ö., Design: P.Ö.U., M.Ş., M.F.Ö., Data Collection or Processing: P.Ö.U., E.D., Z.Ü.T., Analysis or Interpretation: P.Ö.U., E.D., M.Ş., Z.Ü.T., M.F.Ö., Literature Search: P.Ö.U., E.D., M.Ş., Writing: P.Ö.U., E.D., M.Ş., Z.Ü.T., M.F.Ö.

**Conflict of Interest:** No potential conflict of interest was reported by the authors.

**Financial Disclosure:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

## References

1. Alm PA. Stuttering, emotions, and heart rate during anticipatory anxiety: A critical review. J Fluency Disord 2004; 29:123-33.
2. Doruk A, Tuerkbay T, Yelboğa Z, et al. Autonomic Nervous System Imbalance in Young Adults with Developmental Stuttering. Klinik Psikofarmakoloji Bulteni 2008; 18:274-81.
3. Peters HF, Hulstijn W. Stuttering and anxiety: The difference between stutterers and nonstutterers in verbal apprehension and physiologic arousal during the anticipation of speech and non-speech tasks. J Fluency Disord 1984; 9:67-84.
4. Weber CM, Smith A. Autonomic correlates of stuttering and speech assessed in a range of experimental tasks. J Speech Lang Hear Res 1990; 33:690-706.
5. Ozdemir B, Doruk A, Celik C, et al. Evaluation of autonomic nervous system function with tilt table testing in young adults with persistent developmental stuttering. Bulletin of Clinical Psychopharmacology 2010; 20:45-9.
6. Tarkowski Z, Paprzycki P. Reaction of the cardiovascular system during anticipation of speech and while speaking in stutterers and non-stutterers. J Pre Clin Clin Res 2009; 3:118-21.
7. Conture EG, Kelly EM, Walden TA. Temperament, speech and language: An overview. J Commun Disord 2013; 46:125-42.
8. Choi D, Conture EG, Walden TA, Jones RM, Kim H. Emotional diathesis, emotional stress, and childhood stuttering. J Speech Lang Hear Res 2016; 59:616-30.
9. Walsh B, Smith A, Christ SL, Weber C. Sympathetic nervous system activity in preschoolers who stutter. Front Hum Neurosci 2019; 13:1-16.
10. Riley G, Bakker K. SSI-4: Stuttering severity instrument. PRO-ED, an International Publisher 2009.
11. Mutlu Aİ, Tirank ŞB, Gündüz B. Reliability and Validity of the Turkish Version of the Stuttering Severity Instrument, Fourth Edition (SSI-4-TR/ KEŞİDA4) Between 6 And 16 Years Old School Children. IDUHES 2020; 3:135-44.
12. Karabulut M. Sağlıklı Çocuklarda kalp hızı değişkenliği. Fırat Med J 2015; 20:152-5.
13. Ramaekers D, Ector H, Aubert AE, Rubens A, Van de Werf F. Heart rate variability and heart rate in healthy volunteers. Is the female autonomic nervous system cardioprotective? Eur Heart J 1998; 19:1334-41.
14. Silvetti MS, Drago F, Ragonese P. Heart rate variability in healthy children and adolescents is partially related to age and gender. Int J Cardiol 2001; 81:169-74.
15. Fantoni C, Raffa S, Regoli F, et al. Cardiac resynchronization therapy improves heart rate profile and heart rate variability of patients with moderate to severe heart failure. J Am Coll Cardiol 2005; 46:1875-82.
16. Adamson PB, Smith AL, Abraham WT, et al. Continuous autonomic assessment in patients with symptomatic heart failure: prognostic value of heart rate variability measured by an implanted cardiac resynchronization device. Circulation 2004; 110:2389-94.
17. Raj SR, Roach DE, Koshman ML, Sheldon RS. Standard deviation of sequential five-minute RR interval means (SDANN) is a prognostic marker, but not necessarily an autonomic marker. J Am Coll Cardiol 2006; 48:1285-6.

J Pediatr Res 2024;11(1):52-7
DOI: 10.4274/jpr.galenos.2024.13911

Original Article

# The Relationship Between Premature Adrenarche and Markers of Inflammation in Complete Blood Count

Ferda Evin[1], İlkay Ayrancı[1], Mehtap Ertekin[2]

[1]İzmir Bakırçay University Çiğli Training and Research Hospital, Clinic of Pediatric Endocrinology, İzmir, Turkey
[2]İzmir Bakırçay University Çiğli Training and Research Hospital, Clinic of Pediatric Hematology, İzmir, Turkey

**ABSTRACT**

**Aim:** Premature adrenarche (PA) has been associated with metabolic and polycystic ovary syndrome (PCOS) and, thus, with an increased risk for type 2 diabetes and cardiovascular diseases in later life. Mean platelet volume (MPV), neutrophil/lymphocyte ratio (NLR), and platelet/lymphocyte ratio (PLR) are parameters used to show inflammation. This study planned to evaluate systemic inflammation in children with PA using MPV, NLR, and PLR.

**Materials and Methods:** The study included 40 female patients diagnosed with PA and 40 healthy female individuals as a control group. The patient and control groups' MPV, NLR, and PLR values were compared.

**Results:** The mean age of the PA group was 7.18±0.66 years, and the mean age of the control group was 7.09±1.08 years. The mean MPV and platelet distribution width (PDW) values in the PA group were significantly higher than those in the control group (10.25±0.87 vs 9.52±0.79, p<0.001 and 15.43±1.31 vs 14.35±1.84, p=0.04, respectively). However, in the PA group, NLR and PLR were not significantly different from the values in the control group (p>0.05). The results of multivariate logistic regression analysis revealed that the MPV [odds ratio (OR); 95% confidence interval (CI): 0.331 (0.174-0.630); p=0.001], and PDW [OR; 95%: CI: 0.612 (0.425-0.884); p=0.008] were associated with PA in the patient group.

**Conclusion:** Our results demonstrated that PA patients had significantly higher MPV levels and PDW than the healthy controls. Hence, recognition of early markers in adolescence might reveal primary pathogenetic alterations predictive of the later development of PCOS and/or metabolic syndrome.

**Keywords:** Premature adrenarche, mean platelet volume, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, cardiovascular diseases, inflammation

## Introduction

Adrenarche represents a pivotal stage in adrenal cortex development, heralding an upsurge in adrenal androgen precursors. This transformative process typically unfolds in mid-childhood, around 5-8 years old in humans (1). Central to assessing adrenarche are key serum markers such as dehydroepiandrosterone (DHEA) and DHEA sulfate conjugate (DHEAS) (2). While the adrenocorticotropic hormone plays a pivotal role, intrinsic and external factors can influence adrenal androgen (AA) secretion, necessitating a holistic understanding (3).

**Address for Correspondence**
Ferda Evin, İzmir Bakırçay University Çiğli Training and Research Hospital, Clinic of Pediatric Endocrinology, İzmir, Turkey
**Phone:** +90 553 307 35 90 **E-mail:** ferdaevin88@gmail.com **ORCID:** orcid.org/0000-0001-7169-890X
**Received:** 04.02.2024 **Accepted:** 15.02.2024

Premature adrenarche (PA) emerges as a distinctive phase marked by premature androgenic signs before the age of 8 in girls or 9 in boys, coupled with elevated adrenal androgen precursors for their prepubertal age (4,5). In recent times, the spotlight on PA has intensified, linking it to factors such as small birth size, metabolic concerns, and polycystic ovary syndrome (PCOS) (6). This correlation raises concerns about heightened risks of cardiovascular diseases (CVD) and diabetes later in life (7,8). Early puberty has also been associated with increased body mass index (BMI) and heightened obesity risks in women in adulthood (8).

Shifting our focus to hemostasis and thrombosis, platelets, which are pivotal in these processes, have their function influenced by factors such as platelet size (9). Mean platelet volume (MPV), easily measurable in a complete blood count, has emerged as an indicator of platelet size. Recent studies hint at its potential association with chronic diseases such as diabetes and CVD. Increased MPV has been shown to correlate with elevated risks of adverse cardiovascular events, mortality in acute coronary syndrome patients, and occurrences of hypertension and ischemic stroke in the general population (10,11). Additionally, parameters derived from the complete blood count, such as the platelet/lymphocyte ratio (PLR) and the neutrophil/lymphocyte ratio (NLR), indicate inflammation and have been reported to rise in various metabolic and endocrinological conditions (12,13).

Despite the significance of PA, studies exploring its long-term cardiovascular risk profiles and adult outcomes are rare (7,8). Understanding the impact of PA on MPV may provide valuable insights into potential early markers of cardiovascular risk in this population, paving the way for targeted interventions and improved management strategies in order to mitigate long-term health implications. This study endeavored to bridge this gap by investigating MPV, NLR, and PLR in patients with PA, so as to give insights into their relationship to cardiovascular risks and overall health outcomes.

## Materials and Methods

### Study Population and Data Collection

A meticulous retrospective examination of the medical records of 40 female patients diagnosed with PA at İzmir Bakırçay University Çiğli Training and Research Hospital between January, 2022 and December, 2023 was carried out. Ethical approval, aligning with the Declaration of Helsinki, was obtained from the İzmir Bakırçay University Non-invasive Clinical Research Ethics Committee (approval no.: 1239, dated: 10.18.2023). Informed consent, acknowledging the study's objectives and potential medical data publication, was secured from the parents of all of the patients.

The inclusion criteria were females diagnosed with PA between 6 and 8 years of age, excluding those showing clinical signs of adrenal androgen activity before the age of 8. DHEAS values of >40 µg/dL were considered adrenarche (14). The control group comprised healthy children matched for age and gender during routine health visits. Auxological data calculations were carried out using an automated calculator (15). A consistent pediatric endocrinologist, adhering to Tanner's criteria (16), assessed pubertal status, while bone age was determined by referencing Greulich and Pyle's Radiographic Atlas of Skeletal Development.

Various parameters were analyzed, including complete blood count results, luteinizing hormone, DHEAS, estradiol, total testosterone, lipid profile, and procalcitonin. Hormone levels were assessed through the chemiluminescence method utilizing a Beckman Coulter DxI® 600 analyzer. Automated devices, specifically the Technicon H-1 System from Technicon Co, Tournai, Belgium, were employed to obtain erythrocyte indices, platelet counts, and MPV values. Complete blood count measurements also provided the number of leukocytes, lymphocytes, neutrophils, platelets, MPV, and platelet distribution width (PDW). The determination of the NLR entailed the division of the neutrophil count by the lymphocyte count, and for the PLR, the platelet count was divided by the lymphocyte count.

### Statistical Analysis

The analysis of data was carried out using SPSS for Windows, version 25.0 (IBM Inc., Armonk, NY, USA), providing a robust platform for comprehensive data exploration. Group comparisons were executed utilizing the independent samples t-test for variables conforming to normal distributions, while the Mann-Whitney U test was employed for distributions with skewed data. The significance threshold was set at $p<0.05$, signifying statistical significance, and no adjustments were made for multiple statistical tests, ensuring a nuanced interpretation of the findings.

