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Aims and Scope

The Journal of Pediatric Research is the official publication of Ege University and Ege Children's Foundation. It is a peer-reviewed journal published quarterly in March, June, September and December in English.

The Journal of Pediatric Research is a peer-reviewed, open-access journal, which publishes original research articles, invited review articles, clinical reports and case reports in all areas of pediatric research.

The journal publishes original research and review articles, which summarize recent developments about a particular subject based on standards of excellence and expert review and case reports. The target audience includes specialists in general pediatrics and pediatric subspecialties (Emergency Medicine, Allergy and Immunology, Endocrinology, Gastroenterology, Hepatology and Nutrition, Genetics, Cardiology, Hematology-Oncology, Infectious Diseases, Metabolism, Nephrology, Neurology, Rheumatology, Pulmonology, Social Pediatrics, Newborn, Critical Care Medicine, Ethics and Health Service Research), as well as relevant specialities such as Pediatric Surgery, Child and Adolescent Psychiatry, Pedodontics, Pediatric Nursing and Family Physicians.

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Original Articles

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Editorial

Dear JPR Readers,

We are pleased to share with you the third issue of "The Journal of Pediatric Research" in 2023. Here comes the autumn when leaves change color, trees become bare, humans and animals alike prepare for the cold winter that lies ahead.

French philosopher, author, and journalist Albert Camus once described the season as "the second spring, where every leaf is a flower".
During the autumn months, allergies, flus, colds, and other ailments are common in the children.

While this season has an earthy beauty to it, it can also teach the children some valuable lessons about life. Here are some things children can learn from this season: "Accept change, appreciate what you have, let go, live life to the fullest, accept bad times as well as the good". Children need simple tools to understand complex phenomena. We can use the metaphor of the trees and their falling leaves to teach a little child some valuable life lessons.

Third issue of JPR includes 10 interesting articles with different topics. First article entitled "Evaluation of Electrocardiographic Markers for the Risk of Cardiac Arrhythmia in Children with Obesity" concluded that the electrocardiographic risk markers used to predict cardiac arrhythmias were found to be increased in those children with obesity; Increased body weight and adiposity may have unfavorable effects on the cardiac conduction system. In the other study entitled "Evaluation of Vascular Involvement in Children with Celiac Disease" authors found that arterial stiffness and carotid intima media thickness measurements were higher in the celiac disease patients compared to the healthy controls. In another study in this issue, authors evaluated the neurodevelopmental status for urea cycle disorders, and found that peak ammonia levels and the frequency of hyperammonemic episodes have effects on neurological outcomes and there are still poor neurocognitive outcomes despite extracorporeal detoxification.

I would like to acknowledge the authors, the reviewers, editorial team and Galenos Publishing House for their support in the preparation of this issue. We look forward to your scientific contributions in our future issues.

Wish you beautiful autumn season full of joy, health and science.

Kind Regards,

Prof. Dr. Zülal Ülger Tutar

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Original Article

Evaluation of Electrocardiographic Markers for the Risk of Cardiac Arrhythmia in Children with Obesity

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ABSTRACT

Aim: This study was conducted to examine the electrocardiographic markers used in the risk assessment of cardiac arrhythmia in children with obesity.

Materials and Methods: In this prospective study, 60 children aged 3-17 years with exogenous obesity and 60 age and sex-matched healthy controls were included. Demographic data, assessment of atrial and ventricular arrhythmia risk markers in electrocardiography, and standard echocardiography measurements were performed. Values of $p<0.05$ were considered significant.

Results: The mean ages of the study and control groups were 11.51±3.48 years and 10.74±3.72 years, respectively. Both groups had 30 males and 30 females. The study group had significantly higher average mean body mass index (BMI) compared to the control group. In electrocardiographic examinations, P-wave dispersion, QT dispersion (QTd), corrected QTd (QTcd), Tpeak-Tend (Tp-e), Tp-e/QT, and Tp-e/QTc values were significantly higher in the obese group compared to the control group. In echocardiographic examinations, the dimensions of the heart chambers and vascular structure and wall thicknesses were found to be significantly higher in those children with obesity.

Conclusion: The electrocardiographic risk markers used to predict cardiac arrhythmias were found to be increased in those children with obesity. This may suggest that increased body weight and adiposity may have unfavorable effects on the cardiac conduction system.

Keywords: Child, electrocardiography, heart, obesity

Introduction

Childhood obesity, defined by the World Health Organization (WHO) as "abnormal or excessive fat accumulation in the body to the extent that it impairs health", is a global disease with potentially devastating consequences (1). Obesity-related cardiovascular diseases (coronary heart disease, cardiomyopathy, heart failure, cardiac arrhythmias, and heart valve diseases) and impairments (left ventricular hypertrophy, left ventricular dilatation, left atrial dilatation, and blocks) may develop (2). Obesity also paves the way for disruption in the myocardial metabolism and arrhythmia in the heart with changes in fat metabolism. Sinus arrhythmia, bradycardia, sinus

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block, supraventricular beats, ventricular ectopic beats, and intraventricular blocks can be seen in obesity (3).

P-wave dispersion (Pd) is defined as the difference between the longest and shortest P-wave intervals measured in a standard 12-lead electrocardiogram (ECG) (4). It is associated with the inhomogeneous spread of sinus impulses in patients and it is an important non-invasive ECG marker recommended in the evaluation of the risk of atrial arrhythmia (5). QT dispersion (QTd) is a parameter which shows ventricular repolarization heterogeneity (6). It has been shown that QT, corrected QT (QTc), and QTd predict ventricular arrhythmic events and sudden death (7). The Tpeak-Tend interval (Tp-e), an interval from the peak of the T-wave to the end of the T-wave, corresponds to the transmural distribution of ventricular repolarization (8). Tp-e is a new parameter which shows ventricular repolarization and predicts ventricular arrhythmias and sudden death even in those with normal QTc. Both Tp-e and Tp-e/QT ratios have been associated with malignant arrhythmias (9). This study was conducted in order to evaluate cardiac arrhythmia risk markers on the ECGs of children with obesity.

Materials and Methods

Study Group

Our study was conducted prospectively, cross-sectionally, and observationally in children aged 3-17 years between June, 2020 and December, 2020. Sixty children who were followed up with a diagnosis of exogenous obesity were included in the patient group. The exclusion criteria were defined as having a chronic disease other than exogenous obesity and having congenital or acquired heart disease. Sixty healthy children without chronic diseases, who were matched with the study group in terms of age and sex, were included in the control group. Ethical approval was obtained for our study from the Necmettin Erbakan University Meram Faculty of Medicine, Non-Pharmaceutical and Medical Device Research Ethics Committee (decision number: 2020/2605, dated: 19.06.2020).

Clinical and Laboratory Evaluations

Anthropometric measurements [body weight, height, and body mass index (BMI)], age, sex, blood pressure, and physical examinations were evaluated in the study and control groups. Complete blood count, biochemistry, and hormonal tests, which were taken routinely in the patient group, were evaluated. No blood tests were performed in the control group.

Electrocardiographic Evaluations

Electrocardiographic records were obtained using a 12-lead Nihon Kohden Cardiofax S (Tokyo, Japan) electrocardiograph (25 mm/sec velocity and 10 mm/mV amplitude). The ECG images were transferred to a computer in a way that their planarity would not be impaired. The images were evaluated by an experienced physician using a program. Heart rate and PR interval calculations were performed by averaging three consecutive measurements from the DII lead. P-wave duration measurements were made from at least nine leads. The time between the starting and ending points of the P-wave from the isoelectric line was evaluated as the P-wave interval, and then the longest (Pmax) and the shortest (Pmin) P-wave intervals were also determined. Pd was evaluated as the difference between the longest (Pmax) and shortest (Pmin) P-wave intervals (4). QT duration measurements were also made from at least nine leads. The time between the onset of the QRS complex and the junction of the T wave with the isoelectric line was measured as the QT interval. QTd was calculated as the difference between the longest (QTmax) and shortest (QTmin) QT intervals. QTc was calculated according to heart rate using Bazett's formula ($QTc=QT/\sqrt{RR}$) (10). QTc dispersion (QTcd) was determined as the difference between the longest (QTcmax) and the shortest (QTcmin) QTc intervals.

The Tp-e interval was determined by measuring the time between the peak of the T wave and the junction with the isoelectric line. The precordial lead (V5 or V6) which best showed the left ventricle was used when measuring. The tangent method was used when the cut-off point of the end of the T-wave with the isoelectric line could not be evaluated. Tp-e/QT and Tp-e/QTc ratios were calculated by measuring QT and QTc from the same lead (precordial lead) where the Tp-e time was measured.

Echocardiographic Evaluations

The Vivid S5 N (GE, Horten, Norway) echocardiograph and 3S probe were used for echocardiography (ECHO) examinations. A standard examination was performed by an experienced pediatric cardiologist with the techniques specified in the guidelines of the American and European Society of Echocardiography, which are accepted in international practice (11,12). During the ECHO examination of the patients, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left atrium (LA), aortic root (Ao), end-diastolic

interventricular septum thickness (IVSd), end-diastolic left ventricular posterior wall thickness (LVPWd), ejection fraction (EF), and fractional shortening (FS) measurements were made.

Statistical Analysis

The IBM SPSS for Windows® 22.0 program was used for the statistical analysis of the data. Frequencies, ratios, mean and standard deviations of different variables were determined using descriptive statistics. The Kolmogorov-Smirnov test was used to evaluate the conformity of continuous data to the normal distribution. Continuous data which did not show normal distribution were tested for their conformity to the normal distribution through data transformation. In the analysis of normally distributed continuous variables, the t-test was used in independent paired groups to determine differences between the groups, and the Mann-Whitney U test was used in the analysis of independent paired groups of continuous variables which did not show normal distribution. A p-value of <0.05 was considered statistically significant in the analyses.

Results

Characteristics of the Groups

The mean age of the study group and control group was 11.51±3.48 years and 10.74±3.72 years, respectively. The male/female ratio was 30/30 in both groups. There was no significant difference between the study and control groups in terms of age and sex (p>0.05). However, body weight (p<0.001), height (p=0.001), BMI (p<0.001), and BMI-standard deviation scores (SDS) (p<0.001) were found to be significantly higher in the study group compared to the control group. In addition, systolic and diastolic blood pressures were found to be significantly higher in the study group compared to the control group (p<0.001). The general characteristics of the groups are summarized in Table I.

Laboratory Findings

Laboratory findings were obtained from the files of the study group. No blood was collected from the control group. Therefore, a comparison between the two groups could not be made. The blood values of the study group are shown in Table II together with the laboratory reference values.

Table I. Comparison of the general characteristics of the patient and control groups

	Obese subjects (n=60)	Lean subjects (n=60)	p-value
	Mean ± SD	Mean ± SD	
Age (years)	11.51±3.48	10.74±3.72	0.246
Gender (female/male)	30/30	30/30	0.999
Weight (kg)	74.30±25.73	37.00±15.31	<0.001
Height (cm)	151.78±19.66	139.57±18.87	0.001
Body mass index (kg/m²)	31.09±4.83	18.06±3.44	<0.001
Body mass index-SDS	2.42±0.37	0.00±1.07	<0.001
Systolic blood pressure (mmHg)	119.25±12.06	102.42±10.95	<0.001
Diastolic blood pressure (mmHg)	76.72±8.57	66.00±9.37	<0.001

SD: Standard deviation, SDS: Standard deviation scores

Table II. Laboratory values of the patient group

Laboratory values	Mean	SD	Median	Highest	Lowest	Higher compared with reference (%)	Reference values
Glucose (mg/dL)	91.52	7.35	91.35	109.80	71.00	8.3	60-100
Insulin (mU/L)	26.42	11.90	24.80	59.26	7.78	49.1	2.6-24.9
HbA1C (%)	5.17	0.32	5.20	6.00	4.40	1.7	4-6
Cholesterol (mg/dL)	160.08	33.80	159.45	242.70	90.90	10.3	0-200
Triglyceride (mg/dL)	157.80	110.26	136.70	722.30	48.80	42.3	0-150
VLDL (mg/dL)	30.09	19.86	26.90	140.20	9.76	39.2	0-30
HDL (mg/dL)	43.88	9.15	44.70	61.50	16.70	67.2	0-40

Table II. Continued

Laboratory values	Mean	SD	Median	Highest	Lowest	Higher compared with reference	Reference values
LDL (mg/dL)	85.23	28.64	86.36	160.46	10.77	26.7%	0-100
AST (U/L)	26.83	14.30	22.90	93.00	10.50	8.3%	0-41
ALT (U/L)	33.03	26.89	23.20	174.30	10.00	21.6%	0-40

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HbA1c: Hemoglobin A1c, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, SD: Standard deviation

Electrocardiographic Parameters

The Pd, QTd, QTcd, Tp-e durations, and Tp-e/QT and Tp-e/QTc ratios were found to be significantly higher in the study group compared to the control group ($p<0.001$). Heart rate and PR interval time were found to be similar between the groups ($p>0.05$). No patients had arrhythmia or a long QTc interval. The electrocardiographic measurement findings and statistical comparisons are given in Table III.

Echocardiographic Parameters

The dimensions of the cardiac chambers and vessels (LVESD, LVEDD, Ao, LA) and the wall thicknesses (IVSd, LVPWd) of the study group were found to be significantly greater ($p<0.001$). However, there was no significant difference between the groups in terms of the LA/Ao ratio or systolic functions (EF and FS) ($p>0.05$) (Table IV).