## Results

The patient cohort included forty females diagnosed with PA, while the control group comprised forty healthy girls. The PA group's mean age was 7.18±0.66 years; and in the healthy control group, it was 7.09±1.08 years. The demographic characteristics of the PA patients are given

in Table I. Notably, 26.3% of these patients exhibited pubic hair, 16.3% displayed axillary hair, 6.3% had adult-type body odor, and 1.3% presented with acne. No family history of PA was reported among the patients. BMI standard deviation scores of 5 patients were ≥2. During examination, 62.5% of the patients displayed axillary hair growth. Tanner staging indicated that 12.5% were at stage 1, 25% were at stage 2, and 12.5% were at stage 3 for pubic hair growth, with all patients being at stage 1 for breast development.

The laboratory findings for the PA patients, outlined in Table II, included a median bone age of 7.8 (5-10.5) years. The median difference between chronological and bone age was 0.86 (-1.89-3.34) years.

Complete blood count parameters in Table III revealed no significant differences between the groups regarding red blood cell count, white blood cell count, hemoglobin and hematocrit levels, mean erythrocyte volume, red cell distribution width, or platelet count (p>0.05). However, MPV and PDW values were significantly higher in the PA group compared to the control group (p<0.001 and 0.04, respectively). In contrast, PLR and NLR did not show any significant differences between the two groups (p>0.05).

Results from multivariate logistic regression analysis indicated that MPV [odds ratio (OR); 95% confidence interval (CI): 0.331 (0.174-0.630); p=0.001] and PDW [OR; 95% CI: 0.612 (0.425-0.884); p=0.008] were associated with PA in the patient group.

**Table I.** The demographic characteristics of premature adrenarche patients

| Characteristics | Premature adrenarche patients (n=40) |
|---|---|
| Height SDS | 1.11±1.23 |
| Weight SDS | 0.73±1.22 |
| Body mass index SDS | 0.85±1.11 |
| Systolic blood pressure percentile | 49.93±26.07 |
| Diastolic blood pressure percentile | 61.31±20.08 |

SDS: Standard deviation score

**Table II.** The laboratory findings of the premature adrenarche patients

| Characteristics | Premature adrenarche patients (n=40) |
|---|---|
| HOMA-IR | 1.78±1.13 |
| HDL cholesterol (mg/dL) | 53.05±10.03 |
| LDL cholesterol (mg/dL) | 86.62±20.32 |
| Triglyceride (mg/dL) | 75.25±33.34 |
| LH (mlU/mL) | 0.3 (0.03-0.8) |
| FSH (mlU/mL) | 1.29±0.69 |
| Estradiol (pg/mL) | 5 (5-18.4) |
| DHEAS (µg/dL) | 85.84±41.25 |
| Procalcitonin (ng/mL) | 0.03 (0.01-0.11) |

HOMA-IR: Homeostasis Model Assessment Insulin Resistance, HDL: High density lipoprotein, LDL: Light density lipoprotein, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, DHEAS: Dehydroepiandrosterone-sulfate

**Table III.** Complete blood parameters in the premature adrenarche and control groups

| | Premature adrenarche group (n=40) | Control groups (n=40) | p value |
|---|---|---|---|
| WBC (/µL) | 7,198.25±1,987.32 | 7,029.5±1,612.42 | 0.678 |
| RBC (×10⁶/µL) | 4.88±0.06 | 4.94±0.06 | 0.357 |
| HGB (g/dL) | 12.99±0.10 | 12.91±0.21 | 0.461 |
| HCT (%) | 38.76±0.34 | 39.01±0.11 | 0.654 |
| MCV (fL) | 80.16±0.23 | 80.28±0.36 | 0.823 |
| RDW (%) | 13.54±0.09 | 13.42±0.11 | 0.654 |
| PLT (/µL) | 328,300±80,921 | 330,650±76,577 | 0.894 |
| MPV (fL) | 10.25±0.87 | 9.52±0.79 | **<0.001** |
| PDW (%) | 15.43±1.31 | 14.35±1.84 | **0.004** |
| NLR | 1.42±1.17 | 1.30±0.58 | 0.562 |
| PLR | 113.01±34.69 | 113.22±31.79 | 0.977 |

WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean erythrocyte volume, MCH: Mean erythrocyte hemoglobin, MCHC: Mean erythrocyte hemoglobin concentration, RDW: Red cell distribution width, PLT: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio

Correlation analysis revealed a weak positive correlation between MPV and PDW (r=0.252, p=0.024) and a moderate correlation between NLR and PLR (r=0.435, p<0.001). However, no correlations were detected between MPV, NLR, PLR, and DHEAS levels.

## Discussion

In this study, we investigated the correlation between inflammatory markers in a complete blood count and PA. Our findings indicated that individuals with PA exhibited significantly elevated levels of MPV and PDW compared to their healthy counterparts. This investigation was prompted by the understanding that PA represents an early stage in the development of metabolic syndrome and PCOS. Thus, identifying early markers in adolescence might reveal fundamental pathogenetic alterations predictive of later PCOS and/or metabolic syndrome development.

MPV, a parameter gauging the average platelet size in the blood, has been extensively studied for its potential role in assessing and predicting CVD risk, particularly in patients with acute coronary syndrome (10,11). Girls with a history of PA often experience a hyperandrogenic hormonal environment, potentially heightening their cardiovascular risk (17). An earlier study highlighted initial subclinical deterioration in cardiac function and atherosclerotic changes among girls with PA. The authors indicated that PA elevated the likelihood of coronary heart disease, which was attributed to heightened epicardial adipose tissue and carotid intima-media thickness measurements in individuals with PA (18). The augmented risk of coronary heart disease in children with PA was linked to excess adipose tissue in adulthood, suggesting that a process which begins with childhood obesity may contribute to CVD later in life, alongside PA and adult obesity (19). Bolat et al. (20) explored the relationship between platelet aggregation and PA, reporting increased collagen-induced platelet aggregation in girls with PA, potentially associated with an elevated risk of CVD. Moreover, a significant number of girls with a history of PA later develop findings of PCOS, including hyperandrogenism, anovulatory menstrual cycles, and insulin resistance (21). Coviello et al.'s (22) recent findings indicated that adolescents with PCOS exhibited a notably higher occurrence of metabolic syndrome components in comparison to their healthy peers, suggesting a potential relationship between CVD and PA, which carries a high risk of developing PCOS. Our results aligned with these associations, revealing significantly higher levels of MPV and PDW in individuals with PA - both potential inflammation markers for assessing and predicting CVD risk - when compared to healthy controls. However, the PLR and the NLR, recognized as potential inflammation markers for cardiac and non-cardiac diseases in recent years (23), were similar between our study group and our healthy controls.

Prepubertal children with PA commonly develop hyperinsulinemia. Consistent with other research, Liimatta et al. (8) demonstrated that insulin resistance persists into young adulthood in women with a history of PA. Their study revealed that the risk of impaired glucose metabolism persists into adulthood and is strongly associated with central obesity (8). Ibáñez et al. (24) reported similar findings in oral glucose tolerance tests (OGTT) among Catalan girls with premature pubarche. Despite being mostly lean, there was an observed increase in central fat mass associated with hyperinsulinemia (24). In contrast, in a study by Meas et al. (25) where girls with premature pubarche and control subjects within normal BMI limits completed OGTTs, it was found that there was normal glucose tolerance in all subjects, with no significant differences in the plasma glucose or serum insulin profiles between the study groups (25). In our study, insulin resistance in girls with PA was evaluated when measured by HOMA-IR, and no significant increase in insulin resistance was detected.

### Study Limitations

Acknowledging this study's limitations provides context for interpreting the findings and guides future research endeavors. The inclusion of a relatively small sample size from a single institution may restrict the generalizability of the findings to the broader population. Additionally, since the number of male patients with PA was insufficient, only female cases were included in this study. This study's cross-sectional nature hinders establishing a cause-and-effect relationship between PA and the observed markers of inflammation. Cross-sectional studies offer associations but need to elucidate the temporal sequence of events. Continued research with larger cohorts and longitudinal designs will enhance the robustness of our understanding of PA and its cardiovascular implications**.**

## Conclusion

In summary, this study provides a foundational framework to investigate deeper into the complex interplay between PA and cardiovascular well-being. The highlighted inflammatory markers, notably MPV and PDW, present promising avenues for early risk assessment and targeted interventions. As ongoing research advances our understanding of the intricate mechanisms connecting adrenal androgen production to cardiovascular outcomes,

there is potential for uncovering more nuanced insights. These revelations, in turn, could inform the development of preventive strategies and tailored healthcare approaches for those individuals dealing with PA. Moreover, the correlations observed between MPV and PDW underscore the potential interconnectedness of these markers in the context of PA. This research lays the groundwork for prospective studies which may reveal the complexities of these relationships, offering valuable insights into refining cardiovascular risk management strategies in this specific population.

### Ethics

**Ethics Committee Approval:** Ethical approval, aligning with the Declaration of Helsinki, was obtained from the İzmir Bakırçay University Non-invasive Clinical Research Ethics Committee (dated: 10.18.2023, approval no.: 1239).

**Informed Consent:** Informed consent, acknowledging the study's objectives and potential medical data publication, was secured from the parents of all of the patients.

### Authorship Contributions

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

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Financial Disclosure:** The authors disclose that this research study was not funded by any specific grants from public, commercial, or not-for-profit sectors. The study was conducted independently without any financial support or sponsorship that could potentially introduce bias or influence the research outcomes.

## References

1. Silverman SH, Migeon C, Rosemberg E, et al. Precocious growth of sexual hair without other secondary sexual development; premature pubarche, a constitutional variation of adolescence. Pediatrics 1952; 10:426-32.
2. Voutilainen R, Jääskeläinen J. Premature adrenarche: etiology, clinical findings, and consequences. J Steroid Biochem Mol Biol 2015; 145:226-36.
3. Voutilainen R, Riikonen R, Simell O, Perheentupa J. The effect of acth therapy on serum dehydroepiandrosterone, androstenedione, testosterone and 5α -dihydrotestosterone in infants. J Steroid Biochem 1987; 28:193-6.
4. Ibañez L, Virdis R, Potau N, et al. Natural history of premature pubarche: an auxological study. J Clin Endocrinol Metab 1992; 74:254-7.
5. Williams RM, Ward CE, Hughes IA. Premature adrenarche. Arch Dis Child 2012; 97:250-4.
6. Ibañez L, Potau N, Virdis R, et al. Postpubertal outcome in girls diagnosed of premature pubarche during childhood: increased frequency of functional ovarian hyperandrogenism. J Clin Endocrinol Metab 1993; 76:1599-603.
7. Sopher AB, Jean AM, Zwany SK, et al. Bone age advancement in prepubertal children with obesity and premature adrenarche: possible potentiating factors. Obesity 2011; 19:1259-64.
8. Liimatta J, Utriainen P, Laitinen T, Voutilainen R, Jääskeläinen J. Cardiometabolic risk profile among young adult females with a history of premature adrenarche. J Endocr Soc 2019; 3:1771-83.
9. Veninga A, Handtke S, Aurich K, et al. GPVI expression is linked to platelet size, age, and reactivity. Blood Adv 2022; 6:4162-73.
10. Brahmbhatt KJ, Chaudhary B, Raval DM, et al. Association of mean platelet volume with vascular complications in the patients with type 2 diabetes mellitus. Cureus 2022;19;14:e29316.
11. Li J, Zhang J, Chen Y, et al. Mean platelet volume modifies the contribution of homocysteine to cardiovascular disease: a real-world study. Nutr Metab Cardiovasc Dis 2023; 33:194-202.
12. Rajamanickam R, Shanmugavelu L, Subramanian S, et al. Hashimoto's thyroiditis in south ındian centre. Indian J Pediatr 2016; 83:1227-31.
13. Yilmaz H, Ucan B, Sayki M, et al. Usefulness of the neutrophil-to-lymphocyte ratio to prediction of type 2 diabetes mellitus in morbid obesity. Diabetes Metab Syndr 2015; 9:299-304.
14. Guran T, Firat I, Yildiz F, et al. Reference values for serum dehydroepiandrosterone-sulphate in healthy children and adolescents with emphasis on the age of adrenarche and pubarche. Clin Endocrinol 2015; 82:712-8.
15. Demir K, Özen S, Konakçı E, et al. A comprehensive online calculator for pediatric endocrinologists: çedd çözüm/tpeds metrics. J Clin Res Pediatr Endocrinol 2017; 9:182-4.
16. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969; 44:291-303.
17. Livadas S, Bothou C, Macut D. Premature adrenarche and its association with cardiovascular risk in females. Curr Pharm Des 2020; 26:5609-16.
18. Çelik N, Alp H, Çamtosun E, Alp E, Çelik S, Berk E. The association between premature adrenarche and cardiovascular risk may be greater than expected. Horm Res Paediatr 2017; 87:7-14.
19. Prentice P, Viner RM. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. Int J Obes 2013; 37:1036-43.
20. Bolat A, Zeybek C, Gürsel O, Akın O, Taşçılar ME. The relationship between premature adrenarche and platelet aggregation. J Clin Res Pediatr Endocrinol 2023; 27;15:55-61.
21. Báñez L, Dimartino-Nardi J, Potau N, Saenger P. Premature adrenarche--normal variant or forerunner of adult disease? Endocr Rev 2000; 21:671-96.
22. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol Metab 2006; 91:492-7.

23. Onalan E, Gozel N, Donder E. Can hematological parameters in type 2 diabetes predict microvascular complication development? Pakistan J Med Sci 2019; 35:1511-5.

24. Ibáñez L, Ong K, De Zegher F, Marcos MV, Del Rio L, Dunger DB. Fat distribution in non-obese girls with and without precocious pubarche: central adiposity related to insulinaemia and androgenaemia from prepuberty to postmenarche. Clin Endocrinol 2003; 58:372-9.

25. Meas T, Chevenne D, Thibaud E, et al. Endocrine consequences of premature pubarche in post-pubertal caucasian girls. Clin Endocrinol 2002; 57:101-6.

J Pediatr Res 2024;11(1):58-65
DOI: 10.4274/jpr.galenos.2023.95770

Original Article

# Comparison of Anxiety of the Children of Healthcare Workers and Non-Healthcare Workers During the COVID-19 Pandemic

Emine Burçe Dörtkardeşler[1], Begüm Şahbudak[2], Zümrüt Şahbudak Bal[3]

[1]Ege University Faculty of Medicine, Department of Pediatrics, Division of General Pediatrics, İzmir, Turkey
[2]Manisa City Hospital, Clinic of Child and Adolescent Psychiatry, Manisa, Turkey
[3]Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Infectious Disease, İzmir, Turkey

**ABSTRACT**

**Aim:** The children of healthcare workers (HCWs) constitute a group highly vulnerable to anxiety disorders during the coronavirus disease-2019 (COVID-19) pandemic. This study aimed to compare the prevalence and severity of anxiety between the children of HCWs and non-HCWs while identifying factors contributing to this anxiety.

**Materials and Methods:** A cross-sectional study was conducted involving 334 children aged between 6 and 17 years to assess their anxiety levels. The risk factors related to anxiety were determined using binary logistic regression analysis.

**Results:** Significant risk factors for anxiety included having parents who were HCWs, having parents with psychiatric disorders, changes in household members, and following news updates (p=0.045, 0.022, 0.021, and 0.024, respectively). Children of HCWs working in COVID-19 clinics exhibited a higher prevalence of moderate to severe anxiety than the other groups (p=0.036). Additionally, prolonged screen time and changes in sleep duration were more common in children with moderate to severe anxiety.

**Conclusion:** The results suggest that the children of HCWs may be more prone to anxiety symptoms than the children of parents in other professions.

**Keywords:** Anxiety, COVID-19, pandemic, children, healthcare workers

## Introduction

Coronavirus disease-2019 (COVID-19) emerged as a global health crisis, affecting nations worldwide (1). Following the first reported cases, stringent quarantine measures were enacted to curb the infection's spread worldwide. The COVID-19 pandemic also significantly impacted mental health. Early pandemic research in China found that 16.5-28.8% of respondents had moderate to severe depression and anxiety (2). Increased anxiety stemmed from the virus's uncertain incubation, asymptomatic transmission, shortages of medical supplies and staff, and overexposure to COVID-19 news (3,4).

Healthcare workers (HCWs) are especially vulnerable to pandemic-related mental health issues. Their sleep quality is poorer than that of the general public. Among HCWs, women and frontline HCWs report anxiety symptoms

**Address for Correspondence**
Begüm Şahbudak, Manisa City Hospital, Clinic of Child and Adolescent Psychiatry, Manisa, Turkey
**Phone:** +90 505 441 16 14 **E-mail:** begumsahbudak@gmail.com **ORCID:** orcid.org/0000-0003-4928-780X
**Received:** 23.08.2023 **Accepted:** 13.11.2023

more frequently (2-5). The main stressors include virus transmission fears, handling fears and anxiety, and HCW shortages (1,6).

Children and adolescents are especially vulnerable to pandemic-related mental health issues. Factors such as separation from caregivers, infection risk, hospital isolation, and loss can amplify their anxiety. Parents play a pivotal role in easing their children's distress. Their own stress and ability to handle their children's anxiety directly influence their child's anxiety levels (7,8). School closures resulted in less physical activity, altered eating patterns, and more screen time, increase anxiety and distress in children (9-11).

This study aims to evaluate the anxiety levels of children during the COVID-19 pandemic, identify its underlying causes, and compare the severity of anxiety between children of HCWs and those of other professions.

## Materials and Methods

This study employed a cross-sectional, case-control design. A Google form survey was constructed for this study to be completed by the parents and children. Ethics committee approval was obtained from the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 20-7T/51, date: 08.07.2020), and written informed consent was obtained from all participating children and their legal guardians, either via email or in writing. Participants were recruited through notices posted on hospital boards, explicitly targeting children aged 6-17 years and their parents. The participants were without any history of psychiatric disorders. HCWs, encompassing doctors, nurses, health technicians, and secretaries, were identified as potential participants. Those with a familial COVID-19 history were excluded from this study to avoid potential confusion. Four hundred and eighty-two participants and their children completed the online forms. However, 81 were excluded due to incomplete form filling, under cut-off points for anxiety, or a COVID-19 family history. Of these excluded participants, 56 had a familial COVID-19 history, while 25 left form responses incomplete. Sixty-seven participants (30 children of HCWs and 37 children of other professions) were excluded from this study due to providing no or almost no responses to the questions in the The Diagnostic and Statistical Manual of Mental Disorders (DSM) Level 1 Anxiety section, as completed by their parents. Consequently, the final sample consisted of 334 parents and their children who consented to participate between the dates of July to November, 2020.

**Sociodemographic data form:** This survey includes multiple-choice and open-ended questions. The collected demographic data included the children's pre-existing medical conditions, parents' age, profession, education level, residence, alterations in household members, and the current living conditions of the child. For HCW parents, their workplace information was also collected. The survey queried total screen time, durations of physical activity and sleep, and eating habit changes.

**The Diagnostic and Statistical Manual of Mental Disorders 5 - Level 1 Cross-Cutting Symptom Measure Anxiety Disorders Part:** The anxiety section of the scale contains three questions assessing symptoms over two weeks. Scoring options were as follows: never (0), almost never (1), mild (2), moderate (3), and severe (4). Answering as mild or above on any question prompted further evaluation with the Level 2 DSM-5 Anxiety Scale. The parent form evaluated anxiety symptoms in children aged 6-17.

**The Diagnostic and Statistical Manual of Mental Disorders 5 - Level 2 Anxiety Scale:** This scale was utilized to evaluate the anxiety levels of the children. It is a 4-point Likert-type scale with 26 items. The scale is scored as follows: never (1 point), almost never (2 points), sometimes (3 points), often (4 points) and almost always (5 points). This scale assessing anxiety symptoms over the past seven days consists of a 10-item parent form completed by parents or legal guardians of the children aged 6-17 years and a 13-item self-report form for adolescents aged 11-17 years to complete themselves. A cut-off score of 55 was set for anxiety: 55-59.9 denotes mild anxiety, 60-69.9 signifies moderate anxiety, and scores above 70 indicate severe anxiety (12).

### Statistical Analysis

The statistical analysis was conducted using IBM SPSS 25.0 software. The Kolmogorov-Smirnov test and Shapiro-Wilk tests assessed the normality of the quantitative data. Parametric methods were used for normally distributed variables, while non-parametric methods were applied to those not normally distributed. The Mann-Whitney U test was used to compare independent groups, the Pearson chi-square test was used for categorical data. Quantitative variables are depicted as median and interquartile range, while categorical variables are expressed as frequencies. We used a logistic regression model to identify the predictors of anxiety in children. Analysis was conducted at a 95% confidence level, with p-values less than 0.05 considered statistically significant.

## Results

The participant characteristics are presented in Table I. No significant differences were found between the groups

regarding ages and living environments. However, there were significant disparities concerning parental education levels, income levels, and gender. Anxiety severity rates, compared by age, are displayed in Table II, and the participant data for those aged 6-17 years are summarized in Table III.

We conducted a logistic regression model with factors significantly associated with anxiety in children (Table IV). The model fit was good (Hosmer and Lemeshow test, p=0.865). According to this regression analysis, all factors were found to impact children's anxiety. The most significant factor was change of people at home. The self-assessment data for participants aged 11-17 years are summarized in Table V. In regression analysis (Hosmer and Lemeshow Test p=0.731), change of people at home was the only factor detected as increasing anxiety in adolescents (Table VI).