Table III. Electrocardiographic features of the obese and control groups

	Obese subjects (n=60)	Lean subjects (n=60)	p-value
Rate (/min)	94.5±18.63	89.63±16.06	0.128
PR (ms)	123.43±15.87	121.8±13.62	0.546
Pmin (ms)	56±7.9	56.67±6.8	0.621
Pmax (ms)	89.33±7.75	81.07±6.54	<0.001
Pd (ms)	33.33±4.27	24.4±3.01	<0.001
Qtmin (ms)	291.27±26.92	297.13±25.44	0.222
Qtmax (ms)	329.67±26.6	323.2±25.49	0.177
QTd (ms)	38.4±5.9	26.07±3.86	<0.001
Qtcmin (ms)	361.09±22.71	359.67±23.35	0.736
Qtcmax (ms)	408.94±22.76	391.36±24.41	<0.001
QTcd (ms)	47.85±8.04	31.69±5.41	<0.001
Tp-e (ms)	65.57±5.08	58.07±4.2	<0.001
Tp-e/QT	0.21±0.02	0.19±0.02	<0.001
Tp-e/QTc	0.17±0.01	0.15±0.02	<0.001

Pd: P dispersion, Pmax: Maximum P-wave duration, Pmin: Minimum P-wave duration, QTcd: QTc dispersion, QTd: QT dispersion, QTcmax: Maximum QTc duration, QTcmin: Minimum QTc duration, QTmax: Maximum QT duration, QTmin: Minimum QT duration

Table IV. Echocardiographic features of the obese and control groups

	Obese subjects (n=60)	Lean subjects (n=60)	p-value
LVEDD (mm)	44.26±5.04	39.4±4.87	<0.001
LVESD (mm)	26.59±3.51	23.39±3.25	<0.001
IVSd (mm)	8.12±1.33	7.12±1.67	<0.001
LVPWd (mm)	8.5±1.37	7.13±1.07	<0.001
LA (mm)	30.5±5.39	26.18±3.74	<0.001

Table IV. Continued

	Obese subjects (n=60)	Lean subjects (n=60)	p-value
AO (mm)	24.03±3.85	22.00±2.85	<0.001
LA/AO	1.27±0.14	1.25±1.15	0.542
EF (%)	69.42±8.71	71.27±3.42	0.128
FS (%)	39.92±3.02	39.47±5.73	0.837

AO: Aortic root, EF: Ejection fraction, FS: Fractional shortening, IVSd: End-diastolic interventricular septum thickness, LA: Left atrium diameter, LA/AO: Left atrium diameter to aortic root ratio, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, LVPWd: End-diastolic left ventricular posterior wall thickness

Discussion

The prevalence of obesity is increasing worldwide and it constitutes an important economic and public health problem due to its morbidity and mortality (13). The fact that obesity is observed in childhood means that cardiovascular diseases will begin at a younger age, the burden of chronic disease will begin to appear in the most productive years of people, and it will worsen public health (14). In previous studies, it was shown that obesity increases the risk of atrial fibrillation (AF), the risk of AF increases again in adults who were obese in childhood, and it was shown that losing body weight reduces the risk of AF (15,16).

The prevalence of childhood hypertension in children with obesity increases as blood pressure shifts towards higher levels as body weight increases (17). A close relationship has been shown between BMI and blood pressure levels in overweight adolescents. In one study, it was found that every 10-unit increase in BMI was associated with an increase of 10 mmHg in systolic blood pressure and 3 mmHg in diastolic blood pressure (18). Body weight, BMI, and BMI-SDS values were higher in our obese group in comparison to the control group, which were similar in terms of age and sex. Similar to previous studies, both systolic and diastolic blood pressures were found to be significantly higher in the obese group in our study.

Pd is associated with the inhomogeneous spread of sinus impulses in patients and it is an important ECG marker in the evaluation of the risk of atrial arrhythmia (5). Although a significant increase in Pd was observed in studies conducted in adult patients with obesity, there have been conflicting results in studies conducted in children. In the study of Akyüz et al. (19) performed with 67 children with obesity and 70 controls, no significant difference was found between the two groups in terms of Pd. Another pediatric age group study was conducted with 30 children with obesity and 30 controls, and Pd in the obese group was found to be significantly higher than in the control group (51.33±11.67 ms vs. 39.67±11.59 ms, p<0.05) (20). In another study performed with 59 children with obesity and 38 healthy volunteers, Pd was found to be significantly higher in the patient group (21). In our study, Pd was found to be significantly higher in obese children. Accordingly, as Pd is an indicator of susceptibility to atrial arrhythmia, children with obesity should be followed up losely. Also, the importance of losing body weight should be emphasized to families and children in order to reduce risk.

It has been shown that QT, QTc, and QTd predict ventricular arrhythmic events and sudden death (7). Studies have been conducted on QT, QTc, QTd, and QTcd in children with obesity and these values increased with obesity. In a study conducted with 81 children with obesity and 82 normal-weight children in our country, a significant increase was found in QTd and QTcd (22). Olivares López et al. (23) found the mean QTc values to be significantly longer in the obese group in a limited study conducted with 13 patients with obesity and 17 overweight individuals. In another study, it was shown that QTcd was found to be significantly higher in the obese group (24). In the study conducted by Ozkan et al. (25) with 45 children with obesity and 87 normal-weight children, they found the mean QTc values to be significantly higher in the obese group.

Tp-e interval is a relatively new ECG parameter which shows ventricular repolarization and predicts ventricular arrhythmias and sudden death, even in those with normal QTc. Both the Tp-e interval and Tp-e/QT ratio have been associated with malignant arrhythmias (9). There are insufficient data in the literature on these parameters because they have only just begun to be studied, even in adults with obesity. In one study conducted with adult patients, it was shown that Tp-e interval and Tp-e/QT ratio values were higher in the obese group, but no significant difference was found between the groups (26). Another study in the adult age group was conducted with 41 individuals with extreme obesity and 41 healthy people, and they found that the Tp-e interval and ratios of Tp-e/QT and Tp-e/QTc were significantly increased in the patient group (7).

In another study conducted with 126 healthy children aged 9-12 years, the Tp-e/QT ratio and anthropometric values were compared, and a significant linear correlation was found between the Tp-e/QT ratio and BMI (27). Türe et al. (28) showed that in children with obesity, heart rate, PR interval, Pd, QRS duration, QTd, QTcd, left ventricular hypertrophy, and Tp-e interval were found to be statistically different from healthy controls. In our study, the Tp-e interval, and ratios of Tp-e/QT and Tp-e/QTc were found to be significantly higher in the obese group. The fact that transmural repolarization indicators (Tp-e interval, Tp-e/QT ratio, Tp-e/QTc ratio) were found to be higher in our patient group together with other ventricular repolarization parameters (QTd and QTcd) compared to the control group supported the increased risk of ventricular arrhythmia in children with obesity. To the best of our knowledge, there are a limited number of studies on the ECG parameters of children with obesity. Thus, our study may contribute to the literature.

In one study conducted on children, LVEDD was found to be significantly increased in the obese group compared to the control group (20). In our study, it was observed that LVEDD was significantly higher in the obese group compared to the control group, which is consistent with the literature. In many studies in the literature, IVSd and LVPWd were evaluated in children with obesity and it was observed that these parameters were higher in the obese groups. Studies conducted with various numbers of patients with obesity showed that IVSd and LVPWd were significantly higher in children with obesity (20,29,30). In our study, in accordance with the literature, IVSd and LVPWd were significantly higher in the obese group.

LA size is an important determinant of cardiovascular health in adults. In a study performed with 991 children, a relationship was found between BMI and LA size (31). In the study of Hurtado-Sierra (32) on 142 participants including 53 obese and 39 overweight volunteers, LA size was found to be significantly higher in the obese group. In our study, LA diameter was found to be significantly higher in the obese group compared with the control group. Üner et al. (20) and Ghandi et al. (30) both observed that there were no significant differences in terms of systolic functions between the obese and control groups. In our study, in line with the literature, it was observed that systolic functions were preserved in the obese group and also, they were similar to the control group. According to our echocardiographic findings, it was observed that the width of the heart/vessels and wall thicknesses were higher in childhood obesity. We think that this is due to increased body mass, fat proportion, and arterial blood pressure.

Study Limitations

The cardiac evaluations of our patients were cross-sectionally performed at the time of diagnosis. Our data were obtained in the early period of obesity; therefore, long-term cardiac changes in the patients could not be followed. For this reason, we think that comparing long-term and post-weight loss cardiac evaluations will yield more meaningful results.

Conclusion

It was observed that in addition to increased body weight, BMI, and BMI-SDS in children with obesity, heart/vessel widths, wall thicknesses, and blood pressure also increased. Electrocardiographic risk markers for the prediction of atrial (Pd) and ventricular (QTd, QTcd, Tp-e, Tp-e/QT, and Tp-e/QTc) cardiac arrhythmias were found to be higher in our children with obesity group. This suggests that increased body weight and adiposity may affect the electrical conduction system of the heart, and thus it is important to be vigilant in terms of heart rhythm problems which may develop in children with obesity. It is important to explain to patients and their relatives that losing body weight can reduce these risks. However, further studies are needed on this issue.

Ethics

Ethics Committee Approval: Ethical approval was obtained for our study from the Necmettin Erbakan University Meram Faculty of Medicine, Non-Pharmaceutical and Medical Device Research Ethics Committee (decision number: 2020/2605, dated: 19.06.2020).

Informed Consent: Informed consent was obtained from all individuals included in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.Ş., M.E.A., T.B., Design: H.Y.D., F.Ş., B.S.E., T.B., Data Collections or Processing: H.Y.D., F.Ş. M.E.A. T.B., Analysis or Interpretation: F.Ş., M.B.O., B.S.E., T.B., Literature Search: H.Y.D., F.Ş., M.B.O, Writing: H.Y.D., F.Ş.

Conflict of Interest: None of authors have any conflicts of interest to report.

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References

1. WHO. Guideline Report of the commission on ending childhood obesity.World Health Organization (WHO), Geneva, Switzerland, 2016.
2. Helvacı A, Tipi FF, Belen E. Cardiovascular Diseases Releated with Obesity. Eur Arc Med Res 2014; 30(Suppl 1):5-14.
3. Pdziński W, Urban M, Grójec M, et al. Assessment of the character and incidence of electrocardiographic changes in children with simple obesity. Acta Physiol Pol 1985; 36:366-72.
4. Perzanowski C, Ho AT, Jacobson AK. Increased P-wave dispersion predicts recurrent atrial fibrillation after cardioversion. J Electrocardiol 2005; 38:43-6.
5. Sert A, Aslan E, Buyukınan M, Pirgon O. Correlation of P-wave dispersion with insulin sensitivity in obese adolescents. Cardiol Young 2017; 27:229-35.
6. Psallas M, Tentolouris N, Cokkinos A, Papadogiannis D, Cokkinos DV, Katsilambros N. QT dispersion: comparison between diabetic and non-diabetic individuals and correlation with cardiac autonomic neuropathy. Hellenic J Cardiol 2006; 47:255-62.
7. Inanir M, Sincer I, Erdal E, Gunes Y, Cosgun M, Mansiroglu AK. Evaluation of electrocardiographic ventricular repolarization parameters in extreme obesity. J Electrocardiol 2019; 53:36-39.
8. Gupta P, Patel C, Patel H, et al. T(p-e)/QT ratio as an index of arrhythmogenesis. J Electrocardiol 2008; 41:567-74.
9. Guo D, Zhou J, Zhao X, et al. L-type calcium current recovery versus ventricular repolarization: preserved membrane-stabilizing mechanism for different QT intervals across species. Heart Rhythm 2008; 5:271-9.
10. Bazett HC. An analysis of the time relations of electrocardiograms. Heart 1920; 7:353-70.
11. Kampmann C, Wiethoff CM, Wenzel A, et al. Normal values of M mode echocardiographic measurements of more than 2000 healthy infants and children in central Europe. Heart 2000; 83:667-72.
12. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18:1440-63.
13. Prospective Studies Collaboration, Whitlock G, Lewington S, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet 2009; 373:1083-96.
14. Ward ZJ, Long MW, Resch SC, Giles CM, Cradock AL, Gortmaker SL. Simulation of Growth Trajectories of Childhood Obesity into Adulthood. N Engl J Med 2017; 377:2145-153.
15. Adelborg K, Ängquist L, Ording A, et al. Levels of and Changes in Childhood Body Mass Index in Relation to Risk of Atrial Fibrillation and Atrial Flutter in Adulthood. Am J Epidemiol 2019; 188:684-93.
16. Nalliah CJ, Sanders P, Kottkamp H, Kalman JM. The role of obesity in atrial fibrillation. Eur Heart J 2016; 37:1565-72.
17. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res 2015; 116:991-1006.
18. Kelly RK, Magnussen CG, Sabin MA, Cheung M, Juonala M. Development of hypertension in overweight adolescents: a review. Adolesc Health Med Ther 2015; 6:171-87.
19. Akyüz A, Alpsoy S, Akkoyun DC, et al. Effect of overweight on P-wave and QT dispersions in childhood. Turk Kardiyol Dern Ars 2013; 41:515-21.
20. Üner A, Doğan M, Epcacan Z, Epçaçan S. The effect of childhood obesity on cardiac functions. J Pediatr Endocrinol Metab 2014; 27:261-71.
21. Temiz F, Güneş H, Güneş H. Evaluation of Atrial Electromechanical Delay in Children with Obesity. Medicina (Kaunas) 2019; 55:228.
22. Yıldırım Yıldız N, Uçar T, Ramoğlu MG, et al. Does obesity influence ventricular repolarisation in children? Cardiol Young 2021; 31:568-76.
23. Olivares López JL, Vázquez Olivares M, Fleta Zaragozano J, Moreno Aznar LA, Bueno Sánchez M. Trastornos electrocardiográficos y ecocardiográficos en niños con sobrepeso y obesidad [Electrocardiographic and echocardiographic findings in children with overweight and obesity]. Med Clin (Barc) 2005; 125:93-4.
24. Nigro G, Russo V, Di Salvo G, et al. Increased heterogenity of ventricular repolarization in obese nonhypertensive children. Pacing Clin Electrophysiol 2010; 33:1533-9.
25. Ozkan EA, Khosroshahi HE, Serin Hİ, et al. Evaluation of QTc Value and Relation between QTc Interval and Cardiovascular Risk Factors in Obese Children. Am J Health Res 2015; 3:194-7.
26. Braschi A, Abrignani MG, Francavilla VC, Francavilla G. Novel electrocardiographic parameters of altered repolarization in uncomplicated overweight and obesity. Obesity (Silver Spring). 2011; 19:875-81.
27. Castro-Torres Y, Carmona-Puerta R, Chávez-González E, González-Rodríguez EF. Tpeak-Tend, Tpeak-Tend dispersion and Tpeak-Tend/QT in children and its relationship with clinical variables. Colomb Med (Cali) 2019; 50:252-60.
28. Türe M, Akin A, Unal E, et al. The association between electrocardiographic data and obesity in children and adolescents. Minerva Pediatr (Torino) 2021.
29. Wong CY, O'Moore-Sullivan T, Leano R, Byrne N, Beller E, Marwick TH. Alterations of left ventricular myocardial characteristics associated with obesity. Circulation 2004; 110:3081-7.
30. Ghandi Y, Sharifi M, Habibi D, Dorreh F, Hashemi M. Evaluation of left ventricular function in obese children without hypertension by a tissue Doppler imaging study. Ann Pediatr Cardiol 2018; 11:28-33.
31. Ayer JG, Sholler GF, Celermajer DS. Left atrial size increases with body mass index in children. Int J Cardiol 2010; 141:61-7.
32. Hurtado-Sierra D, Colin-Ramírez E, Lena-Hernández EL, et al. Subclinical cardiac alterations detected by echocardiography in Mexican school children with overweight and obesity. Arch Cardiol Mex 2019; 89:202-11.