**Table I.** Demographic characteristics of the participants

| | **HCWs parents n=118 (35.3%)** | **Parents with other profession n=216 (64.7%)** | **p value** |
|---|---|---|---|
| Child age (months) [median (IQR†)] | 112.97 (73.66) | 120.34 (73.24) | 0.396* |
| Maternal age (years) [median (IQR†)] | 40.50 (6) | 40.00 (6) | 0.195* |
| Paternal age (years) [median (IQR†)] | 42.00 (7) | 42.00 (7) | 0.581* |
| **Child age** | | | |
| 6-10 years, n (%) | 76 (64.4) | 123 (56.9) | 0.184** |
| 11-17 years, n (%) | 42 (35.6) | 93 (43.1) | |
| **Gender** | | | |
| Female, n (%) | 70 (59.3) | 102 (47.2) | **0.034**** |
| Male, n (%) | 48 (40.7) | 114 (52.8) | |
| **Education level of mother** | | | |
| First-degree or high school, n (%) | 16 (13.6) | 89 (41.2) | **<0.001**** |
| College or higher, n (%) | 102 (86.4) | 127 (58.8) | |
| **Education level of father** | | | |
| First-degree or high school, n (%) | 18 (15.3) | 85 (39.5) | **<0.001**** |
| College or higher, n (%) | 100 (84.7) | 130 (60.5) | |
| **Place of residence** | | | |
| Urban area, n (%) | 112 (94.9) | 199 (92.1) | 0.337** |
| Rural area, n (%) | 6 (5.1) | 17 (7.9) | |
| **Number of children** | | | |
| 1 or 2 children, n (%) | 112 (94.9) | 192 (88.9) | 0.066** |
| 3 children or more, n (%) | 6 (5.1) | 24 (11.1) | |

†Interquartile range, *Mann-Whitney-U test, **Pearson chi-square test, HCWs: Health care workers

**Table II.** Anxiety severity of children compared by ages (Parents' assessment and Self-Assessment)

| | **6-10 years (Parents' assessment) n=199 (59.6%)** | **11-17 years (Parents' assessment) n=135 (40.4%)** | **11-17 years (Self-assessment) n=135 (40.4%)** |
|---|---|---|---|
| No anxiety, n (%) | 90 (45.2) | 67 (49.6) | 80 (59.3) |
| Mild, n (%) | 42 (21.1) | 22 (16.3) | 22 (16.3) |
| Moderate, n (%) | 58 (29.1) | 31 (23.0) | 26 (19.3) |
| Severe, n (%) | 9 (4.5) | 15 (11.1) | 7 (5.2) |
| Total, n (%) | 109 (54.8) | 68 (50.4)* | 55 (40.7)* |

*p<0.001 (Pearson chi-square test)

When comparing the anxiety of children of HCWs working in COVID-19 clinics, non-COVID-19 clinics, and other professions, it was found that 25 (65.8%) out of the 38 children of HCWs working in COVID-19 clinics exhibited anxiety symptoms. Anxiety symptoms were reported in 47 (58.8%) out of the 80 children of HCWs working in non-COVID-19 clinics, and in 105 (48.6%) out of the 216 children of other professions (Table VII). Thus, the children of HCWs working in COVID-19 clinics exhibited anxiety symptoms more frequently than others (p=0.036).

In the HCWs group, 53.4% were doctors, 23.7% were nurses, 6.8% were technicians, and 16.1% were from other departments such as psychology and pharmacy.Among children of doctors, 66.7% reported experiencing anxiety, compared to 57.1% among nurses' children and 51.9% among children from other departments. However, these differences were not statistically significant (p=0.372).

The relationship between anxiety and screen time, physical activity duration, weight changes, and changes in sleep time were also evaluated (Table VIII).

**Table III.** Comparison of children 6-17 years old with anxiety and controls (According to parents' assessment)

| | **Anxiety 177 (53.0)** | **Controls 157 (47.0)** | **p value** |
|---|---|---|---|
| **Gender, n (%)** | | | |
| Female | 100 (58.1) | 72 (41.9) | 0.052* |
| Male | 77 (47.5) | 85 (52.5) | |
| **Age, n (%)** | | | |
| 6-10 years | 109 (54.8) | 90 (45.2) | 0.429* |
| 11-17 years | 68 (50.4) | 67 (49.6) | |
| **Education level of mothers, n (%)** | | | |
| First-degree or high school | 54 (51.4) | 51 (48.6) | 0.698* |
| College or higher | 123 (53.7) | 106 (46.3) | |
| **Education level of fathers, n (%)** | | | |
| First-degree or high school | 51 (49.5) | 52 (50.5) | 0.373* |
| College or higher | 126 (54.8) | 104 (45.2) | |
| **Place of residence, n (%)** | | | |
| Urban area | 169 (54.3) | 142 (45.7) | 0.070* |
| Rural area | 8 (34.8) | 15 (65.2) | |
| **Profession of parents, n (%)** | | | |
| Healthcare workers | 72 (61.0) | 46 (39.0) | **0.030*** |
| Other professions | 105 (48.6) | 111 (51.4) | |
| Having a chronic disease, n (%) | 29 (44.6) | 36 (55.4) | 0.132* |
| Psychiatric disorders in parents, n (%) | 17 (77.3) | 5 (22.7) | **0.018*** |
| Following the news, n (%) | 123 (57.5) | 91 (42.5) | **0.028*** |
| Change of people at home, n (%) | 18 (81.8) | 4 (18.2) | **0.005*** |

*Pearson chi-square test

**Table IV.** Logistic regression model on factors associated with anxiety in children

| | **p value** | **Odds ratio** | **95% CI (Lower-Upper)** |
|---|---|---|---|
| HCW parents | **0.045*** | 1.616 | 1.010-2.586 |
| Psychiatric disorders in parents | **0.022*** | 3.392 | 1.192-9.655 |
| Following the news | **0.024*** | 1.713 | 1.073-2.734 |
| Change of people in the house | **0.021*** | 3.750 | 1.223-11.496 |

*p<0.005 (Binary Logistic Regression)
CI: Confidence interval

**Table V.** Comparison of 11-17 years aged children with anxiety and controls (According to self-assessment)

| | **Anxiety 55 (40.7)** | **Controls 80 (59.3)** | **p value** |
|---|---|---|---|
| **Gender, n (%)** | | | |
| Female | 28 (42.4) | 38 (57.6) | 0.697 |
| Male | 27 (39.1) | 42 (60.9) | |
| **Education level of mothers, n (%)** | | | |
| First-degree or high school | 29 (42.6) | 39 (57.4) | 0.650 |
| College or higher | 26 (38.8) | 41 (61.2) | |
| **Education level of fathers, n (%)** | | | |
| First-degree or high school | 27 (39.1) | 42 (60.9) | 0.643 |
| College or higher | 28 (43.1) | 37 (56.9) | |
| **Place of residence, n (%)** | | | |
| Urban area | 53 (44.5) | 66 (55.5) | **0.014** |
| Rural area | 2 (12.5) | 14 (87.5) | |
| **Profession of parents, n (%)** | | | |
| HCWs | 22 (52.4) | 20 (47.6) | 0.274 |
| Other professions | 35 (37.6) | 58 (62.4) | |
| Having a chronic disease, n (%) | 4 (17.4) | 19 (82.6) | **0.012** |
| Psychiatric disorders in parents, n (%) | 4 (50.0) | 4 (50.0) | 0.715 |
| Following the news, n (%) | 49 (47.6) | 54 (52.4) | **0.004** |
| Change of people at home, n (%) | 6 (85.7) | 1 (14.3) | **0.018** |

Pearson chi-square test
HCWs: Healthcare workers

**Table VI.** Logistic regression model on factors associated with 11-17 aged children's anxiety (According to self-assessment)

| | **p value** | **Odds ratio** | **95% CI (Lower-Upper)** |
|---|---|---|---|
| Place of residence (Urban area) | 0.100 | 2.120 | 0.865-5.198 |
| Following the news | 0.064 | 1.545 | 0.976-2.447 |
| Change of people at home | **0.017*** | 3.911 | 1.280-11.950 |
| Having a chronic disease | 0.140 | 0.656 | 0.374-1.148 |

CI: Confidence interval

**Table VII.** Comparison of anxiety according to parents' professions

| | **Anxiety n=177 (53.0)** | | | | **Controls n=157 (47.0)** | **p value** |
|---|---|---|---|---|---|---|
| | **Mild** | **Moderate** | **Severe** | **Total** | | |
| **HCWs (COVID-19 clinics), n (%)** | 9 (23.7) | 11 (28.9) | 5 (13.2) | 25 (65.8) | 13 (34.2) | 0.036* |
| **HCWs (non-COVID-19 clinics), n (%)** | 24 (30.0) | 19 (23.8) | 4 (5.0) | 47 (58.8) | 33 (41.3) | |
| **Other professions, n (%)** | 31 (14.4) | 59 (27.3) | 15 (6.9) | 105 (48.6) | 111 (51.4) | |

*Pearson chi-square test
HCWs: Healthcare workers, COVID-19: Coronavirus disease-2019

**Table VIII.** Children's daily habits related to anxiety

| | **Anxiety** | | | **Controls, n (%) 157 (47.0%)** | **p value*** |
|---|---|---|---|---|---|
| | **Mild, n (%) 64 (19.1)** | **Moderate, n (%) 89 (26.6)** | **Severe, n (%) 24 (7.1)** | | |
| **Screen time** | | | | | |
| <1 hour | 1 (1.6) | 3 (3.4) | 0 (0.0) | 19 (12.1) | **0.005*** |
| 1-3 hours | 22 (34.4) | 19 (21.3) | 4 (16.7) | 44 (28.0) | |
| >3 hours | 41 (64.1) | 67 (75.3) | 20 (83.3) | 94 (59.9) | |
| **Change in sleep time** | | | | | |
| Same | 36 (56.3) | 48 (53.9) | 5 (20.8) | 107 (68.2) | **<0.001*** |
| Increased | 20 (31.3) | 23 (25.8) | 10 (41.7) | 23 (14.6) | |
| Decreased | 8 (12.5) | 18 (20.2) | 9 (37.5) | 27 (17.2) | |
| **Change in weight** | | | | | |
| Same | 26 (40.6) | 37 (41.6) | 6 (25.0) | 71 (45.2) | 0.081* |
| Increased | 30 (46.9) | 49 (55.1) | 13 (54.2) | 68 (43.3) | |
| Decreased | 8 (12.5) | 3 (3.4) | 5 (20.8) | 18 (11.5) | |
| **Physical activity time** | | | | | |
| <1 hour | 33 (51.6) | 43 (48.3) | 17 (70.8) | 77 (49.0) | 0.588* |
| 1-3 hour | 24 (37.5) | 34 (38.2) | 6 (25.0) | 60 (38.2) | |
| >3 hours | 7 (10.9) | 12 (13.5) | 1 (4.2) | 20 (12.7) | |

*Pearson chi-square test

We identified a correlation between increased screen time and anxiety (p=0.005). Moreover, when comparing changes in sleep duration-increased, decreased, or unchanged-we observed that individuals with higher anxiety levels reported no change in sleep duration (p<0.001).

## Discussion

During COVID-19, children faced heightened psychological distress due to factors such as school closures, reduced physical activity, altered daily routines, and social isolation. Children of HCWs also grappled with fears of losing their parents and heightened separation anxiety. Our study, seemingly the first of its kind, compared anxiety in HCWs' children to those of other professionals during the pandemic, finding a higher anxiety prevalence in HCWs' children. Contributing factors included the parents' job, their history of psychiatric disorders, news exposure, and caregiver changes (13,14).

Comparing age groups (6-11 and 12-17), we observed slightly higher anxiety levels in the 6-11 age group, though the difference is not statistically significant. In contrast, another research reported greater psychological distress in high school students (15). Also, in one review, it was found that there were higher prevalence rates of psychiatric disorders in adolescents than in children (16). However, in our research, adolescents reported less anxiety than their parents perceived. This discrepancy could be due to adolescents' tendency to avoid reporting internalizing symptoms.

Our study found that the children of HCWs, those with parents having psychiatric disorders, those exposed to pandemic news, and those with changes in caregivers showed higher anxiety rates. Changes in the household were the most significant factor contributing to anxiety. Many HCWs were separated from their families to minimize infection risks, leading to increased household shifts for their children. The critical role of a stable family environment in maintaining mental health is well-documented (16). Changes in caregivers within households lead to feelings of isolation among children of HCWs.

Studies show higher psychiatric symptom rates in females, aligning with our findings of increased anxiety in girls during the pandemic (15-18). Prior research highlights urban living, a history of psychiatric disorders, and female

gender as anxiety risk factors (19). Our study also noted raised anxiety levels in urban children, especially evident in self-reports from those aged 11-17.

Studies investigating the mental health of HCWs during the pandemic reported anxiety rates ranging from 20% to 60.2% (5,20-23). A positive correlation was found between the anxiety scores of the HCWs and their children (20). Consistent with this, we found a higher rate of anxiety among children of HCWs compared to others. Moreover, our findings showed a higher prevalence of anxiety in the children of HCWs than in a previously reported study (20). These results align with the notion that frontline HCWs and their children are at increased risk of psychiatric disorders (5,18,21,24). We observed more common anxiety, particularly moderate and severe cases, in the children of frontline HCWs compared to second-line HCWs and other professions.

Sleeping problems and changes are common among children during the pandemic (9). Previous studies have reported sleep disorders and decreased social interaction during the pandemic (25-27). However, we found that children with higher levels of anxiety reported no change in their sleep duration. Interestingly, pandemic-related home refinement was reported to improve sleep quality (9), suggesting that pandemic-induced anxiety symptoms might not be associated with changes in sleep patterns.

### Study Limitations

While our study contributes to the literature, it has limitations. Our results rely on self-reported data, potentially compromising objectivity. The small sample size further restricts our findings. Social distancing measures necessitated an online survey, possibly limiting participant numbers. Using online recruitment due to COVID-19 might also have affected our ability to carry out objective psychiatric evaluations.

## Conclusion

Our research underscores the need to support HCWs' children's mental health, as they are especially vulnerable during the COVID-19 pandemic. Clinicians should recognize that these children are a particularly vulnerable group and may require special attention in terms of mental health support. Healthcare providers should screen for these risk factors when assessing children's mental health during and after the pandemic. Clinicians should consider age and gender when assessing and addressing anxiety in children, tailoring interventions accordingly. This study emphasizes that changes in the household, particularly related to HCWs separated from their families to minimize infection risks, had a significant impact on children's anxiety. Clinicians should discuss screen time and sleep habits with children and their parents, providing guidance on healthy routines and strategies to manage anxiety-related sleep disturbances. Given the challenges of conducting in-person assessments during the pandemic, clinicians should continue to explore and utilize online mental health services to reach and support children who may be experiencing anxiety.

This study's surprising finding of heightened anxiety in adolescents in rural areas suggests that mental health services should be accessible and tailored to the specific needs of rural communities. A multidisciplinary approach involving healthcare providers, psychologists, educators, and community support services may be necessary to address the complex mental health needs of children during and after the pandemic.

### Acknowledgments

The authors wish to thank the children, their parents, and the HCWs who participated in this research project.

### Ethics

**Ethics Committee Approval:** Ethics committee approval was obtained from the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 20-7T/51, date: 08.07.2020).

**Informed Consent:** Written informed consent was obtained from all participating children and their legal guardians, either via email or in writing.

### Authorship Contributions

Concept: E.B.D., B.Ş., Z.Ş.B., Design: E.B.D., B.Ş., Z.Ş.B., Data Collection or Processing: E.B.D., B.Ş., Z.Ş.B., Analysis or Interpretation: E.B.D., B.Ş., Z.Ş.B., Literature Search: E.B.D., B.Ş., Z.Ş.B., Writing: E.B.D., B.Ş., Z.Ş.B.

**Conflict of Interest:** No potential conflict of interest was reported by the authors.

**Financial Disclosure:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

## References

1. Chen Q, Liang M, Li Y, et al. Mental health care for medical staff in China during the COVID-19 outbreak. Lancet Psychiatry 2020; 7:e15-e16.
2. Wang C, Pan R, Wan X, et al. Immediate Psychological Responses and Associated Factors during the Initial Stage of the 2019

Coronavirus Disease (COVID-19) Epidemic among the General Population in China. Int J Environ Res Public Health 2020; 17:1-25.

3. Huang Y, Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 outbreak in China: a web-based cross-sectional survey. Psychiatry Res 2020; 288:1-6.
4. Dong L, Bouey J. Public Mental Health Crisis during COVID-19 Pandemic, China. Emerg Infect Dis 2020; 26:1616-8.
5. Lai J, Ma S, Wang Y, et al. Factors Associated With Mental Health Outcomes Among Health Care Workers Exposed to Coronavirus Disease 2019. JAMA Netw Open 2020; 3:e203976.
6. Joob B, Wiwanitkit V. Medical personnel, COVID-19 and emotional impact. Psychiatry Res 2020; 288:112952.
7. Liu JJ, Bao Y, Huang X, Shi J, Lu L. Mental health considerations for children quarantined because of COVID-19. Lancet Child Adolesc Health 2020; 4:347-9.
8. Loades ME, Chatburn E, Higson-Sweeney N, et al. Rapid Systematic Review: The Impact of Social Isolation and Loneliness on the Mental Health of Children and Adolescents in the Context of COVID-19. J Am Acad Child Adolesc Psychiatry 2020; 59:1218-1239.e3.
9. Altena E, Baglioni C, Espie CA, et al. Dealing with sleep problems during home confinement due to the COVID-19 outbreak: Practical recommendations from a task force of the European CBT-I Academy. J Sleep Res 2020; 29:e13052.
10. The Lancet Child Adolescent Health. Pandemic school closures: risks and opportunities. Lancet Child Adolesc Health 2020; 4:341.
11. Wang G, Zhang Y, Zhao J, Zhang J, Jiang F. Mitigate the effects of home confinement on children during the COVID-19 outbreak. Lancet 2020; 395:945-7.
12. Diagnostic and Statistical Manual of Mental Disorders, 5th Ed., Text Revision (DSM-5-TR®). DSM-5-TR Online Assessment Measures. American Psychiatric Association, 2013.
13. Dubey S, Biswas P, Ghosh R, et al. Psychosocial impact of COVID-19. Diabetes Metab Syndr 2020; 14:779-88.
14. Mahajan C, Kapoor I, Prabhakar H. Psychological Effects of COVID-19 on Children of Health Care Workers. Anesth Analg. 2020; 131: e169-e170.
15. Qin Z, Shi L, Xue Y, et al. Prevalence and Risk Factors Associated With Self-reported Psychological Distress Among Children and Adolescents During the COVID-19 Pandemic in China. JAMA Netw Open 2021; 4:e2035487.
16. Marques de Miranda D, da Silva Athanasio B, Sena Oliveira AC, Simoes-E-Silva AC. How is COVID-19 pandemic impacting mental health of children and adolescents? Int J Disaster Risk Reduct 2020; 51:1-8.
17. Duan L, Shao X, Wang Y, et al. An investigation of mental health status of children and adolescents in china during the outbreak of COVID-19. J Affect Disord 2020; 275:112-8.
18. Şahin MK, Aker S, Şahin G, Karabekiroğlu A. Prevalence of Depression, Anxiety, Distress and Insomnia and Related Factors in Healthcare Workers During COVID-19 Pandemic in Turkey. J Community Health 2020; 45:1168-77.
19. Özdin S, Bayrak Özdin Ş. Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: The importance of gender. Int J Soc Psychiatry 2020; 66:504-11.
20. Şahin B, Hoşoğlu E, Bedia Ö. Anxiety symptoms in healthcare workers and their children during the COVID-19 pandemic in Turkey. Namık Kemal Tıp Dergisi 2020; 8:321-30.
21. Al Ammari M, Sultana K, Thomas A, Al Swaidan L, Al Harthi N. Mental Health Outcomes Amongst Health Care Workers During COVID 19 Pandemic in Saudi Arabia. Front Psychiatry 2021; 11:1-11.
22. Shechter A, Diaz F, Moise N, et al. Psychological distress, coping behaviors, and preferences for support among New York healthcare workers during the COVID-19 pandemic. Gen Hosp Psychiatry 2020; 66:1-8.
23. Rossi R, Socci V, Pacitti F, et al. Mental Health Outcomes Among Frontline and Second-Line Health Care Workers During the Coronavirus Disease 2019 (COVID-19) Pandemic in Italy. JAMA Netw Open 2020; 3:e2010185.
24. Elbay RY, Kurtulmuş A, Arpacıoğlu S, Karadere E. Depression, anxiety, stress levels of physicians and associated factors in Covid-19 pandemics. Psychiatry Res 2020; 290:1-5.
25. Singh S, Roy D, Sinha K, Parveen S, Sharma G, Joshi G. Impact of COVID-19 and lockdown on mental health of children and adolescents: A narrative review with recommendations. Psychiatry Res 2020; 293:1-10.
26. Jiao WY, Wang LN, Liu J, et al. Behavioral and Emotional Disorders in Children during the COVID-19 Epidemic. J Pediatr 2020; 221:264-266.e1.
27. Guessoum SB, Lachal J, Radjack R, et al. Adolescent psychiatric disorders during the COVID-19 pandemic and lockdown. Psychiatry Res 2020; 291:1-6.

J Pediatr Res 2024;11(1):66-74
DOI: 10.4274/jpr.galenos.2024.92053

Original Article

# An Evaluation of Quality of Life in Children and Adolescents in an Inpatient Oncology Unit: A 6-month Follow-up Study

Birsen Şentürk Pilan[1], İpek İnal Kaleli[1], Serpil Erermiş[1], Mehmet Kantar[2], Eda Ataseven[2], Burcu Özbaran[1], Tezan Bildik[1]

[1]Ege University Faculty of Medicine, Department of Child and Adolescent Psychiatry, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Pediatrics, Pediatric Hematology and Oncology, İzmir, Turkey

**ABSTRACT**

**Aim:** Childhood cancers are life-threatening diseases which are universally distressing and potentially traumatic for children and their families at the time of diagnosis, during treatment, and beyond.

**Materials and Methods:** Thirty-nine child patients between the ages of 0-18 years receiving treatment in a pediatric oncology hospital for various pediatric cancers who consented to participate in this study were recruited. The participants were assessed via Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version-Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition/K-SADS-PL-DSM-5 for ages 6-18 by a trained and certified child and adolescent psychiatrist. The clinical assessments of patients aged 0-5 years were completed by a trained child and adolescent psychiatrist in agreement with the DSM-5 and the standard principles of psychiatric interview for the pediatric population. The previous and current psychiatric diagnoses of the participants were recorded. The Quality-of-Life Scale for Children was administered to the participants and their caregivers at the first interview and at the 6$^{th}$ month of follow-up.