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Original Article

Evaluation of Vascular Involvement in Children with Celiac Disease

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ABSTRACT

Aim: Celiac disease is associated with an increased risk of cardiovascular disease due to inflammation and autoimmunity involved in its pathophysiology. We aimed to evaluate vascular involvement in children with celiac disease based on their augmentation index, carotid pulse wave velocity, carotid intima-media thickness, echocardiographic findings, and blood pressure.

Materials and Methods: This cross-sectional and controlled study was performed at a single center between 2018 and 2019. The study population consisted of 44 patients with celiac disease who had been on a gluten-free diet for at least one year.

Results: We compared celiac patients with a healthy group. While the celiac patients had significantly higher carotid intima media thickness and carotid pulse wave velocity values, there was no difference in the augmentation index values. There was no significant difference in carotid artery intimal medial thickness, augmentation index and carotid pulse wave velocity values between the diet-compliant and non-compliant groups.

Conclusion: Although hypertension was not detected, arterial stiffness and carotid intima media thickness measurements were higher in the celiac disease patients compared to the healthy controls. This showed that these parameters can be used in early vascular damage assessment. These measurements, which are non-invasive and repeatable, can be a guide for the monitoring of the development of preclinical atherosclerosis in the follow-up of the pediatric patients diagnosed with celiac disease.

Keywords: AIx, celiac disease, children, cIMT, PWV

Introduction

Celiac disease (CD) is an immune-mediated condition characterized by inflammation triggered by dietary gluten and related prolamins and associated small intestinal villous atrophy (1,2). Genetic, immunologic and environmental factors are thought to be responsible for the development of CD (3,4).

CD is diagnosed using specific antibodies, such as tissue-transglutaminase antibody immunoglobulin-A antibody (tTG IgA), and screening followed by confirmatory small intestine biopsies (5). The only treatment is a life-long gluten-free diet (GFD). A GFD eliminates the gliadin and glutamine protein fractions contained in wheat, rye, and barley. Serological test values return to normal in 6-12 months after starting a strict GFD diet (6).

The risk of cardiovascular disease (CVD) in CD patients is still debated. Inflammation is associated with an increased risk of atherosclerosis (7,8). Atherosclerosis is

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the most important risk factor for CVD and it begins in childhood. Patients at high risk of CVD can be identified by determining arterial thickness and elasticity (9-11). In the review study by Bernardi et al. (12), it was concluded that celiac patients should be screened for CVD risk. As subclinical atherosclerosis is reversible when detected and intervened early, the early diagnosis of arterial injury is important to prevent future vascular risk. Early diagnosis is possible by measuring increased carotid artery intimal medial thickness (cIMT), carotid pulse wave velocity (PWV) and the augmentation index (AIx). cIMT is evaluated by ultrasonography (13). Arterial stiffness can be assessed by the measurement of carotid PWV and AIx, which are the main indices for estimating arterial elasticity (14). These methods are repeatable, reliable, easy, and noninvasive techniques which can detect any increased arterial stiffness and thickness at an early stage (15).

CD is associated with an increased risk of CVD due to inflammation and autoimmunity (16-21). Additionally, the GFD contains high levels of fat, sugar and salt, and children with CD are exposed to excessive hypercaloric and hyperlipidemic products. Moreover, it is an unbalanced diet. A GFD may have a negative impact on cardiometabolic and cardiovascular risk factors, including obesity, increased serum lipid levels, insulin resistance, and cardiometabolic and cardiovascular risk factors leading to metabolic syndrome and atherosclerosis (22). In this study, we aimed to evaluate vascular involvement in children with CD based on their AIx, cIMT, carotid PWV, echocardiographic findings, and blood pressure.

Materials and Methods

This cross-sectional and controlled study was performed at a single center between 2018 and 2019. The study population consisted of 44 previously diagnosed biopsy-proven CD patients who had been on a GFD for at least one year.

There were no known co-morbidities such as hypertension (HT), dyslipidemia, systemic autoimmune disease, active infection, Mediterranean fever syndrome or selective immunoglobulin-A deficiency. The control group consisted of healthy children (n=35) of similar age and gender to the patient group who were admitted to the pediatric nephrology outpatient clinic. This study was approved by the Medical Research Ethics Committee of Ege University, Medical School (date: 17/12/2020, number: 20-12.1T/36). A written informed consent form was signed by the patients' parents or caregivers.

CD is diagnosed by a detailed clinical story, physical examination, laboratory tests and upper gastrointestinal endoscopy and biopsy.

The patients' age at the time of diagnosis, follow-up time, complaints, age, gender and family history were recorded. Their weight, height, body mass index (BMI) and standard deviation scores (SDS) at the initial and final visits were also recorded. All patients underwent weight measurement with a digital scale and height measurement with a stadiometer. SDS calculated according to Turkish growth charts were used to evaluate weight, height and BMI values in different age and gender groups (23).

IgA, tTG IgA, sedimentation rate and C-reactive protein were assessed after at least eight hours of fasting during the final visit. Samples were analyzed at a local laboratory. Tissue transglutaminase IgA antibody levels were measured by enzyme-linked immunosorbent assay.

Adherence to a GFD was assessed based on tTG IgA antibody levels. Tissue-transglutaminase IgA levels higher than 10 U/mL were considered positive and non-compliant to GFD.

Blood pressure was measured in the resting position using an Omron automatic blood pressure device and a suitable sized cuff. Average diastolic and systolic blood pressure values over 95% according to age, sex, and height after at least three measurements were considered as HT (24). Patients with HT underwent a 24-hour ambulatory blood pressure monitoring by the pediatric nephrology clinic. Echocardiogram (ECHO) was performed on those who had not had a cardiac evaluation within the previous six months.

The patients' carotid PWV, AIx and cIMT were measured. Carotid PWV and AIx were calculated using Vicorder three times (Skidmore Medical Limited, Bristol, UK). AIx was calculated as the difference between the first and second systolic peaks of the central aortic waveforms and defined as the percentage of the wavelength. For cIMT measurements, a Siemens Acuson Antares device and VFX-7-13-megahertz linear probe were used. Carotid intima-media thickness measurements were performed approximately 1 cm proximal from the bifurcation. All these measurements were performed by an experienced pediatric nephrologist.

Statistical Analysis

Statistical analysis was performed using basic statistical methods. The distribution of the data was calculated using the Kolmogorov-Smirnov normality test. The Independent

Samples t-test was used to compare numerical data between groups. Pearson's test was used to calculate the correlation between cIMT, carotid PWV and AIx with laboratory parameters. The significance level was accepted as $p<0.05$. The required sample size was calculated with the G-Power program to determine the difference in the evaluated parameters of both groups. The sample size was calculated to be a minimum of 8 people in each group and a total of 16 people. Data were analyzed using IBM SPSS 22.0 (IBM Corp., Armonk, NY) software package.

Results

Forty-four patients (28 females and 16 males) with CD having a mean age of 13.16±4.97 years were included in this study. Thirty-five age-matched (mean 14.00±4.01 years) subjects were selected as healthy controls. Of the healthy control group, 18 were female and 17 were male.

When the anthropometric measurements of the celiac group and the control group were compared, the values of the control group were found to be significantly higher than the celiac group (mean weight: 54.80±18.40 kg vs 42.34±15.19 kg, mean height: 156.74±16.52 cm vs 146.04±20.72 cm, respectively).

Thirty-six (81.8%) patients had classical (diarrhea, abdominal pain, and abdominal distention), five (11.4%) had atypical (short stature, anemia, and constipation) and three (6.8%) had silent types of CD, respectively.

Based on tTG IgA antibody results, 22 (50%) patients were considered to be compliant with the GFD. The anthropometric measurements of the CD patients are presented in Table I.

The mean of three blood pressure measurements indicated stage 2 HT in only one of the CD patients who was 19 years old. Stage 1 HT was detected as a result of twenty-four hours of ambulatory blood pressure monitoring. There were no pathological findings of HT in his ECHO evaluation. The other patients had normal blood pressure and ECHO results.

There was no significant difference between the anthropometric measurements, biochemical parameters, cIMT, AIx and carotid PWV values in the diet-compliant and non-compliant groups (Table II).

We compared the celiac patients and the healthy group. While the celiac patients had significantly higher cIMT and carotid PWV values, there was no difference in the AIx values (Table III).

The celiac patients had no significant relationship between their tTG IgA antibody and carotid PWV, cIMT and AIx levels. However, there was a significant negative relationship between their AIx and height ($r=-0.575$, $p<0.001$), weight ($r=-0.609$, $p<0.001$) and BMI ($r=-0.459$, $p=0.002$) and positive relationships between their carotid PWV and weight ($r=0.362$, $p=0.016$) and BMI ($r=0.387$, $p=0.01$) in those children with CD. There was a significant positive relationship between their cIMT and height SD values ($r=0.558$, $p=0.001$).

Table I. Anthropometric measurements of the CD patients (n=44) at the time of diagnosis

	Minimum	Maximum	Mean ± SD
Height (cm)	81.0	160.0	126.32±25.28
Height SD	-4.12	2.74	-1.14±1.55
Weight (kg)	11.0	61.0	29.15±14.13
Weight SD	-3.30	1.50	-1.11±1.32
BMI (kg/m²)	13.22	22.32	16.89±2.48
BMI SD	-2.80	1.44	-0.53±1.09

CD: Celiac disease, SD: Standard deviation

Table II. Comparative analysis of the demographic, anthropometric and biochemical data of tTG IgA antibody positive and negative CD patients

	Patients with tTG IgA antibody (+) Mean ± SD (n=22)	Patients with tTG IgA antibody (-) Mean ± SD (n=22)	p-value
Age (year)	13.50±5.61	13.50±4.38	0.99
Weight (kg)	41.21±16.34	43.47±14.25	0.62
Weight SD	-0.47±1.27	-0.53±1.42	0.90
Height (cm)	145.00±23.31	147.09±18.25	0.74
Height SD	-0.78±1.38	-0.55±1.53	0.66
BMI (kg/m²)	18.52±3.14	19.42±3.77	0.40
BMI SD	-0.14±1.17	-0.36±1.34	0.64

CD: Celiac disease, SD: Standard deviation, tTG IgA: Tissue-transglutaminase antibody immunoglobulin-A antibody

Table III. Comparative analysis of the cIMT, AIx, and carotid PWV values of the CD patients and the healthy control group on the final visit

	CD patients Mean ± SD (n=44)	Mean ± SD (n=35)	p-value
cIMT	0.51±0.05	0.45±0.08	0.001
Carotid PWV	4.82±0.42	4.59±0.29	0.001
AIx	25.93±15.27	22.54±7.96	0.238

CD: Celiac disease, SD: Standard deviation, cIMT: Carotid artery intimal medial thickness, AIx: Augmentation index, PWV: Pulse wave velocity

Discussion

There is limited data on vascular involvement in pediatric CD patients. Our study is the first report evaluating preclinical atherosclerosis in pediatric celiac patients using carotid PWV, AIx, cIMT measurements; ECHO and blood pressure values.

Inflammation plays an important role in the pathogenesis of atherosclerosis. Chronic inflammation may be responsible for the development of atherosclerosis in pediatric CD. However, the autoimmune nature of CD has also targeted other organ systems, including the cardiovascular system. Some studies in adult CD patients have suggested that CD is associated with early atherosclerosis (25,26). In a multicenter study conducted in 2013, 14% (n=114) of CD patients were shown to have at least one traditional CVD risk factor and screening for CVD risk factors in CD was recommended both at the time of diagnosis and during follow-up (8). A significant increase in PWV and cIMT was observed in adults with CD compared to the control group (25-27). All these findings suggest that preclinical atherosclerosis may be common in adults with CD. Atherosclerosis is known to start in childhood; however, symptoms usually appear at older ages. Studies have demonstrated that an earlier onset increases the chance of atheromatous plaque formation. Therefore, the early detection and intervention of the development of atherosclerosis in childhood are crucial in terms of decreasing the mortality and morbidity which can be observed in older ages. In a pediatric population study, cIMT and PWV values were not different in CD patients compared with healthy controls (28). In the present study, we found that cIMT and carotid PWV values were significantly higher in the CD patients (mean 0.51±0.05 vs. 0.45±0.08, mean 4.82±0.42 vs. 4.59±0.29, respectively). In our study, another important finding was the insignificant increase in AIx in CD patients compared to the healthy controls. Multicenter and larger sample size studies may be needed to obtain a statistically significant difference regarding this.

There is a correlation between the presence of CD-specific antibodies and disease severity. In contrast to three other studies, no significant difference was found between tTG IgA antibody positive and negative subjects in terms of carotid PWV, AIx and cIMT in this study. It might be considered that the normal ranges of serologic tests should be reviewed (25,28,29).

There was a positive relationship between the cIMT and tTG IgA antibody levels in a study conducted in pediatric age group CD patients (28). However, in our study, CD patients had no significant relationship between tTG IgA antibody and carotid PWV, cIMT, or AIx levels. Therefore, arterial thickness and elasticity may be affected by uncontrolled or undiagnosed CD as well as other unknown factors. There was a significant negative relationship between AIx and height ($r=-0.575$, $p<0.001$), weight ($r=-0.609$, $p<0.001$) and BMI ($r=-0.459$, $p=0.002$). There was a significant positive relationship between cIMT and height SD values ($r=0.558$, $p=0.001$). There was a significant positive relationship between carotid PWV with weight and BMI values ($r=0.362$, $p=0.016$, $r=0.387$, $p=0.010$, respectively) as was previously shown in the pediatric CD patients (28).

The blood pressure values of the CD patients, except for one, were within the normal range. A 19-year-old male patient with stage 1 HT had diet compliance problems. We learned that this patient had been smoking a packet of cigarettes per day for the last three years. Uncontrolled CD and smoking were the probable cause of HT in this subject. The development of HT may be related to tTG IgA antibody positivity and result in inflammatory activity by causing atherosclerosis.

Study Limitations

Our study had a few limitations such as the small number in the patient group, the lack of cIMT, AIx and carotid PWV measurements before starting a GFD and the lack of CVD risk factors data for both the CD patients and the healthy controls.

Conclusion

In conclusion, although HT was not detected, arterial stiffness and cIMT measurements were higher in the CD

patients compared to the healthy controls. This shows that such parameters can be used in early vascular damage assessment. These measurements, which are non-invasive and repeatable, can be a guide for monitoring the development of preclinical atherosclerosis in the follow-up of CD patients.

Ethics

Ethics Committee Approval: This study was approved by the Medical Research Ethics Committee of Ege University, Medical School (date: 17/12/2020, number: 20-12.1T/36).

Informed Consent: A written informed consent form was signed by the patients' parents or caregivers.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

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References

1. Schuppan D, Dennis MD, Kelly CP. Celiac disease: epidemiology, pathogenesis, diagnosis, and nutritional management. Nutr Clin Care 2005; 8:54-69.
2. Ludvigsson JF, Green PH. Clinical management of coeliac disease. J Intern Med 2011; 269:560-71.
3. Mäki M, Mustalahti K, Kokkonen J, et al. Prevalence of Celiac disease among children in Finland. N Engl J Med 2003; 348:2517-24.
4. Walker MM, Murray JA, Ronkainen J, et al. Detection of celiac disease and lymphocytic enteropathy by parallel serology and histopathology in a population-based study. Gastroenterology 2010; 139:112-9.
5. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012; 54:136-60.
6. No authors listed. NIH Consensus Development Conference on Celiac Disease. NIH Consens State Sci Statements 2004; 21:1-23.
7. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem 2001; 47:403-11.
8. Norsa L, Shamir R, Zevit N, et al. Cardiovascular disease risk factor profiles in children with celiac disease on gluten-free diets. World J Gastroenterol 2013; 19:5658-64.
9. Simons PC, Algra A, Bots ML, Grobbee DE, van der Graaf Y. Common carotid intima-media thickness and arterial stiffness: indicators of cardiovascular risk in high-risk patients. The SMART Study (Second Manifestations of ARTerial disease). Circulation 1999; 100:951-7.
10. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995; 26:1235-41.
11. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation 2006; 113:657-63.
12. Bernardi N, Sciatti E, Pancaldi E, et al. Coeliac and cardiovascular disease: a possible relationship between two apparently separate conditions. Monaldi Arch Chest Dis 2022;93.
13. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39:257-65.
14. Zhang Y, Agnoletti D, Protogerou AD, et al. Characteristics of pulse wave velocity in elastic and muscular arteries: a mismatch beyond age. J Hypertens 2013; 31:554-9.
15. Bruno RM, Bianchini E, Faita F, Taddei S, Ghiadoni L. Intima media thickness, pulse wave velocity, and flow mediated dilation. Cardiovasc Ultrasound 2014; 12:34.
16. Ludvigsson JF, Montgomery SM, Ekbom A, Brandt L, Granath F. Small-intestinal histopathology and mortality risk in celiac disease. JAMA 2009; 302: 1171-8.
17. Ludvigsson JF, James S, Askling J, Stenestrand U, Ingelsson E. Nationwide cohort study of risk of ischemic heart disease in patients with celiac disease. Circulation 2011; 123:483-90.
18. Ludvigsson JF, de Faire U, Ekbom A, Montgomery SM. Vascular disease in a population-based cohort of individuals hospitalised with coeliac disease. Heart 2007; 93:1111-5.
19. Peters U, Askling J, Gridley G, Ekbom A, Linet M. Causes of death in patients with celiac disease in a population-based Swedish cohort. Arch Intern Med 2003; 163:1566-72.
20. Rubio-Tapia A, Ludvigsson JF, Choung RS, et al. Increased mortality among men aged 50 years old or above with elevated IgA anti-transglutaminase antibodies: NHANES III. BMC Gastroenterol 2016; 16:136.
21. Potter MDE, Brienesse SC, Walker MM, Boyle A, Talley NJ. Effect of the gluten-free diet on cardiovascular risk factors in patients with coeliac disease: A systematic review. J Gastroenterol Hepatol 2018;33:781-91.
22. Anania C, Pacifico L, Olivero F, Perla FM, Chiesa C. Cardiometabolic risk factors in children with celiac disease on a gluten-free diet. World J Clin Pediatr 2017; 6:143-8.
23. Neyzi O, Bundak R, Gökçay G, et al. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol 2015; 7:280-93.
24. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004; 114(2 Suppl 4th Report):555-76.

25. Korkmaz H, Sozen M, Kebapcilar L. Increased arterial stiffness and its relationship with inflammation, insulin, and insulin resistance in celiac disease. Eur J Gastroenterol Hepatol 2015; 27:1193-9.

26. Sari C, Bayram NA, Doğan FE, et al. The evaluation of endothelial functions in patients with celiac disease. Echocardiography 2012; 29:471-7.

27. De Marchi S, Chiarioni G, Prior M, Arosio E. Young adults with coeliac disease may be at increased risk of early atherosclerosis. Aliment Pharmacol Ther 2013; 38:162-9.

28. Demir AM, Kuloğlu Z, Yaman A, Fitöz S, Nergizoğlu G, Kansu A. Carotid intima-media thickness and arterial stiffness as early markers of atherosclerosis in pediatric celiac disease. Turk J Pediatr 2016; 58:172-9.

29. Caliskan Z, Demircioglu K, Sayar S, et al. Lipid profile, atherogenic indices, and their relationship with epicardial fat thickness and carotid intima-media thickness in celiac disease. North Clin Istanb 2019; 6:242-7.

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Original Article

Assessment of the Prognostic Power of Preoperative Laboratory Biomarkers in Predicting Pediatric Complicated Appendicitis and the Outcomes of the Relevant Surgical Intervention

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ABSTRACT

Aim: The purpose of this study was to assess the prognostic power of preoperative laboratory biomarkers in pediatric age group individuals diagnosed with appendicitis in the emergency department in distinguishing complicated appendicitis from non-complicated appendicitis and in predicting postoperative outcomes.

Materials and Methods: The population of this descriptive, cross-sectional, retrospective study consisted of children (younger than 18 years of age) who applied to our hospital's emergency department between January, 2020 and October, 2021 and underwent surgical intervention with a diagnosis of acute appendicitis. Preoperative laboratory test results, intraoperative surgical outcomes, lengths of hospital stay and postoperative complications data were recorded in the patient follow-up forms and analyzed.

Results: The intraoperative and pathological data revealed that 179 (37.8%) and 294 (62.1%) patients had complicated and non-complicated appendicitis, respectively. An analysis of the complete blood count results indicated that the C-reactive protein (CRP) level, and CRP-to-albumin ratio (CAR), neutrophil count, leukocyte count, monocyte-to-lymphocyte ratio (MLR), and neutrophil-to-lymphocyte ratio (NLR) were significantly higher, whereas the sodium and albumin levels were significantly lower in those patients with complicated appendicitis than in those with non-complicated appendicitis. Among the parameters investigated, the NLR, CAR values, and the presence of hyponatremia were found to be significantly associated with the length of hospital stay and postoperative complication rates in those patients with complicated appendicitis.

Conclusion: The findings of our study show that leukocyte counts, neutrophil counts, NLR values, CRP, sodium, and direct bilirubin levels measured preoperatively in the emergency department can be used to identify pediatric patients with complicated appendicitis. In addition, MLR and CAR values, as new biomarkers, can provide guidance in emergency interventions and also predict postoperative outcomes.

Keywords: Appendicitis, complicated, pediatric, laboratory, biomarkers

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Introduction

The incidence of appendicitis is higher in developing and newly industrialized countries and in the 15-19 years old age range (1). Acute appendicitis, which is among the diagnoses to be considered in children admitted to the emergency department with abdominal pain, is the most common cause of emergency surgery in the child age group (2). The higher risk of complications such as gangrenous, necrotizing, or perforated appendicitis necessitates more prudence in the pediatric patient group, in patients under 5 years of age in particular (3,4). Both the inability of the pediatric age group to describe their symptoms well enough and delays in admission and diagnosis due to the Coronavirus disease-2019 pandemic have led to an increase in the risk of perforation in recent years (5). Given that complicated appendicitis is associated with higher complication and morbidity rates than uncomplicated appendicitis, early operative intervention is required in the treatment of complicated appendicitis, especially in the presence of a perforation (6,7).

The literature data indicate that blood tests are routinely performed in children diagnosed with acute appendicitis, which include the measurement of markers which can be potentially used to predict complicated appendicitis. Among these markers are the leukocyte and neutrophil counts and the neutrophil-to-lymphocyte ratio (NLR), which are measured within the scope of the complete blood count, and C-reactive protein (CRP) levels (8-10). In recent years, it has been reported that hyponatremia, CRP-to-albumin ratio (CAR) and monocyte-to-lymphocyte ratio (MLR) can also be used as markers for complicated appendicitis in children (11-14). There are numerous studies in the literature on new biomarkers, yet only a limited number of these studies address pediatric patients or the relationship between these biomarkers and postoperative outcomes.

In this context, the aim of this study was to assess the prognostic power of those parameters measured preoperatively within the scope of the hemogram and biochemistry tests in pediatric age group individuals diagnosed with appendicitis in the emergency department in distinguishing complicated appendicitis from non-complicated appendicitis and in predicting postoperative outcomes.

Material and Methods

This study was designed as a descriptive, cross-sectional, retrospective study. The study protocol was approved by University of Health Sciences Turkey, Dr. Behçet Uz Child Disease and Surgery Training and Research Hospital, Clinical Research Ethics Committee (decision no: 621, date: October 10th, 2021).

The study population consisted of patients younger than 18 who applied to the emergency department between January, 2020 and October, 2021 and who underwent surgical intervention at the pediatric surgery clinic with a diagnosis of acute appendicitis based on biochemical tests and ultrasonographic findings. Between January, 2020 and October, 2021, 491 of the 1,091 patients admitted to the pediatric surgery clinic with a diagnosis of acute abdomen were operated on for acute appendicitis.

Patients who underwent emergency appendectomy were taken to the ward after staying in the pediatric surgery intensive care unit for 6-8 hours. Those with metabolic, endocrine and hematological diseases and those who used drugs were excluded from this study because these can be a determinant in biochemical and hematological parameters. Those patients with metabolic and endocrine diseases, those categorized as class IV based on the American Society of Anesthesiologists (ASA) scores and negative appendectomies (n=30, 6%) were excluded from this study. A total of 473 patients were included in this study.

The patients were divided into two groups based on their histopathological data as the complicated (gangrenous, perforated) and the non-complicated (phlegmonous) appendicitis groups. The demographic characteristics of the children such as gender, age, weight, clinical characteristics such as ASA scores, laboratory test results of venous blood samples taken during admission to the emergency department, length of hospital stay, and postoperative wound infection, abscess development, coagulation disorders, and transfusion data were obtained from the files and automation system of the patients and recorded in their patient follow-up forms.

Statistical Analysis

The descriptive statistics obtained from the research data were tabulated as mean ± standard deviation values in cases of continuous variables determined to conform to the normal distribution, as median, maximum and minimum values in cases of continuous variables determined not to conform to the normal distribution, and as numbers and percentage values in cases of categorical variables. The normal distribution characteristics of the numerical variables were analyzed with Anderson-Darling, Kolmogorov-Smirnov and Shapiro-Wilk tests.

In comparisons of two independent groups, the independent samples t-test was used in cases of numerical variables which conformed to the normal distribution, and the Mann-Whitney U test was used in cases of numerical variables which did not conform to the normal distribution. In comparisons of differences between categorical variables according to groups, Pearson's chi-squared test, Fisher's exact test and Fisher-Freeman-Halton test were used.

Univariate and multiple logistic regression analyses were used to determine those variables which can predict complicated appendicitis. Those variables with a p-value of less than 0.250 were further analyzed with a multivariate model.

The prognostic powers of the NLR, MLR, and CAR variables in differentiating complicated acute appendicitis from non-complicated appendicitis were analyzed using receiver operating characteristic (ROC) curve analysis. The optimal cut-off values of the variables which can be used to predict complicated appendicitis were determined with Youden's index using the DeLong method in MedCalc Statistical Software Trial version software, along with 95% confidence interval (CI) and area under the curve (AUC) values.

All other statistical analyses were performed using the Jamovi version 2.2.5.0 (The Jamovi Project, 2021) and JASP version 0.16.1 (JASP Team, 2022) software packages. The probability (p) statistics of ≤0.05 were deemed to indicate statistical significance.

Results

The mean age of the 473 patients who were admitted to the emergency department and diagnosed with acute appendicitis and who underwent surgical procedure by the pediatric surgery clinic, of whom 151 (31.9%) were female, and 322 (68.1%) were male, was 10.1±3.7 years. The rate of the patients with a body mass index score of ≥30 (obese) was 12.9%. Based on the histopathological data, it was determined that 179 (37.8%) patients had complicated and 294 (62.1%) had non-complicated appendicitis (Table I).

There were significant differences between the groups in terms of the preoperative laboratory test results of the patients (Table II). Accordingly, neutrophil counts (p<0.001), leukocyte counts (p<0.001), NLR (p=0.021), CRP levels (p<0.001) and CAR values (p<0.001) were significantly higher, whereas lymphocyte percentage and hemoglobin and serum sodium and albumin levels were significantly lower in those patients with complicated appendicitis compared to those with non-complicated appendicitis.