**Results:** While no significant differences were observed in the quality of life of children with a novel pediatric cancer diagnosis and children with cancer recurrence/ongoing treatment per their own reports, the parents reported significant improvement in the quality of life of those children who had a novel cancer diagnosis after six months.

**Conclusion:** The parents' and their children's reports were highly correlated, and this association remained significant in multiple linear regression analyses for both the initial interviews and the follow-ups. The parents' reports on their children's quality of life appear to be reliable in accurately predicting their children's quality of life in the clinical setting.

**Keywords:** Pediatric oncology, quality of life, child and adolescent mental health, psycho-oncology

## Introduction

Childhood cancers are life-threatening diseases which are universally distressing and potentially traumatic for children and their families at the time of diagnosis, during treatment, and beyond. A population-based registry study conducted between 2001 and 2010 revealed age-standardized incidence rates for childhood cancers to be 140.6 per million person-years in the 0-14 year-old

**Address for Correspondence**
İpek İnal Kaleli, Ege University Faculty of Medicine, Department of Child and Adolescent Psychiatry, İzmir, Turkey
**Phone:** +90 536 856 07 96 **E-mail:** ipekinal93@gmail.com **ORCID:** orcid.org/0000-0003-2148-2363
**Received:** 22.12.2023 **Accepted:** 29.02.2024

demographic. Leukemia, central nervous system (CNS) tumors, and lymphomas were the most commonly reported cancers in the pediatric population respectively (1). Similarly, with a relatively younger population, a registry-based study conducted in Turkey reported 2,000 new cases of pediatric cancer each year. In contrast to the world statistics, the most common childhood cancers reported in Turkey were leukemias (31%), lymphomas (19%), CNS neoplasms (13%), and neuroblastomas (7%), which is in line with the reported frequencies in developing countries for lymphomas and CNS tumors; ranking as the second and third most common pediatric cancers, respectively (2). Dramatic improvements in survival rates have occurred as a result of increased aggressive multimodal therapies with 5-year survival rates reaching 74.4% in Turkey (3), but still 10-15% lower than the 5-year survival rates reported in developed countries (4).

Pediatric cancers remain among the leading causes of death in children (2,5), and despite developments in treatment methods and a global increase in survival rates, treatment process and complications negatively affect quality of life (QoL), especially in the pediatric population (6,7). The social and psychological distress experienced and expressed by the child and their caregivers vary depending on the type of cancer, the social environment, and the medical care provided, with all playing a role in the perceived distress. Thus, the health-related QoL, defined by the perceptions of the effects of a disease and its treatment on a patient over time, may worsen in children diagnosed with cancer and their families during and after treatment (8,9). While a multitude of studies have assessed the QoL after treatment, it is equally important to appraise the QoL during the treatment process in vulnerable populations, such as the pediatric population.

The present study aimed (1) to identify differences in QoL of children with a novel cancer diagnosis and those with recurrence or ongoing treatment, (2) to examine changes in QoL over time for both groups of children, and (3) to evaluate the level of agreement between parental reports on QoL of their children with the children's own reports to assess if the parent reports could be used in the place of the children's in situations where children may not be able to express their needs effectively.

## Materials and Methods

### Study Design

Forty-one child patients and their parents were recruited into this study. The patients were between the ages of 0-18 years receiving treatment in a pediatric oncology hospital for various pediatric cancers and all consented to participate in this study. The participants were assessed via Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS)-Present and Lifetime Version-Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition/K-SADS-PL-DSM-5 for ages 6-18 years by a trained and certified child and adolescent psychiatrist. A trained child and adolescent psychiatrist completed the clinical assessments of those patients aged 0-5 years in agreement with DSM-5 and the standard principles of psychiatric interview for the pediatric population. The previous and current psychiatric diagnoses of the participants were recorded. The Quality-of-Life Scale for Children (PedsQL) was administered to the patients and their parents at their first and their 6$^{th}$-month follow-up interviews.

Of the 41 patients recruited initially, two were unable to complete the second interview or the QoL assessment performed at six months and were excluded. Thus, a total of 39 patients and their parents were included in this study. Sixteen participants had been recently admitted to the hospital with a novel oncologic diagnosis (diagnosis made less than 6 months prior to the initial interview) and formed the novel diagnosis group, and 23 patients had been diagnosed more than 6 months prior with a history of either readmission for ongoing treatment or recurrence and formed the recurrence group.

This study was conducted at Ege University Faculty of Medicine and Ege University Faculty of Medicine Medical Research Ethics Committee approved the study regarding ethical principles (approval no.: 19-3.1T/45, date: 20.03.2019). Children and their parents were verbally informed about the study's design and verbal assent or written informed consent forms were obtained from the children and their parents, where appropriate.

### Measures

#### K-SADS

K-SADS is a widely utilized semi-structured psychiatric interview for the diagnosis of various pediatric mental disorders (10). K-SADS is an internationally valid and reliable tool for diagnosing psychiatric disorders of childhood including for the Turkish youth (11).

#### The PedsQL

PedsQL is a brief measure assessing five subdomains: physical functioning, emotional functioning, psychosocial functioning, social functioning, and school functioning to create a composite assessment of health-related QoL (12).

This measure can be completed by either the children and adolescents aged 2-18 years as a self-report questionnaire or by their caregivers (13). In the current study, even though 25.6% of the pediatric population (n=10) were unable to complete the scale due to their age, 74.4% were deemed to be at a capable level and the PedsQL was completed by both the caregivers and the children and both were included in the analysis.

### Statistical Analysis

IBM SPSS Statistics v25.0 was used for the statistical analyses. The distribution of the PedsQL scores were assessed by Shapiro-Wilk. The differences between the children's and their parents' PedsQL scores were assessed with Student's t-test for normally distributed variables and the Mann-Whitney U test for variables with a non-normal distribution. Changes in PedsQL scores during the initial interview and follow-up as paired samples were assessed with the Paired Samples t-test and Wilcoxon Rank-Sum test for normal and non-normal distributions, respectively. Pearson's and Spearman's correlation was utilized to determine the level of agreement between the parents' and children's PedsQL reports. Multiple Linear Regression Analyses were conducted in order to predict the children's PedsQL scores in the initial and the follow-up interviews by using the parents' PedsQL scores as the predictors in the respective interviews, while accounting for group and psychiatric diagnoses. All parametric and non-parametric tests were two-tailed, and p values <0.05 were considered statistically significant for all analyses.

## Results

A total of 39 pediatric patients and their caregivers were included in this study. The mean age of the population was 9.71 (±4.66) years, 38.5% (n=15) were male and 61.5% (n=24) were female. The highest frequencies among the pediatric neoplasms were rhabdomyosarcoma (n=7, 17.9%), osteosarcoma (n=7, 17.9%), medulloblastoma (n=4, 10.3%), and acute lymphoblastic leukemia (n=4, 10.3%) in the study population. The most common psychiatric diagnosis was depression (n=20, 51.3%), followed by adjustment disorder (n=7, 17.9%), anxiety disorder (n=3, 7.7%), and intellectual disability (n=1, 2.6%). Eight participants did not meet any criteria for any psychiatric disorder (20.5%). The sociodemographic data with pediatric oncologic and psychiatric disorders are summarized in Table I.

Nine children were unable to complete the PedsQL. The child and adolescent reported PedsQL scores were not found to be statistically different between the novel diagnosis (n=10) or the recurrence (n=20) groups in either the initial or follow-up interviews (all p>0.05). Only the parents' PedsQL School Functioning sub-scores in the initial interview were higher than the recurrence/ongoing treatment group (p=0.035). No differences in the parents' PedsQL total or subscale scores were found among the groups in the initial interview (all p>0.05). In contrast, the parents' PedsQL total scores (p=0.022) and Emotional Functioning (p=0.022), School Functioning (p=0.041), and Psychosocial Functioning (p=0.002) scores were found to be higher in the novel diagnosis group compared to the recurrence group at the 6-month follow-up interview. The differences in PedsQL scale scores between the novel diagnosis and recurrence groups in the initial and follow-up interviews are summarized in Table II.

The PedsQL scores according to the children did not significantly change between the initial and the follow-up interviews, for either the novel diagnosis or the recurrence groups (all p>0.05). However, the parents' PedsQL scores in the novel diagnosis group were found to be different between the initial and follow-up interviews for all domains except for the School Functioning domain (all p<0.05). The Physical Functioning (p=0.013), Emotional Functioning (p=0.020), Social Functioning (SFS) (p<0.001), Psychosocial Health Total Score (p=0.005) and scale total scores (p=0.002) were significantly higher in the sixth-month interview in comparison to the initial interview for the novel diagnosis group. However, a significant change was not observed for the parents' PedsQL scores of the readmission/ ongoing treatment group with a history of oncologic disease greater than 6 months during their initial and follow-up assessments (Table III).

The parents' scores for PedsQL were found to be highly correlated with the children's PedsQL scores in all subdomains for both interviews (all p<0.05). The correlation between the total scores and the subscale scores in the initial and the follow-up interview of the patients and their caregivers for the PedsQL are summarized in Table IV.

Multiple linear regression analyses were conducted to test whether the parents' PedsQL scores could be used to reliably predict the children's PedsQL scores, while also accounting for group (novel diagnosis vs. recurrence)

and psychiatric diagnosis for both the initial and follow-up interviews. The multiple linear regression model [F (3,26)=5.075, p=0.007, $R^2$=0.369, adjusted $R^2$=0.297] while accounting for group and psychiatric diagnosis and the parents' PedsQL scores as the predictor was significant, and the parents' PedsQL scores were found to be a significant predictor of the children's PedsQL scores at the initial interview (β=0.616, p=0.001).

The multiple linear regression model for the follow-up interview [F (3,25)=42.720, p<0.001, $R^2$=0.837, adjusted $R^2$=0.817] was also significant, and the parents' PedsQL scores were found to be a significant predictor of the children's PedsQL scores (β=0.950, p<0.001), when the group and psychiatric diagnosis variables were accounted for. The results of the multiple linear regression analyses can be found in Table V.