The mean length of hospital stay of the pediatric patients was 3 days (minimum 2 days-maximum 39 days). The rate of complication development in the postoperative period was 9.9%. Among patients with complicated and non-complicated appendicitis, with regards to operation time, length of hospital stay, wound infection, abscess development, coagulation disorders, and the need for transfusion, no significant difference was observed.

Table I. Demographic and clinical characteristics of patients

	Total (n=473)	Groups		p-value
		Complicated appendicitis (n=179)	Non-complicated appendicitis (n=294)	
Age (years)	10.1±3.7	9.3±3.8	10.5±3.6	**<0.001†**
Gender				
Male	322 (68.1%)	119 (66.5%)	203 (69.0%)	0.632*
Female	151 (31.9%)	60 (33.5%)	91 (31.0%)	
Weight (kg)	39.8±17.2	36.0±16.1	42.2±17.4	**<0.001†**
Obesity	61 (12.9%)	20 (11.2%)	41 (13.9%)	0.465*
ASA score				
I	362 (76.5%)	142 (79.3%)	220 (74.8%)	0.172*
II	100 (21.1%)	31 (17.3%)	69 (23.5%)	
III	11 (2.3%)	6 (3.4%)	5 (1.7%)	

Descriptive statistical values are mean ± SD or number of cases (%).
*: Pearson chi-squared, Fisher's Exact or Fisher-Freeman-Halton test, †: Independent Samples t-test
ASA: American Society of Anesthesiologists

Table II. Comparison laboratory parameters according to groups

	Groups		p-value
	Complicated appendicitis (n=179)	Non-complicated appendicitis (n=294)	
WBC count (X10^9/L)	17,633.0±5,569.2	15,374.7±4,948.0	**<0.001***
Neutrophil count (X10^9/L)	14.3±5.2	12.4±4.9	**<0.001‡**
Neutrophils %	81.9 [29.4-92.6]	81.3 [30.7-94.2]	0.663*
Lymphocyte count	1.7 [0.5-5.2]	1.7 [0.4-5.3]	0.555*
Lymphocyte %	9.8 [2.9-44.5]	11.2 [2.7-57.5]	0.008*
Neutrophil/Lymphocyte ratio	8.2 [1.1-30.4]	7.3 [0.5-35.0]	0.021*
Monocyte/Lymphocyte ratio	0.1 [0.0-1.0]	0.1 [0.0-1.0]	**<0.001***
Hemoglobin (g/dL)	12.8±1.1	13.1±1.3	0.021‡
Hematocrit (%)	37.8±2.9	38.3±3.4	0.070‡
Platelet count (X10^9/L)	314.0±81.8	306.1±76.2	0.298‡
PDW (fL)	10.2 [7.6-68.3]	10.5 [7.5-66.8]	0.149*
MPV (fL)	9.4 [7.1-12.9]	9.5 [6.8-13.1]	0.869*
MPV/Platelet ratio	0.0 [0.0-0.1]	0.0 [0.0-0.1]	0.352*
Procalcitonin	0.3 [0.1-0.6]	0.3 [0.1-0.5]	0.520*
Glucose (mg/dL)	105.0 [55.0-264.0]	105.0 [44.0-162.0]	0.582*
Sodium (mEq/L)	136.0 [126.0-143.0]	138.0 [129.0-144.0]	**<0.001***
Potassium (mEq/L)	4.1 [3.1-5.4]	4.2 [3.4-5.5]	0.056*
Chloride (mEq/L)	102.0 [86.0-110.0]	104.0 [94.0-111.0]	**<0.001***
Calcium (mEq/L)	9.4 [8.0-10.4]	9.4 [7.7-10.7]	0.462*
Protein (mg/dL)	7.1 [4.9-8.2]	7.2 [5.6-8.6]	0.016*
Albumin (mg/dL)	4.3 [2.7-5.2]	4.6 [3.0-5.4]	**<0.001***
BUN (mg/dL)	9.7 [3.3-45.3]	10.0 [2.5-35.0]	0.508*
Creatinine (mg/dL)	0.6 [0.4-1.1]	0.6 [0.5-1.0]	0.104*
Alanine aminotransferase (IU/L)	12.0 [5.0-60.0]	14.0 [5.0-381.0]	**0.001***
Aspartate aminotransferase (IU/L)	19.0 [8.0-58.0]	20.0 [7.0-210.0]	0.061*
Total bilirubin (mg/dL)	0.7 [0.2-3.5]	0.6 [0.1-3.3]	0.003*
Direct bilirubin (mg/dL)	0.3 [0.1-2.1]	0.3 [0.1-1.3]	**<0.001***
CRP (mg/dL)	7.9 [0.2-32.4]	1.4 [0.1-33.0]	**<0.001***
CRP/Albumin ratio	1.8 [0.0-12.0]	0.3 [0.0-7.7]	**<0.001***
Prothrombin time (min)	14.4 [11.1-23.4]	14.0 [11.3-26.5]	0.065*
INR	1.3 [1.0-2.0]	1.2 [1.0-2.3]	0.072*
Activated partial thromboplastin time (min)	30.3±3.6	30.4±3.4	0.709‡

Descriptive statistical values are mean ± SD or number of cases (%). Number values in square brackets are minimum and maximum values.
*: Mann-Whitney U test.
†: Pearson Chi-Squared, Fisher's Exact or Fisher-Freeman-Halton test.
‡: Independent Samples t-test.
WBC: White blood cell count, PDW: Platelet distribution width, MPV: Mean platelet volume, BUN: Blood urea nitrogen, CRP: C-reactive protein, INR: International Normalized Ratio, SD: Standard deviation

The results of the univariate logistic regression analysis indicated that age, leukocyte and neutrophil counts, MLR and CAR values, sodium, direct bilirubin, hemoglobin, and CRP levels were significantly correlated with complicated appendicitis ($p<0.05$). Further analysis of these variables with multivariate logistic regression analysis revealed that leukocyte count [odds ratio (OR): 1.01, 95% CI: 1.01-1.02, $p=0.005$], CAR value (OR: 0.64, 95% CI: 0.54-0.77, $p<0.001$), and sodium levels (OR: 1.22, 95% CI: 1.11-1.35, $p<0.001$) were independent risk factors for complicated appendicitis (Table III).

The diagnostic value of these variables in predicting complicated appendicitis was analyzed using ROC analysis. Accordingly, it was determined that NLR values of >6.52, MLR values of >0.09, and CAR values of >1.12 predicted the presence of complicated acute appendicitis (Table IV). Among these variables, CAR values of >1.12 had the highest AUC value and predicted complicated acute appendicitis with a specificity of 79.25% and a sensitivity of 66.48% (AUC=0.789, 95% CI: 0.749-0.825, $p<0.001$) (Figure 1). In addition to NLR values of >6.52, and CAR values of >1.12, serum sodium levels of ≤135 were found to be significantly associated with increased postoperative complication rates and prolonged lengths of hospital stay (Table V).

Table III. Regression analysis for variables of clinical and statistical significance for the prediction of complicated appendicitis

	Univariate logistic regression		Multivariate logistic regression	
	OR [95% CI]	**p-value**	**OR [95% CI]**	**p-value**
Age	1.10 [1.04-1.16]	**<0.001**	1.05 [0.98-1.13]	0.148
Hemoglobin	1.19 [1.02-1.39]	**0.028**	1.01 [0.81-1.25]	0.961
WBC count	1.01 [1-01-1-02]	**<0.001**	1.01 [1-01-1-02]	**0.005**
Neutrophil count	0.93 [0.89-0.96]	**<0.001**	-	-
NLR	0.98 [0.95-1.01]	0.121	1.03 [0.98-1.09]	0.225
MLR	0.03 [0-01-0.16]	**<0.001**	0.39 [0.01-10.69]	0.578
Sodium	1.39 [1.28-1.51]	**<0.001**	1.22 [1.11-1.35]	**<0.001**
Direct bilirubin	0.11 [0.03-0.37]	**<0.001**	0.26 [0.05-1.25]	0.092
CRP	0.85 [0.82-0.88]	**<0.001**	-	-
CRP/Albumin	0.51 [0.43-0.60]	**<0.001**	0.64 [0.54-0.77]	**<0.001**

OR: Odds ratio, CI: Confidence interval, CRP: C-reactive protein, WBC: White blood cell count, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio

Table IV. ROC curve analysis results of some parameters for the prediction of complicated appendicitis

	AUC	Sensitivity	Specificity	Cut-off values	95% CI	p-value
NLR	0.563	66.48	45.92	>6.52	0.517-0.608	**0.018**
MLR	0.659	67.04	58.84	>0.09	0.615-0.702	**<0.001**
CRP/Albumin ratio	0.789	66.48	79.25	>1.12	0.749-0.825	**<0.001**

Area under the ROC curve, CI: Confidence interval, ROC: Receiver operating characteristic, AUC: Area under the curve, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, CRP: C-reactive protein

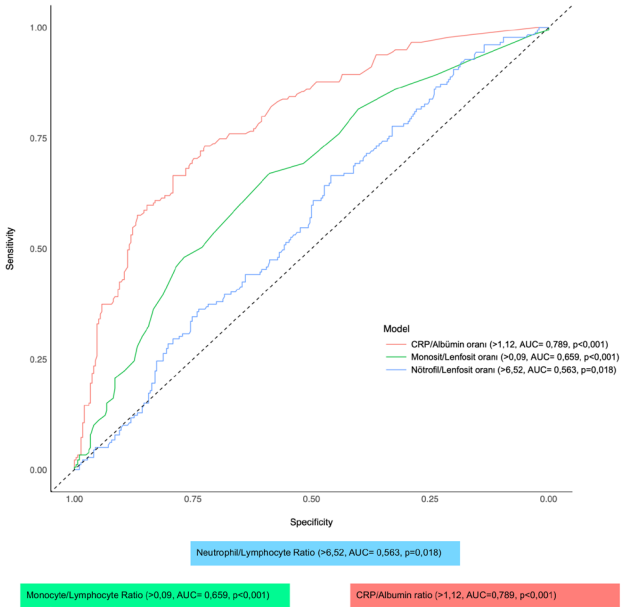

Figure 1. ROC curve analysis of NLR, MLR and CRP/Albumin ratio in the prediction of complicated acute appendicitis
ROC: Receiver operating characteristic, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, CRP: C-reactive protein

Table V. Relationship of ROC curve results with postoperative outcomes in the prediction of complicated acute appendicitis

	NLR >6.52	p-value	CRP/Albumin ratio >1.12	p-value
ICU stay (days)	1.24±1.17	**<0.013**	1.29±0.95	**<0.014**
Hospital stay (days)	5.09±4.62	**<0.01**	6.34±4.75	**<0.01**
Complication rate (%)	12.90	**0.005**	19.44	**<0.01**

ROC: Receiver operating characteristic, NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein, ICU: Intensive care unit

Discussion

Among the variables investigated within the scope of this study, age, leukocyte counts and neutrophil counts were previously suggested as predictors for complicated appendicitis in pediatric patients (3,4). However, the findings of this descriptive, cross-sectional study indicated that complicated appendicitis was more common in younger age groups and that the rate of complicated appendicitis was significantly higher among acute appendicitis patients with higher leukocyte and neutrophil counts, NLR, CRP, CAR values, and lower sodium levels. Therefore, in addition to the commonly proposed predictors of age, leukocyte counts and neutrophil counts, the aforementioned parameters were determined as additional potential biomarkers of complicated appendicitis in the pediatric age group.

MLR, which has recently been started to be used in children, is an inflammatory marker associated with gastrointestinal and lymphoid pathologies. Hence, it has been suggested that the appendix, a lymphoid tissue located in the gastrointestinal tract, may be associated with MLR (15,16). As a matter of fact, there is evidence suggesting that MLR can be used in the diagnosis of pediatric acute appendicitis (17). Accordingly, it has been reported that MLR may be an independent predictor for complicated appendicitis. In parallel, given that increased monocyte expression is distinctive for complicated appendicitis, high MLR values have been indicated as a risk factor for complicated appendicitis (13).

CRP is an acute phase reactant mainly synthesized in liver hepatocytes. It is elevated in areas of infection or inflammation, and it plays an essential role in inflammation processes (18). CRP increase is associated with appendicitis complications such as perforation or appendix abscesses (19). CAR is a new prognostic parameter associated with the severity of inflammation. In this context, it was reported that CAR ≥1.39 might predict complicated appendicitis (20).

Stimuli such as hypovolemia, pain, nausea, vomiting and sequestration into the third space cause the release of non-osmotic vasopressin (ADH) from the posterior pituitary gland. Reabsorption of water by ADH binding to vasopressin-2 receptors in the kidney results in hyponatremia (21,22). In addition, cytokines such as IL-6 and IL-1β are associated with inflammatory conditions by taking a role in non-osmotic ADH secretion have been found to cause hyponatremia (21). Hyponatremia, which was determined to be significantly related to the level of inflammation in children, was also found to be associated with intra-abdominal sepsis, perforated appendicitis and perforated diverticulitis (23). Although the etiology of hyponatremia in appendicitis is still not clearly defined, it is thought that the processes mentioned above may be effective (24).

There are also studies which provided evidence on the use of hyponatremia as a predictor for complicated appendicitis. In a few of these studies, Pham et al. (4), Lindestam et al. (11), and Besli et al. (25) reported that sodium values of ≤135 mmol/L, ≤136 mmol/L, and ≤138 mmol/L, respectively, might predict complicated appendicitis. In a retrospective study including 1,283 pediatric patients, Walsh et al. (26) reported that serum sodium values of

<135 mmol/L predicted complicated appendicitis with a specificity of 95.7% and thus concluded that hyponatremia may be a distinctive indicator of complicated appendicitis. All these studies reveal the strong relationship between hyponatremia and complicated appendicitis in children. Similarly, serum sodium levels of ≤135 mmol/L significantly predicted complicated appendicitis in this study.