**Table I.** Socio-demographic data with oncologic and psychiatric diagnoses of the participants

| | **Novel diagnosis (n=16)** | **Recurrence/ongoing treatment (n=23)** | **Total (n=39)** | **Z/$\chi^2$** | **p value** |
|---|---|---|---|---|---|
| | **M (SD)/n (%)** | **M (SD)/n (%)** | **M (SD)/n (%)** | | |
| **Age** | 7.31±3.94 | 11.39±4.45 | 9.71±4.66 | -2.788 | 0.004[a]* |
| **Gender** | | | | 0.011 | 0.918[b] |
| Male | 6 (37.5) | 9 (39.1) | 15 (38.5) | | |
| Female | 10 (62.5) | 14 (60.9) | 24 (61.5) | | |
| **Age at diagnosis** | 7.31±3.94 | 9.19±4.49 | 8.42±4.32 | -1.274 | 0.207[a] |
| **Oncologic diagnosis** | | | | | |
| Rhabdomyosarcoma | 1 (2.6) | 6 (15.4) | 7 (17.9) | | |
| Osteosarcoma | 3 (7.7) | 4 (10.3) | 7 (17.9) | | |
| Acute lymphoblastic leukemia | 2 (5.1) | 2 (5.1) | 4 (10.3) | | |
| Medulloblastoma | 2 (5.1) | 2 (5.1) | 4 (10.3) | | |
| Lymphoma | 2 (5.1) | 1 (2.6) | 3 (7.7) | | |
| Wilms tumor | 1 (2.6) | 1 (2.6) | 2 (5.1) | | |
| Neuroblastoma | 2 (5.1) | 0 (0) | 2 (5.1) | | |
| Hepatocellular carcinoma | 0 (0) | 2 (5.1) | 2 (5.1) | | |
| Myeloid sarcoma | 1 (2.6) | 0 (0) | 1 (2.6) | | |
| Acute myeloblastic leukemia | 0 (0) | 1 (2.6) | 1 (2.6) | | |
| Rhabdoid/Teratoid sarcoma | 1 (2.6) | 0 (0) | 1 (2.6) | | |
| Pilocytic astrocytoma | 0 (0) | 1 (2.6) | 1 (2.6) | | |
| Anaplastic astrocytoma | 0 (0) | 1 (2.6) | 1 (2.6) | | |
| Ovarian tumors | 1 (2.6) | 0 (0) | 1 (2.6) | | |
| PNET | 0 (0) | 1 (2.6) | 1 (2.6) | | |
| Ewing sarcoma | 0 (0) | 1 (2.6) | 1 (2.6) | | |
| **Psychiatric diagnoses, n (%)** | 10 (25.6) | 21 (53.8) | 31 (79.5) | | |
| No psychiatric diagnosis | 6 (15.4) | 2 (5.1) | 8 (20.5) | | 0.045* |
| Major depressive disorder | 6 (15.4) | 14 (35.9) | 20 (51.3) | | 0.133[c] |
| Generalized anxiety disorder | 0 (0) | 3 (7.7) | 3 (7.7) | | 0.255[c] |
| Adjustment disorder | 3 (7.7) | 4 (10.3) | 7 (17.9) | | 1.000[c] |
| Intellectual disability | 1 (2.6) | 0 (0) | 1 (2.6) | | 0.410[c] |

[a]Mann-Whitney U test, [b]Chi-square test, [c]Fisher's Exact test
*p<0.05
SD: Standard deviation

**Table II.** Children and their parents' PedsQL total and subscores comparison between novel diagnosis and recurrence/ongoing treatment groups for each interview

| **Childrens' PedsQL scores** | **Novel diagnosis (n=10)** | **Recurrence/Ongoing treatment (n=20)** | **Z/t** | **p value** |
|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | | |
| **Initial interview** | | | | |
| Physical functioning | 40.31±26.90 | 42.97±35.25 | -0.110 | 0.914[b] |
| Emotional functioning | 61.50±28.68 | 56.25±24.0 | 0.530 | 0.601[a] |
| Social functioning | 85.0±18.86 | 83.75±13.08 | -0.671 | 0.530[b] |
| School functioning | 67.22±22.38 | 53.16±24.62 | -1.559 | 0.129[b] |
| Total scale score | 60.11±19.37 | 56.72±21.43 | 0.421 | 0.677[a] |
| Psychosocial score | 71.67±20.20 | 64.50±16.66 | -1.343 | 0.183[b] |
| **Follow-up interview** | | | | |
| Physical functioning | 55.90±34.64 | 50.0±33.43 | 0.741 | 0.667[a] |
| Emotional functioning | 73.33±22.36 | 64.75±28.07 | 0.807 | 0.427[a] |
| Social functioning | 87.22±15.83 | 83.0±17.04 | -0.528 | 0.627[b] |
| School functioning | 67.22±18.89 | 45.53±30.04 | 0.085 | 0.59[a] |
| Total scale score | 68.96±15.91 | 59.47±24.15 | 0.086 | 0.293[a] |
| Psychosocial score | 77.04±15.16 | 62.79±19.13 | 0.169 | 0.060[a] |
| **Parents' PedsQL scores** | **Novel diagnosis (n=16)** | **Recurrence/Ongoing treatment (n=23)** | **Z/t** | **p value** |
| | **Mean ± SD** | **Mean ± SD** | | |
| **Initial interview** | | | | |
| Physical functioning | 37.89±27.07 | 43.20±30.38 | 0.404 | 0.578[a] |
| Emotional functioning | 52.96±27.88 | 50.43±22.45 | 0.330 | 0.755[a] |
| Social functioning | 75.0±15.91 | 75.65±16.11 | 0.875 | 0.901[a] |
| School functioning | 62.0±24.28 | 42.14±22.99 | 0.965 | 0.035[a]* |
| Total scale score | 52.33±19.39 | 52.31±18.62 | 0.764 | 0.998[a] |
| Psychosocial score | 62.78±19.19 | 57.10±34.61 | 0.146 | 0.255[a] |
| **Follow-up interview** | | | | |
| Physical functioning | 61.04±20.07 | 52.41±34.61 | 0.957 | 0.345[a] |
| Emotional functioning | 78.0±18.30 | 60.22±25.47 | -2.282 | 0.022[b]* |
| Social functioning | 88.0±12.50 | 79.54±18.05 | -1.190 | 0.249[b] |
| School functioning | 70.5±24.20 | 47.0±30.10 | 2.141 | 0.041[a]* |
| Total scale score | 73.64±11.43 | 59.02±24.79 | 2.415 | 0.022[a]* |
| Psychosocial score | 80.77±12.79 | 62.42±21.14 | 23.284 | 0.002[a]* |

[a]Student's t-test, [b]Mann-Whitney U test, *p<0.05
SD: Standard deviation, PedsQL: Quality-of-Life Scale for Children

**Table III.** Children and their parents' PedsQL total and sub-scores changes in time

| Children's PedsQL scores | Novel diagnosis group (n=10) | | | Recurrence/Ongoing treatment group (n=20) | | |
|---|---|---|---|---|---|---|
| | Initial assessment | 6-month follow-up | p value | Initial assessment | 6-month follow-up | p value |
| | Mean ± SD | Mean ± SD | | Mean ± SD | Mean ± SD | |
| Physical functioning | 40.31±26.90 | 55.90±34.64 | 0.291[a] | 42.97±35.25 | 50.0±33.43 | 0.381[b] |
| Emotional functioning | 61.50±28.68 | 73.33±22.36 | 0.264[a] | 56.25±24.0 | 64.75±28.07 | 0.289[a] |
| Social functioning | 85.0±18.86 | 87.22±15.83 | 0.416[b] | 83.75±13.08 | 83.0±17.04 | 0.875[b] |
| School functioning | 67.22±22.38 | 67.22±18.89 | 0.691[a] | 53.16±24.62 | 45.53±30.04 | 0.393[b] |
| Total scale score | 60.11±19.37 | 68.96±15.91 | 0.282[a] | 56.72±21.43 | 59.47±24.15 | 0.675[a] |
| Psychosocial score | 71.67±20.20 | 77.04±15.16 | 0.594[b] | 64.50±16.66 | 62.79±19.13 | 0.751[a] |
| **Parents' PedsQL scores** | **Novel diagnosis group (n=16)** | | | **Recurrence/Ongoing treatment group (n=23)** | | |
| | Initial assessment | 6-month follow-up | p value | Initial assessment | 6-month follow-up | p value |
| | Mean ± SD | Mean ± SD | | Mean ± SD | Mean ± SD | |
| Physical functioning | 37.89±27.07 | 61.04±20.07 | 0.013[a]* | 43.20±30.38 | 52.41±34.61 | 0.112[a] |
| Emotional functioning | 52.96±27.88 | 78.0±18.30 | 0.020[b]* | 50.43±22.45 | 60.22±25.47 | 0.067[a] |
| Social functioning | 75.0±15.91 | 88.0±12.50 | <0.001[a]* | 75.65±16.11 | 79.54±18.05 | 0.229[b] |
| School functioning | 62.0±24.28 | 70.5±24.20 | 0.484[a] | 42.14±22.99 | 47.0±30.10 | 0.360[a] |
| Total scale score | 52.33±19.39 | 73.64±11.43 | 0.002[a]* | 52.31±18.62 | 59.02±24.79 | 0.123[a] |
| Psychosocial score | 62.78±19.19 | 80.77±12.79 | 0.005[a]* | 57.10±34.61 | 62.42±21.14 | 0.202[a] |

[a]Paired sample t-test, [b]Wilcoxon Rank-Sum test, *p<0.05
SD: Standard deviation, PedsQL: Quality-of-Life Scale for Children

**Table IV.** Levels of agreement for PedsQL total and sub-scores between the children/adolescent's and their parents' reports

| Initial interview | Correlation coefficient (r) | p value |
|---|---|---|
| **PedsQL scores** | | |
| Physical functioning | 0.683[a] | <0.001* |
| Emotional functioning | 0.735[b] | <0.001* |
| Social functioning | 0.451[a] | 0.012* |
| School functioning | 0.693[a] | <0.001* |
| Total scale score | 0.594[b] | <0.001* |
| Psychosocial health total score | 0.632[a] | <0.001* |
| **6-month follow-up interview** | **Correlation coefficient (r)** | **p value** |
| **PedsQL scores** | | |
| Physical functioning | 0.859[a] | <0.001* |
| Emotional functioning | 0.813[a] | <0.001* |
| Social functioning | 0.843[a] | <0.001* |
| School functioning | 0.928[b] | <0.001* |
| Total scale score | 0.908[b] | <0.001* |
| Psychosocial health total score | 0.995[b] | <0.001* |

[a]Spearman correlation, [b]Pearson correlation, *p<0.05
PedsQL: Quality-of-Life Scale for Children

## Discussion

Although the global burden of pediatric cancers has fallen dramatically compared to the previous decade, their detriment to the QoL of children and their parents remains (14). The most common pediatric cancers in our study population were sarcomas, specifically, rhabdomyosarcoma (n=7, 17.9%) and osteosarcoma (n=7, 17.9%). Sarcomas are generally rare but unfortunately lethal, and were overrepresented in our study population (15). Acute lymphoblastic leukemia (n=4, 10.3%) was the second most common, with medulloblastomas (n=4, 10.3%), which is relatively low compared to the extant literature, as childhood leukemias are reported to be the most common childhood cancers (14). Leukemia patients are primarily treated in the pediatric hematology inpatient unit in our facility. Hence, the low numbers observed in our study might be associated with this.