The relationship between complicated appendicitis and bilirubin levels is not as clear as with leukocytosis, CRP, and hyponatremia. Some studies reported that increased total serum bilirubin levels could be used as an indicator of perforated appendicitis in children, whereas others, e.g., Yang et al. (12), could not detect a significant relationship between perforated appendicitis and bilirubin levels (27). In fact, Bonadio et al. (28) stated that serum total bilirubin levels could not correctly distinguish those children with perforated appendicitis from those diagnosed with appendicitis. In contrast, our results of univariate logistic regression analysis reveal a significant correlation between direct bilirubin levels and the risk for complicated appendicitis.

Studies have shown that preoperative laboratory test results can be used to predict postoperative outcomes. With respect to this, it was reported that preoperative CRP values of >10 mg/dL predicted prolonged postoperative lengths of hospital stay, and preoperative NLR >10.5 and hyponatremia predicted the development of a postoperative intra-abdominal abscess in pediatric patients operated on for acute appendicitis (29-34). In addition, preoperative leukocyte counts and CRP levels have been reported as risk factors for postoperative adhesive bowel obstruction in children operated on for complicated appendicitis (35). It has also been reported that mean platelet volume and NLR can be used as biomarkers in order to reduce negative appendectomy rates (8). Furthermore, in an analysis of 35,291 pediatric patients who underwent appendectomy, hyponatremia was found to be significantly correlated with prolonged lengths of hospital stay and 30-day postoperative mortality risks (36). Additionally, NLR >6.52, CAR >1.12, and serum sodium levels of ≤135 mmol/L were found to be significantly correlated with prolonged lengths of hospital stay and postoperative complication rates in this study.

Ischemia-modified albumin (IMA), a marker used for the early detection of ischemia, has also been used in the diagnosis of pediatric appendicitis in recent studies. It was observed that the level of IMA, a protein, was higher in the complicated appendicitis group than in the control group (37,38). Pentraxin 3 (PTX3), which is an acute phase reactant, is another marker which can be used in the diagnosis of pediatric appendicitis. CRP blood values rise within 48 hours of the onset of inflammation, while PTX3 blood values rise within hours. Therefore, it is thought that PTX3 seems to be a more valuable marker (38). However, studies on IMA and PTX3 are few in the pediatric age group, and more studies are needed before they can be used as biomarkers.

Complicated appendicitis is reportedly more common in children and it is associated with higher morbidity and reoperation rates and longer lengths of hospital stay (3,4,6). The benefits of early appendectomy in pediatric perforated appendicitis cases include reduced morbidity and complication rates, shortened recovery times, reduced parental stress, and lower hospital costs (39). In a multicenter study conducted with pediatric non-perforated appendicitis cases, it was determined that each additional hour spent in an emergency department triage until appendectomy increased the perforation risk by 2%. Furthermore, it was stated that a delay of 12 hours would increase the perforation risk by approximately 25% (40). For this reason, it is vital to identify those children at risk of complicated appendicitis as early as possible, to make timely decisions about further examination and treatment, to improve perioperative care, and to take precautions by predicting any complications which may develop. In this way, the length of hospital stay can be shortened, and the related hospital costs can be reduced. In this respect, it is crucial to determine the biomarkers included in the laboratory tests which are the most easily accessible and the simple diagnostic tools used in the emergency room, the place where the patient is first intervened (9).

Study Limitations

Our study is a single-center study conducted only with pediatric patients. Its retrospective design and the number of patients are the limitations of this study. Another limitation of our study was the inability to exclude the presence of an undiagnosed inflammatory disease in the patients.

Conclusion

It is important to distinguish between complicated and non-complicated appendicitis early in children diagnosed with appendicitis in order to conduct further examinations and plan early treatment. Hence, there is a need for suitable biomarkers which can be measured simply and inexpensively within the scope of routine laboratory tests. Increased diagnostic efficiency in emergency departments will have positive effects on hospital costs as well. In addition to preoperatively measured neutrophil counts, leukocyte

counts, MLR, CAR values, CRP, sodium, and direct bilirubin levels may also be used as new biomarkers in predicting the risks of complicated appendicitis. Preoperative NLR, MLR, and CAR values may also provide information on postoperative outcomes.

Ethics

Ethics Committee Approval: The study protocol was approved by University of Health Sciences Turkey, Dr. Behçet Uz Child Disease and Surgery Training and Research Hospital, Clinical Research Ethics Committee (decision no: 621, date: October 10th, 2021).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.A., Concept: C.S.Ö., K.E.Ş., Ö.A., Design: C.S.Ö., K.E.Ş., Data Collection or Processing: C.S.Ö., K.E.Ş., Analysis or Interpretation: Ö.A., Literature Search: C.S.Ö., Ö.A., Writing: C.S.Ö., K.E.Ş.

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References

1. Ferris M, Quan S, Kaplan BS, et al. The Global Incidence of Appendicitis: A Systematic Review of Population-based Studies. Ann Surg 2017; 266:237-41.
2. Kharbanda AB, Stevenson MD, Macias CG, et al. Interrater reliability of clinical findings in children with possible appendicitis. Pediatrics 2012; 129:695-700.
3. Feng W, Zhao XF, Li MM, Cui HL. A clinical prediction model for complicated appendicitis in children younger than five years of age. BMC Pediatrics 2020; 20:401.
4. Pham XBD, Sullins VF, Kim DY, et al. Factors predictive of complicated appendicitis. J Surg Res 2016; 206:62-6.
5. Gerall CD, DeFazio JR, Kahan AM, et al. Delayed presentation and sub-optimal outcomes of pediatric patients with acute appendicitis during the COVID-19 pandemic. J Pediatr Surg 2021; 56:905-10.
6. Troester AM, Utria AF, Goffredo P, Cho E, Zhou P, Hassan I. Pediatric Appendectomy: an Analysis of 22,334 Cases from the Pediatric National Surgical Quality Improvement Program Data. J Gastrointest Surg 2021; 25:523-5.
7. Ingram MC, Harris CJ, Studer A, et al. Distilling the key elements of pediatric appendicitis clinical practice guidelines. J Surg Res 2021; 258:105-12.
8. Almaramhy HH. Acute appendicitis in young children less than 5 years: review article. Ital J Pediatr 2017; 43:15.
9. Hodge SV, Mickiewicz B, Lau M, Jenne CN, Thompson GC. Novel molecular biomarkers and diagnosis of acute appendicitis in children. Biomark Med 2021; 15:1055-65.
10. Begic-Kapetanovic S, Avdagic N, Zaciragic A, Hasic S, Babic N, Hadzimuratovic A. Could the neutrophil-to-lymphocyte ratio serve as a marker in the diagnosis and prediction of acute appendicitis complications in children? Arch Med Sci 2021; 17:1672-8.
11. Lindestam U, Almström M, Jacks J, et al. Low plasma sodium concentration predicts perforated acute appendicitis in children: A prospective diagnostic accuracy study. Eur J Pediatr Surg 2020; 30:350-6.
12. Yang J, Liu C, He Y, Cai Z. Laboratory Markers in the Prediction of Acute Perforated Appendicitis in Children. Emerg Med Int 2019; 2019:4608053.
13. Nissen M, Tröbs RB. The lymphocyte-to-monocyte ratio may distinguish complicated from non-complicated pediatric appendicitis: A retrospective study and literature review. Pediatr Neonatol 2022; 63:146-53.
14. Feng W, Yang QY, Zhao XF, Li MM, Cui HL. The use of the ratio of C-reactive protein to albumin for the diagnosis of complicated appendicitis in children. Am J Emerg Med 2022;52:148-54.
15. Yuan Y, Liu J, Zhou Y, et al. The relationship between monocyte-to-lymphocyte ratio and the risk of gastrointestinal system involvement in children with IgA vasculitis: A preliminary report. Adv Clin Exp Med 2021; 30:999-1005.
16. Tezol O, Bozlu G, Sagcan F, Daloglu FT, Citak C. Value of neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio and red blood cell distribution width in distinguishing between reactive lymphadenopathy and lymphoma in children. Bratisl Lek Listy 2020; 121:287-92.
17. Duman L, Karaibrahimoğlu A, Büyükyavuz Bİ, Savaş MÇ. Diagnostic value of monocyte-to-lymphocyte ratio against other biomarkers in children with appendicitis. Pediatr Emerg Care 2022; 38:739-42.
18. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at sites of inflammation and infection. Front Immunol 2018; 13:754.
19. Sack U, Biereder B, Elouahidi T, Bauer K, Keller T, Tröbs RB. Diagnostic value of blood inflammatory markers for detection of acute appendicitis in children. BMC Surg 2006; 6:6:15.
20. Hou J, Feng W, Liu W et al. The use of the ratio of C-reactive protein to albumin for the diagnosis of complicated appendicitis in children. Am J Emerg Med 2022;52:148-54.
21. Park SJ, Shin JI. Inflammation and hyponatremia: an underrecognized condition? Korean J Pediatr 2013; 56:519-22.
22. Peri A, Thompson CJ, Verbalis JG. Disorders of Fluid and Electrolyte Metabolism. Focus on Hyponatremia. In: Mohottige D, Lehrich RW, Greenberg A (eds). Hypovolemic Hyponatremia Front Horm Res. Basel, Karger, 2019;93-103.
23. Alsaleh A, Pellino G, Christodoulides N, Malietzis G, Kontovounisios C. Hyponatremia could identify patients with intrabdominal sepsis and anastomotic leak after colorectal surgery: a systematic review of the literature. Updates Surg 2019; 71:17-20.
24. Anand S, Krishnan N, Birley JR, Tintor G, Bajpai M, Pogorelić Z. Hyponatremia-A New Diagnostic Marker for Complicated Acute Appendicitis in Children: A Systematic Review and Meta-Analysis. Children (Basel) 2022; 9:1070.

25. Besli GE, Çetin M, Durakbaşa ÇU, Özkanlı Ş. Predictive value of serum sodium level in determining complicated appendicitis risk in children. Haydarpasa Numune Med J 2019; 59:35-40.

26. Walsh A, Lala S, Wells C, Upadhyay V. Hyponatremia an indicator of complicated appendicitis in children: Starship experience. ANZ J Surg. ANZ J Surg 2022; 92:747-52.

27. Pogorelić Z, Lukšić AM, Mihanović J, Đikić D, Balta V. Hyperbilirubinemia as an Indicator of Perforated Acute Appendicitis in Pediatric Population: A Prospective Study. Surg Infect (Larchmt) 2021; 22:1064-71.

28. Bonadio W, Bruno S, Attaway D, Dharmar L, Tam D, Homel P. Lack of utility of measuring serum bilirubin concentration in distinguishing perforation status of pediatric appendicitis. Am J Emerg Med 2017; 35:885-8.

29. Mizrahi N, Samuk I, Ashkenazi S, Chodick G, Freud E, Livni G. Pediatric complex appendicitis are there predictors of postoperative outcomes. Arch Pediatr Surg 2019; 3:53-8.

30. Chen CL, Chao HC, Kong MS, Chen SY. Risks factors for prolonged hospitalization in pediatric appendicitis patients with medical treatment. Pediatr Neonatol 2017; 58:223-8.

31. Obayashi J, Ohyama K, Manabe S, et al. Are there reliable indicators predicting postoperative complications in acute appendicitis? Pediatr Surg Int 2015; 31:1189-93.

32. Obinwa O, Peirce C, Cassidy M, Fahey T, Flynn J. A model predicting perforation and complications in paediatric appendicectomy. Int J Colorectal Dis 2015; 30:559-65.

33. Miguel CD, Serrano AJM, Nunez V, et al. Neutrophil-to-lymphocyte ratio as a predictor of postsurgical intraabdominal abscess in children operated for acute appendicitis. Front Pediatr 2019; 7:424.

34. Serradilla J, Bueno A, De la Torre C, et al. Predictive factors of gangrenous post-appendectomy intra-abdominal abscess. A case-control study. Cir Pediatr 2018; 31:25-8.

35. Feng W, Du XB, Zhao XF, Li MM, Cui HL. Risk factors of postoperative adhesive bowel obstruction in children with complicated appendicitis. Pediatr Surg Int 2021; 37:745-54.

36. Benzon HA, Bobrowski A, Suresh S, Wasson NR, Cheon EC. Impact of preoperative hyponatraemia on paediatric perioperative mortality. Br J Anaesth 2019; 123:618-26.

37. Ulusoy E, Çitlenbik H, Akgül F, et al. Is ischemia-modified albumin a reliable marker in accurate diagnosis of appendicitis in children? World J Surg 2020; 44:1309-15.

38. Falchi D, Bakır AC, Risteski T, et al. Diagnostic value of the ischemia modified albumin and pentraxin 3 in pediatric appendicitis: A meta analysis. J Clin Trials Exp Investig 2022; 1:1-9.

39. Howell EC, Dubina ED, Lee SL. Perforation risk in pediatric appendicitis: assessment and management. Pediatric Health Med Ther 2018; 9:135-45.

40. Meltzer JA, Kunkov S, Chao JH, et al. Association of delay in appendectomy with perforation in children with appendicitis. Pediatr Emerg Care 2019; 35:45-9.

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Original Article

Evaluation of the Neurodevelopmental Status for Urea Cycle Disorders: Based on Clinical Experience

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ABSTRACT

Aim: Urea cycle disorders (UCD) still have poor neurological outcomes despite early diagnosis and treatment. We aimed to present the neurological outcomes of UCD patients and to determine the main simple and accessible factors affecting these outcomes.

Materials and Methods: This was a descriptive cross-sectional study conducted in two pediatric metabolism centers on 29 patients from 25 unrelated families who were diagnosed and followed with UCD based on clinical presentation, neurological parameters, biochemical measurements, and molecular analysis.

Results: Within the study population, the most common diagnosis was argininosuccinate synthase deficiency in 13 (44.82%) patients, followed by N-acetylglutamate synthase deficiency in five patients (17.24%), ornithine transcarbamylase deficiency in four patients (13.79%), arginase 1 deficiency in three patients (10.34%), carbamoyl phosphate synthase 1 deficiency in three patients (10.34%), and argininosuccinate lyase deficiency in one patient (3.44%). Peak ammonia levels were observed to be significantly higher in those patients with delayed milestones and patients who had Denver II <-2 standard deviation score results (p=0.032, p=0.026). Effect sizes were large in both groups. Delayed milestones were noted in 17 (94.4%) of the cases with peak ammonia >500 µmol/L (n=18). Those patients with abnormal neurological parameters had a significantly higher mean number of hyperammonemic episodes per year. Extracorporeal detoxification was given to eight patients, in combination with therapeutic hypothermia in two patients. Rapid regression was observed in brain edema in those who underwent therapeutic hypothermia.

Conclusion: Our study emphasizes the effect of peak ammonia levels and the frequency of hyperammonemic episodes on neurological outcomes. There were still poor neurocognitive outcomes despite extracorporeal detoxification. This highlights the need to reassess current treatment strategies, including the threshold for starting extracorporeal detoxification if ammonia levels exceed 500 µmol/L. The use of therapeutic hypothermia by experienced teams may be promising due to its brain edema-reducing effects.

Keywords: Urea cycle disorders, Hyperammonemia, Citrullinemia, Inborn errors of metabolism, therapeutic hypothermia

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Introduction

The urea cycle is the primary metabolic pathway for nitrogen disposal from the liver. Six enzymes [carbamoyl phosphate synthase 1 (CPS-1, EC 6.3.4.16), ornithine transcarbamylase (OTC, EC 2.1.3.3), argininosuccinate synthase (ASS, EC 6.3.4.5), argininosuccinate lyase (ASL, EC 4.3.2.1), arginase 1 (ARG1, EC 3.5.3.1), N-acetylglutamate synthase (NAGS, EC 2.3.1.1)] and two mitochondrial transporters (aspartate-glutamate carrier and ornithine-citrulline carrier) are coordinated in this pathway (1). OTC, CPS1 and NAGS are mitochondrial enzymes, ASS, ASL, and ARG1 are cytosolic enzymes. Urea cycle disorders (UCD) are inborn metabolism errors and estimated incidence ranges from 1/8,000 to 1/35,000 births (2). UCDs have a highly variably phenotypic spectrum ranging from hyperammonemic encephalopathy, acute liver failure, and spastic paraplegia to asymptomatic. The patients have an increased risk of poor neurological outcome (3). They are often affected by morbidity due to neurocognitive deterioration. Hyperammonemia and its effects on neurological outcome is a common clinical presentation. Disease severity variables such as the age of disease onset, the age of the first hyperammonemic episode (HE), the number of HEs per year, the peak ammonia level, and the plasma amino acid levels affect neurological outcomes (4). However, it is unclear whether the consequences of hyperammonemia alone or brain metabolic changes such as synaptic transmission and glutamine toxicity contribute. Although Zielonka et al. (5) showed a relationship between enzyme activity and neurological outcome in ASSD patients, the correlation of phenotypic severity with genotype or *in vitro* enzyme activity is unclear. In addition, enzyme analysis is not useful in daily practice.

Diagnosis is made by selective metabolic investigation in symptomatic patients, family screening of the index patient, newborn screening, and prenatal testing. The sensitivity of newborn screening in UCD is already low and it is not performed in our country. Guidelines for the diagnosis and management of UCDs have been proposed for the use of single therapeutic tools as well as their combinations (6). UCD guideline recommendations are currently used in our country. However, the UCD guideline is still lacking regarding the impact of adherence to these guidelines on neurological outcomes. Despite the development of extracorporeal detoxification treatments, diet therapy and new ammonia-scavengers, mortality and morbidity in early-onset patients are still poor. In the future, a better understanding of predictive markers is needed in order to provide more information on phenotypes in neurotoxic metabolites-related diseases.

To the best of our knowledge, there is no single, definitive way to predict the neurodevelopmental status of a patient with UCD. Increasing clinical awareness and neonatal screening programs and the development of new diagnostic and therapeutic proposals are expected to provide better outcomes. Based on our clinical experiences, we aimed to present the neurological outcomes of UCD patients from two metabolic centers and to determine key simple and accessible factors affecting neurological outcomes. We believe that simple predictive factors will help clinicians in practice.

Materials and Methods

Study Design and Data Acquisition

Individuals were included based on a retrospective collection of the data of the ASS deficiency (ASSD), NAGS deficiency (NAGSD), OTC deficiency (OTCD), ARG-1 deficiency (ARGD), CPS-1 deficiency (CPS1D) and ASL deficiency (ASLD) patients who had been diagnosed in two metabolism centers in Turkey. The inclusion criteria were rare biallelic variants in *ASS1*, *NAGS*, *OTC*, *ARG1*, *CPS1* and *ASL* genes classified as likely pathogenic or pathogenic according to the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology guidelines. All data were retrieved via standardized proformas agreed on by the participating centers.

The follow-up period was every month until 12 months, every 2 months until 6 years of age, and every 3 months thereafter. The clinical variabilities were categorized according to disease onset as early-onset (EO <28 days), late-onset (LO >28 days) and asymptomatic. For phenotyping, the following variables were analyzed within this study: the individual's genetic ancestry, sex, age at last visit, and clinical status. Data on the following clinical and laboratory characteristics were collected: peak ammonia (first clinical presentation), plasma amino acids, the number of HEs per year, the duration of the HEs, extracorporeal detoxification, kidney dysfunction, and hepatocellular dysfunction. We evaluated the neurological status under the headings of delayed milestones, fine and gross motor skills, tone abnormalities, bedridden status, and the Denver II and Wechsler Intelligence Scale for Children-Revised (WISC-R). Denver II was applied to evaluate children aged 0-6 years in terms of development. Patients with a Denver II test result of <-2SDS were considered abnormal, and those with >-2 standard deviation score (SDS) were considered to be within

normal neurological parameters. The validity and reliability of the test was performed by Eratay et al. (7) in Turkey. Patients between 6 and 16 years of age were evaluated using the WISC-R. This test includes verbal and performance subscales. WISC-R was adapted into Turkish, and it has been used widely for years (8). In addition, detailed data on neurological disease, laboratory values, and the clinical features involved were evaluated and recorded according to Human Phenotype Ontology terminology. An asymptomatic female OTCD carrier was also included in this study.

Therapy monitoring was undertaken during follow-up visits which included the evaluation of clinical parameters, laboratory parameters, and dietary consumption records. The "last three-days dietary consumption" records were requested from the patients/parents. Protein, amino acids, energy, and medication intakes were calculated.

Molecular Analyses

All exons and exon-intron junctions of the genes were evaluated by next-generation sequencing methods. Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Standardized PCR pools were prepared using a NexteraXT sample preparation kit for next-generation sequencing analysis with the Miseq device (Illumina, Inc.).

Sanger sequencing of the genomic variants identified by exome sequencing or targeted gene sequencing was performed for all patients and their families. Sanger sequencing was used to validate the pathogenic variants within families on the 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA). The sequencing results were analyzed using CLC genomic workbench software. For the clinical interpretation of variants, allele frequency data were obtained from various databases, including gnomAD (http://gnomad.broadinstitute.org/) and ExAc (http://exac.broadinstitute.org/). The pathogenicity of variants was assessed using in silico prediction tools, such as PolyPhen-2 (http://genetics.bwh. harvard.edu/pph2), SIFT (http://sift.jcvi.org), MutationTaster (http://www. mutationtaster.org) and the Human Splicing Foundation (http://www.umd.be/hsf/). Variants were classified according to ACMG. All pathogenic variants are described according to the accepted HGVS nomenclature. Nucleotide numbers were derived from complementary DNA sequences.

Statistical Analysis

Statistical analyses of the data were performed using the SPSS software package for Windows software package (ver.18.0; SPSS Inc., Chicago, IL, USA). As descriptive statistics, numbers, and percentages for categorical variables, mean±SD or median ([minimum (min.)-maximum (max.)] were used for numerical variables. The distribution of data was evaluated using the Shapiro-Wilk test. For numerical comparisons, the Student's t-test or Mann-Whitney U test were used to assess differences between two groups according to the normal distribution of the measured parameters. A significance level of $p<0.05$ was set to indicate statistical significance. The effect size [Cohen d ($0.20\geq d<0.50$: small effect, $0.50\geq d<0.80$: medium effect, $d\geq 0.80$: large effect and Rank-Biserial correlation coefficient (≥0.50: large effect)] was calculated for statistically significant results.

This study was performed in accordance with the declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Diyarbakır Gaziyaşargil Training and Research Hospital (approval no: 928, date: 05.11.2021).

Results

Demographic and Clinical Findings

A total of 29 UCD patients (17/12, M/F) from 25 different families from two metabolic centers in the southeastern region of Turkey were included in this study. 86.2% of the individuals in our cohort were born to consanguineous parents. Twenty-six (89.65%) patients were diagnosed by selective metabolic investigation after the onset of their symptoms, whereas three (10.34%) were diagnosed by family screening. Within the study population, the most common diagnosis was ASSD in 13 (44.82%) patients, followed by NAGSD in five patients (17.24%), OTCD in four patients (13.79%), ARG1D in three patients (10.34%), CPS1D in three patients (10.34%), and ASLD in one patient (3.44%).

Sixteen (55.17%) patients were EO, 12 (41.37%) patients were LO and one (3.44%) patient was asymptomatic (Female OTC). The mean age at initial symptom was 9.8±9.68 days (median: 4, min.: 1, max.: 28) in the EO group, 21.27±27.07 months (median: 8, min.: 0.3, max.: 85) in the LO group. The mean age at diagnosis was 11.6±11.72 days (median: 4, min.: 2, max.: 38) in EO patients, and 64.97±65.21 months (median: 38.75, min.: 3, max.: 219) in the LO group. The mean age of the patients at last visit was 6.64±8.2 years (median: 4, min.: 0.3, max.: 43 years). The patients' detailed clinical and molecular characteristics are shown in Table I.

The most common initial symptoms were encephalopathy, tachypnea, and seizures in the EO group and delayed milestones, feeding difficulties, and

Table I. Demographic, clinical and molecular characteristics of UCD patients

Family	Patient	Gender	Con.	Diagnosis	Disease onset	CVVHDF	Latest neurodevelopmental status	Gene	Transcript	DNA change	Protein change	Zygosity	Novel	Pathogenity
F1	P1	M	Yes	ASSD	EO	No	Normal	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F2	P2	F	Yes	ASSD	LO	No	Normal	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F3	P3	M	Yes	ASSD	LO	No	Mild ID	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F4	P4	M	Yes	ASSD	LO	No	Normal	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F5	P5	M	Yes	ASSD	LO	No	Profunda ID, GDD	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F6	P6	M	Yes	ASSD	EO	CVVHDF	Normal	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F6	P7	M	Yes	ASSD	EO	No	Normal	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F7	P8	F	Yes	ASSD	LO	No	Mild ID	*ASS1*	NM_054012.4	c.1085G>T	p.Gly362Val	Homozygous		Pathogenic
F8	P9	F	Yes	ASSD	EO	CVVHDF	GDD	*ASS1*	NM_054012.4	c.1168G>A	p.Gly380Arg	Homozygous		Pathogenic
F9	P10	F	Yes	ASSD	EO	CVVHDF	GDD	*ASS1*	NM_054012.4	c.1168G>A	p.Gly380Arg	Homozygous		Pathogenic
F10	P11	F	Yes	ASSD	EO	CVVHDF	Profunda ID, GDD	*ASS1*	NM_054012.4	c.1087C>T	p.Arg363Trp	Homozygous		Pathogenic
F11	P12	F	Yes	ASSD	EO	No	Profunda ID, GDD	*ASS1*	NM_054012.4	c.1087C>T	p.Arg363Trp	Homozygous		Pathogenic
F12	P13	M	No	ASSD	EO	No	Normal	*ASS1*	NM_054012.4	c.814C>T, c.970+5G>A	p.Arg272Cys, -	Compound heterozygous		Pathogenic/ Pathogenic
F13	P14	F	Yes	NAGSD	EO	No	Normal	*NAGS*	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F14	P15	F	Yes	NAGSD	EO	No	Profunda ID, has ability to walk	*NAGS*	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F15	P16	F	Yes	NAGSD	EO	No	Profunda ID, has ability to walk	*NAGS*	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F16	P17	M	Yes	NAGSD	EO	No	Mild ID	*NAGS*	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F17	P18	M	Yes	NAGSD	EO	CVVHDF	ASD	*NAGS*	NM_153006.3	c.1450T>C	p.Trp484Arg	Homozygous		Pathogenic
F18	P19	M	Yes	OTCD	LO	No	Profunda ID, GDD	*OTC*	NM_000531.6	c.172T>C	p.Trp58Arg	Hemizygous	Novel	Likely Pathogenic
F18	P20	M	Yes	OTCD	LO	No	Normal	*OTC*	NM_000531.6	c.172T>C	p.Trp58Arg	Hemizygous	Novel	Likely Pathogenic
F18	P21	F	No	OTCD	LO	No	Asymptomatic, normal	*OTC*	NM_000531.6	c.172T>C	p.Trp58Arg	Hemizygous	Novel	Likely Pathogenic
F19	P22	M	No	OTCD	LO	No	Normal	*OTC*	NM_000531.6	c.774T>A	p.Asn258Lys	Hemizygous		Likely Pathogenic
F20	P23	M	Yes	ARGD	LO	No	Profunda ID, loss of ability to walk	*ARG1*	NM_000045.4	c.130+1G>A		Homozygous		Pathogenic
F20	P24	F	Yes	ARGD	LO	No	ASD, loss of ability to walk	*ARG1*	NM_000045.4	c.130+1G>A		Homozygous		Pathogenic

Table I. Continued

Family	Patient	Gender	Con.	Diagnosis	Disease onset	CVVHDF	Latest Neurodevelopmental Status	Gene	Transcript	DNA change	Protein change	Zygosity	Novel	Pathogenity
F21	P25	F	Yes	ARGD	LO	No	Mild ID, loss of ability to walk	ARG1	NM_000045.4	c.703G>C	p.Gly235Arg	Homozygous		Pathogenic
F22	P26	M	Yes	CPS1D	EO	CVVHDF	Severe ID, GDD	CPS1	NM_001875.4	c.3037_3039del	p.Val1013del	Homozygous		Pathogenic
F23	P27	M	Yes	CPS1D	EO	No	Delayed milestones	CPS1	NM_001875.4	c.3049C>G	p.Pro1017Ala	Homozygous		Likely Pathogenic
F24	P28	M	No	CPS1D	LO	CVVHDF	Delayed milestones	CPS1	NM_001875.4	c.3002G>T, c.3402G>C	p.Arg1001Leu p.Leu1134Phe	Compound heterozygous	Novel/ Novel	VUS/Likely Pathogenic
F25	P29	M	Yes	ASLD	EO	CVVHDF	Mild ID, DD	ASL	NM_000048.4	c.918+5G>A		Homozygous		Pathogenic

M: Male, F: Female, Con: Consanguinity, UCD: Urea cycle disorder, CVVHDF: Continuous venovenous hemodiafiltration, LO: Late-onset, EO: Early-onset, ASLD: Argininosuccinate lyase deficiency, CPS1D: Carbamoyl phosphate synthase 1 deficiency, ARGD: Arginase 1 deficiency, OTCD: Ornithine transcarbamylase deficiency, NAGSD: N-acetylglutamate synthase deficiency, ASSD: Argininosuccinate synthase deficiency

gait abnormalities in the LO group. In ASS1D, the most common clinical presentation was hyperammonemic encephalopathy in eight patients with EO and delayed milestones in five patients with LO. All NAGSD patients presented with encephalopathy in the neonatal period. All OTCD patients, except the asymptomatic female OTCD patient, presented with varying degrees of intellectual disability (ID). Two ARG1D patients presented with gait abnormalities; one patient presented with autism spectrum disorder, and gait abnormalities developed in the follow-up. Three CPS1D patients, one of whom was LO (P28), presented with encephalopathy and died during a HE due to ventricular arrhythmia. When the neurological parameters of the patients were evaluated according to their disease onset (EO vs. Late-onset), no significant correlation was found. No significant difference was found between the neurocognitive outcomes of cytosolic UCDs and mitochondrial UCDs. There was no significant difference between the peak ammonia levels and the mean number of HEs per year between these two groups.