The most common psychiatric diagnosis was depression (n=20, 51.3%), followed by adjustment disorder (n=7, 17.9%) and anxiety disorder (n=3, 7.7%) in our study. Eight participants did not have any psychiatric disorder (20.5%). Depression is reported frequently in the literature in pediatric

**Table V.** Multiple linear regression results for children and adolescents' PedsQL total scores in the initial and follow-up interviews

| | B | SE | Beta | t | p value | 95% CI | |
|---|---|---|---|---|---|---|---|
| | | | | | | LL | UL |
| **Initial interview** | | | | | | | |
| Group | -4.799 | 6.853 | -0.117 | -0.700 | 0.490 | -18.886 | 9.289 |
| Psychiatric diagnosis (any) | 5.381 | 8.459 | 0.107 | 0.636 | 0.530 | -12.008 | 22.769 |
| Parent PedsQL total scores | 0.671 | 0.173 | 0.612 | 3.869 | 0.001* | 0.314 | 1.027 |
| **Follow-up interview** | | | | | | | |
| Group | 5.081 | 3.990 | 0.115 | 1.273 | 0.215 | -3.137 | 13.299 |
| Psychiatric diagnosis (any) | 0.695 | 4.696 | 0.013 | 0.148 | 0.884 | -8.976 | 10.366 |
| Parent PedsQL total scores | 0.976 | 0.089 | 0.950 | 10.975 | <0.001* | 0.793 | 1.159 |

*p<0.05
SE: Standard error, LL: Lower limit, UL: Upper limit, PedsQL: Quality-of-Life Scale for Children, CI: Confidence interval

cancer patients and survivors, as well as adjustment disorder (16). In a meta-analysis, as many as 24.6% of adult patients with cancer were reported to have some depressive disorder, while adjustment disorder rates were reported to be 15.4% (17). The possible overrepresentation of depressive disorders in our study sample could be due to the younger age of our population, which is frequently associated with higher mental distress (16).

Pediatric QoL as measured by PedsQL was not found to be different between those children who had recently been diagnosed with cancer and those with ongoing treatment, in either the first or the second interviews, and it was found not to have changed over time as no statistical differences were found between the initial and the follow-up interviews (all p>0.05). However, the parents' PedsQL School Functioning sub-scores of the novel diagnosis group in the initial interview were higher than the recurrence/ongoing treatment group (p=0.035), and parents' PedsQL total scores (p=0.022) and Emotional Functioning (p=0.022), School Functioning (p=0.041), and Psychosocial Functioning (p=0.002) scores were found to be higher in the novel diagnosis group compared to the recurrence group at the 6-month follow-up interview. Indeed, this difference was found to be attributable to an increase in pediatric QoL by the parents, as the parents' PedsQL scores in the novel diagnosis group were found to have improved between the initial and follow-up interviews for all domains except for the School Functioning domain which was found to be higher in the initial interview. A similar improvement was not detected for the recurrence/ongoing treatment group. Mental health as a composite of the biopsychosocial construct of health in general has a great impact on the perceived QoL. Psychological distress, even in the absence of a life-threatening chronic disease, is associated with poorer reports of QoL (18). Furthermore, cancer as a chronic disease and the chemotherapeutics employed in its treatment are associated with persistent physical, neurocognitive and psychosocial difficulties in addition to their deleterious effects on mental health, during and after treatment (19-21).

The increases in QoL in six months compared to the baseline assessment were different for the novel diagnosis group and recurrence/ongoing treatment group in our study. The parents' attitudes towards their children and their emotional coping capabilities may have impacted their perception of their children's QoL. High levels of parental distress are associated with poorer mental health in children (22). This association can be explained through the complex relationship between genetic susceptibility, the dynamic process of behavioral/emotional learning, and the capacity for the provision of emotional support by the caregiver under duress (23,24). Adjustment to the diagnosis and circumstances probably plays an integral role in the perceived improvement in QoL in our sample.

Although the proxy reports of the parents concerning their children's QoL might differ from the children's accounts, the parental and child reports of health-related QoL are generally reported to be in statistical agreement with one another. However, this correlation between reported QoL seems to be higher for physical performance rather than for emotional and social functioning in other research (9,25). This was not the case for our study, as the PedsQL scores of the patients and their parents were high in agreement.

### Study Limitations

The inclusion of both children and their parents, assessments made with a semi-structured clinical tool, and conducting interviews at two points in time are the important strengths of this study. However, the modest sample size is a major limitation for the present study, as this study was conducted in a pediatric oncology inpatient unit. Another significant limitation was the restriction of the children's reports on their own QoL. Further studies concerning the treatment process, taking the patients' own experiences, as well as their caregivers' perspectives and experiences into account should be conducted with greater sample sizes in order to minimize biases.

## Conclusion

The present study highlights that there are no significant differences in the QoL of those children with a novel diagnosis of pediatric cancer and those with cancer recurrence/ongoing treatment, according to their own reports. In contrast, the parents reported significant improvements in the QoL of their children with a novel cancer diagnosis after six months. The same improvement was not seen in the recurrence/ongoing treatment group, indicating that adjustment to the diagnosis and circumstances is an important component of QoL. Furthermore, the parents' reports on their children's QoL were highly correlated with the children's own reports. This association remained significant in multiple linear regression analyses in which the novel diagnosis and recurrence, and psychiatric disorders were taken into account, at both the initial and follow-up interview conducted after 6 months. Thus, the parents' reports of their children's QoL can be used in the clinical setting to accurately predict the children's QoL, especially in circumstances where the children are unable to verbalize their own needs.

### Acknowledgments

The authors would like to thank RA Ömer Faruk Dadaş for his valuable guidance in the statistical analysis of the data and interpretation of the results.

### Ethics

**Ethics Committee Approval:** This study was conducted at Ege University Faculty of Medicine and Ege University Faculty of Medicine Medical Research Ethics Committee approved the study regarding ethical principles (approval no.: 19-3.1T/45, date: 20.03.2019).

**Informed Consent:** Children and their parents were verbally informed about the study's design and verbal assent or written informed consent forms were obtained from the children and their parents, where appropriate.

### Authorship Contributions

Surgical and Medical Practices: B.Ş.P., S.E., B.Ö., Concept: B.Ş.P., S.E., M.K., E.A., B.Ö., T.B., Design: B.Ş.P., S.E., M.K., E.A., B.Ö., T.B., Data Collection and/or Processing: B.Ş.P., İ.İ.K., Analysis and/or Interpretation: B.Ş.P., İ.İ.K., B.Ö., Literature Search: B.Ş.P., İ.İ.K., E.A., T.B., Writing: B.Ş.P., İ.İ.K., T.B.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Financial Disclosure:** The authors disclose that this research study was not funded by any specific grants from public, commercial, or not-for-profit sectors. The study was conducted independently without any financial support or sponsorship that could potentially introduce bias or influence the research outcomes.

## References

1. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001–10: a population-based registry study. Lancet Oncol 2017; 18:719-31.
2. Kutluk MT, Yesilipek A. Pediatric cancer registry Turkey: 2009-2016 (TPOG & TPHD). J Clin Oncol 2017; 35:e22015.
3. Kebudi R, Turkish Pediatric Oncology Group. Pediatric oncology in Turkey. J Pediatr Hematol Oncol 2012; 34(Suppl 1):S12-4.
4. Jemal A, Ward EM, Johnson CJ, et al. Annual Report to the Nation on the Status of Cancer, 1975-2014, Featuring Survival. J Natl Cancer Inst 2017; 109:djx030.
5. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022; 72:7-33.
6. Racine NM, Khu M, Reynolds K, Guilcher GMT, Schulte FSM. Quality of life in pediatric cancer survivors: contributions of parental distress and psychosocial family risk. Curr Oncol 2018; 25:41-8.
7. Landolt MA, Vollrath M, Niggli FK, Gnehm HE, Sennhauser FH. Health-related quality of life in children with newly diagnosed cancer: a one year follow-up study. Health Qual Life Outcomes 2006; 4:1-8.
8. Fardell JE, Vetsch J, Trahair T, et al. Health-related quality of life of children on treatment for acute lymphoblastic leukemia: A systematic review. Pediatr Blood Cancer 2017; 64. doi: 10.1002/pbc.26489.
9. Eiser C, Morse R. The measurement of quality of life in children: past and future perspectives. J Dev Behav Pediatr JDBP 2001; 22:248-56.
10. Kaufman J, Birmaher B, Brent D, et al. Schedule for Affective Disorders and Schizophrenia for School-Aged Children: Present and Lifetime Version (K-SADS-PL) DSM-5. J Am Acad Child Adolesc Psychiatry 1997; 36:980-8.

11. Ünal F, Öktem F, Çetin Çuhadaroğlu F, et al. [Reliability and Validity of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5 November 2016-Turkish Adaptation (K-SADS-PL-DSM-5-T)]. Turk Psikiyatri Derg 2019; 30:42-50.

12. Varni JW, Seid M, Kurtin PS. PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. Med Care 2001; 39:800-12.

13. Cakin Memik N, Ağaoğlu B, Coşkun A, Uneri OS, Karakaya I. [The validity and reliability of the Turkish Pediatric Quality of Life Inventory for children 13-18 years old]. Turk Psikiyatri Derg Turk J Psychiatry 2007; 18:353-63.

14. Wu Y, Deng Y, Wei B, et al. Global, regional, and national childhood cancer burden, 1990-2019: An analysis based on the Global Burden of Disease Study 2019. J Adv Res 2022; 40:233-47.

15. Burningham Z, Hashibe M, Spector L, Schiffman JD. The Epidemiology of Sarcoma. Clin Sarcoma Res 2012; 2:1-16.

16. Park EM, Rosenstein DL. Depression in adolescents and young adults with cancer. Dialogues Clin Neurosci 2015; 17:171-80.

17. Mitchell AJ, Chan M, Bhatti H, et al. Prevalence of depression, anxiety, and adjustment disorder in oncological, haematological, and palliative-care settings: a meta-analysis of 94 interview-based studies. Lancet Oncol 2011; 12:160-74.

18. Bastiaansen D, Ferdinand RF, Koot HM. Predictors of quality of life in children and adolescents with psychiatric disorders. Child Psychiatry Hum Dev 2020; 51:104-113.

19. Anderson F, Kunin-Batson A. Neurocognitive late effects of chemotherapy in children: the past 10 years of research on brain structure and function. Pediatr Blood Cancer 2009; 52:159-64.

20. Patenaude AF, Kupst MJ. Psychosocial functioning in pediatric cancer. J Pediatr Psychol 2005; 30:9-27.

21. Speechley KN, Barrera M, Shaw AK, Morrison HI, Maunsell E. Health-related quality of life among child and adolescent survivors of childhood cancer. J Clin Oncol Off J Am Soc Clin Oncol 2006; 24:2536-43.

22. Maurice-Stam H, Oort FJ, Last BF, Brons PPT, Caron HN, Grootenhuis MA. Longitudinal assessment of health-related quality of life in preschool children with non-CNS cancer after the end of successful treatment. Pediatr Blood Cancer 2008; 50:1047-51.

23. Malpert AV, Kimberg C, Luxton J, et al. Emotional distress in parents of long-term survivors of childhood acute lymphoblastic leukemia. Psychooncology 2015; 24:1116-23.

24. Hamner T, Latzman RD, Latzman NE, Elkin TD, Majumdar S. Quality of life among pediatric patients with cancer: contributions of time since diagnosis and parental chronic stress. Pediatr Blood Cancer 2015; 62:1232-6.

25. Pierce L, Hocking MC, Schwartz LA, Alderfer MA, Kazak AE, Barakat LP. Caregiver distress and patient health-related quality of life: psychosocial screening during pediatric cancer treatment. Psychooncology 2017; 26:1555-61.