Laboratory Findings

The mean peak ammonia level of the EO patients was 1,217.19±595.94 µmol/L (range: 378-2,300), and for the LO patients, it was 419.77±257.19 µmol/L (range: 80-875). Peak ammonia levels were significantly higher in the EO patients ($p<0.001$). According to the disease groups, the highest peak ammonia levels were seen in NAGSD, CPS1D and ASS1D patients, respectively. There was no significant difference between the mean peak ammonia levels of the cytosolic and mitochondrial UCD group ($p=0.89$). Peak ammonia levels were observed to be significantly higher in those patients with delayed milestones and those patients who had Denver II <-2SDS results ($p=0.032$, $p=0.026$). Effect sizes were large in both groups [Cohen d: -1.04 (confidence interval (CI): -2.10 - 0.09), -1.03 (CI: -2.05 - 0.05); respectively (CI=95th percentile CI)]. Delayed milestones were noted in 17 (94.4%) of those cases with peak ammonia >500 µmol/L (n=18). The comparison of neurological status parameters with clinical and laboratory features is presented in Table II.

Seventy-one HEs were recorded. The mean age at the time of HE was 10.4±7.8 months (two days-16 years). The mean HE per year was significantly higher in those cases with abnormal neurological parameters ($p=0.019$). Patients with delayed milestones, abnormal gross motor skills, tone abnormalities, and bedridden status had a significantly higher mean number of HEs per year. Also, the effect sizes were large for the abnormal gross motor skills, tone abnormalities and bedridden groups (Rank biserial

correlation coefficients: 0.58, 0.57 and 0.97, respectively). There were abnormal gross motor skills in 11 (84.61%) patients with mean number of HEs per year ≥1 and bedridden status in 4 (57.14%) patients with a mean number of HEs per year ≥2.

The mean peak plasma glutamine level was 1,293±492.4 µmol/L (N: 123-809, range: 280-2,045). There was no significant relationship between plasma glutamine levels and neurological parameters, peak ammonia, the mean number of HEs per year, or continuous venovenous hemodiafiltration (CVVHDF). The mean initial plasma citrulline level was 875.2±799.6 µmol/L (N: 9-52, range: 224-2,700) in ASSD patients. Peak citrulline levels were significantly higher in ASSD patients who had abnormal neurological parameters ($p<0.031$). Although we could not find a significant relationship between the citrulline subgroups and delayed milestones in ASSD patients, delayed milestones occurred in all subjects with a citrulline levels over 500 µmol/L. Abnormal fine and gross motor skills and tone abnormalities were observed in all cases with a citrulline level over 1.000 µmol/L.

Treatment

In the emergency management, according to the diagnosis groups and ammonia levels, protein intake was stopped, and an appropriate dose of intravenous (IV) glucose support to prevent catabolism, IV sodium benzoate,

Table II. Comparison of neurological status parameters with disease characteristics

		Delayed Milestones		Fine Motor Skills		Gross Motor Skills		Tone Abnormalities	
		No (n=6)	**Yes (n=23)**	**Normal (n=7)**	**Abnormal (n=22)**	**Normal (n=16)**	**Abnormal (n=13)**	**No (n=15)**	**Yes (n=14)**
Disease Onset	Early Onset	3 (50%)	13 (56.5%)	3 (42.9%)	10 (45.5%)	9 (56.3%)	7 (53.8)	7 (46.7%)	6 (42.9%)
	Late Onset	3 (50%)	10 (43.5%)	4 (57.1%)	12 (54.5%)	7 (43.8%)	6 (46.2)	8 (53.3%)	8 (57.1%)
Disease Group	Cytosolic UCDs (n=17)	3 (50%)	14 (60.9%)	4 (57.1%)	13 (59.1%)	9 (56.3%)	8 (61.5)	6 (40%)	6 (42.9%)
	Mitochondrial UCDs (n=12)	3 (50%)	9 (39.1%)	3 (42.9%)	9 (40.9%)	7 (43.8%)	5 (38.5)	9 (60%)	8 (57.1%)
Peak Ammonia (µmol/L)		384.67±318.53	983.65±620.38*	517.86±374.05	968.5±646	718±423.39	1034.15±778.67	707.87±436.24	1022.43±749.41
Peak Ammonia Levels (µmol/L)	50-200	2 (33.3%)	2 (8.7%)	1 (14.3%)	3 (13.6%)	2 (12.5%)	2 (15.4)	2 (13.3%)	2 (14.3%)
	201-500	3 (50%)	4 (17.4%)	4 (57.1%)	3 (13.6%)	4 (25%)	3 (23.1%)	4 (26.7%)	3 (21.4%)
	501-1000	1 (16.7%)	9 (39.1%)*	1 (14.3%)	9 (40.9%)	8 (50%)	2 (15.4%)	7 (46.7%)	3 (21.4%)
	>1001	0	8 (34.8%)*	1 (14.3%)	7 (31.8%)	2 (12.5%)	6 (46.2%)	2 (13.3%)	6 (42.9%)
Mean Number of HEs per Year		0.52±0.52	1.45±1.1 *	0.34±0.18	1.55±1.08	0.75±0.58	1.91±1.23*	0.73±0.6	1.84±1.21*
Elevated Glutamine (µmol/L)	809-1000	2 (33.3%)	10 (43.5%)	2 (28.6%)	10 (45.5)	6 (37.5%)	6 (46.2%)	5 (33.3%)	7 (50%)
	1001-2000	3 (50%)	13 (56.5%)	4 (57.1%)	12 (54.5)	9 (56.3%)	7 (53.8%)	9 (60%)	7 (50%)
	>2000	1 (16.7%)	0	1 (14.3%)	0	1 (6.3%)	0	1 (6.7%)	0
Elevated Citrulline (µmol/L)	52-500	3 (100%)	5 (45.5%)	3 (75%)	5 (50%)	7 (77.8%)	1 (20%)	7 (77.8%)	1 (20%)
	500-1000	0	2 (18.2%)	1 (25%)	1 (10%)	2 (22.2%)	0 (0%)	2 (22.2%)	0
	1001-2000	0	3 (27.3%)	0 (0%)	3 (30%)	0	3 (60%)*	0	3 (60%)*
	>2001	0	1 (9.1%)	0 (0%)	1 (10%)	0	1 (20%)*	0	1 (20%)*
CVVHDF	No	5 (83.3%)	16 (69.9%)	7 (100%)	14 (63.6%)	13 (81.3%)	8 (61.5%)	12 (80%)	9 (64.3%)
	Yes	1 (16.7%)	7 (30.4%)	0	8 (36.4%)	3 (18.8%)	5 (38.5%)	3 (20%)	5 (35.7%)
Length of Stay CVVHDF	0-12 hours	1 (100%)	2 (28.6%)	2 (100%)	1 (20%)	2 (66.7%)	1 (20%)	2 (66.7%)	1 (20%)
	12-24 hours	0	3 (42.9%)	0	2 (40%)	1 (33.3%)	2 (40%)	1 (33.3%)	2 (40%)
	>24 hours	0	2 (28.6%)	0	2 (40%)	0	2 (40%)	0	2 (40%)
Diet Therapy	Protein controlled diet	3 (60%)	5 (21.7%)	2 (33.3%)	6 (27.3%)	5 (33.3%)	3 (23.1%)	4 (28.6%)	4 (28.6%)
	Calculated diet	2 (40%)	18 (78.3%)	4 (66.7%)	16 (72.7%)	10 (66.7%)	10 (76.9%)	10 (71.4%)	10 (71.4%)

Table II. Continued

		Bedridden		Denver II		WISC-R	
		No (n=21)	**Yes (n=7)**	**> -2 SDS (n=8)**	**< -2 SDS (n=11)**	**Normal (n=8)**	**Abnormal (n=11)**
Disease Onset	Early Onset	11 (52.4%)	5 (71.4%)	5 (62.5%)	8 (72.7%)	5 (62.5%)	8 (72.7%)
	Late Onset	10 (47.6%)	2 (28.6%)	3 (37.5%)	3 (27.3%)	3 (37.5%)	3 (27.3%)
Disease Group	Cytosolic UCDs (n=17)	12 (57.1%)	5 (71.4%)	3 (37.5%)	7 (63.7%)	3 (37.5%)	7 (63.7%)
	Mitochondrial UCDs (n=12)	9 (42.9%)	2 (28.6%)	5 (62.5%)	4 (36.4%)	5 (62.5%)	4 (36.4%)
Peak Ammonia (µmol/L)		712±465.57	1372.86±796.4		655.88±318.172	1291.73±760.88*	655.88±318.172
Peak Ammonia Levels (µmol/L)	50-200	4 (19%)	0	1 (12.5%)	2 (18.2%)	1 (12.5%)	2 (18.2%)
	201-500	5 (23.8%)	1 (14.3%)	2 (25%)	0	2 (25%)	0
	501-1000	8 (38.1%)	2 (28.6%)	4 (50%)	2 (18.2%)*	4 (50%)	2 (18.2%)*
	>1001	4 (19%)	4 (57.1%)	1 (12.5%)	7 (63.6%)*	1 (12.5%)	7 (63.6%)*
Mean Number of HEs per Year		0.8±0.59	2.85±0.93*		0.738±0.625	1.691±1.288	0.738±0.625
Elevated Glutamine (µmol/L)	809-1000	11 (52.4%)	1 (14.3%)	3 (37.5%)	7 (63.6%)	3 (37.5%)	7 (63.6%)
	1001-2000	9 (42.9%)	6 (85.7%)	4 (62.5%)	4 (36.4%)	4 (62.5%)	4 (36.4%)
	>2000	1 (4.8%)	0	1 (0.12%)	0	1 (0.12%)	0
Elevated Citrulline (µmol/L)	52-500	7 (77.8%)	1 (20%)	3 (100)	3 (33.3%)	3 (100)	3 (33.3%)
	500-1000	2 (22.2%)	0	0	2 (22.2%)	0	2 (22.2%)
	1001-2000	0	3 (60%)*	0	3 (33.3%)	0	3 (33.3%)
	>2001	0	1 (20%)*	0	1 (11.1%)	0	1 (11.1%)
CVVHDF	No	18 (85.7%)	3 (42.9%)*	7 (87.5%)	5 (45.5%)	7 (87.5%)	5 (45.5%)
	Yes	3 (14.3%)	4 (57.1%)	1 (12.5%)	6 (54.5%)	1 (12.5%)	6 (54.5%)
Length of Stay CVVHDF	0-12 hours	2 (66.7%)	1 (25%)	0	2 (33.3%)	0	2 (33.3%)
	12-24 hours	1 (33.3%)	2 (50%)	0	3 (50%)	0	3 (50%)
	>24 hours	0	1 (25%)	1 (100%)	1 (16.7%)	1 (100%)	1 (16.7%)
Diet Therapy	Protein controlled diet	7 (35%)	1 (14.3%)	2 (25%)	3 (27.3%)	2 (25%)	3 (27.3%)
	Calculated diet	13 (65%)	6 (85.7%)	6 (75%)	8 (72.7%)	6 (75%)	8 (72.7%)

*p<0.05, HE: Hyperammonenic episodes, CVVHDF: Continuous venovenose hemodiafiltration, UCD: Urea cycle disorders, WISC-R: Wechsler Intelligence Scale for Children-Revised, SDS: Standard deviation score

IV L-arginine, carbamyl-glutamate, and extracorporeal detoxification treatments were applied. Drug dosages were adjusted in accordance with the current guidelines. In maintenance treatment, a combination of a low protein diet containing essential amino acid supplements, citrulline and/or arginine supplementation, and ammonia scavengers (sodium benzoate, sodium phenylbutyrate, carbamylglutamate) were recommended. The three-day dietary consumption records of the patients were arranged according to the patients' statements, and their natural and synthetic protein intakes were calculated. In accordance with the recommendations, dietary therapy was applied to all patients except for the female OTCD carrier. The calculated diet (amino acids restricted formula and natural protein restricted diet) was applied to 20 patients (71.42%), and a protein-controlled diet (natural protein intake restricted diet without amino acid restricted formula) was applied to eight patients (28.57%). The compliance rate with the diet, evaluated according to the dietary consumption records, was 96.42%. Thirteen (81.25%) of the EO patients and seven (58.33%) of the LO patients received a calculated diet. The EO patients on the calculated diet had lower natural protein

and higher caloric intake than the LO patients. There was no significant relationship between the diet groups and the neurological parameters (p=0.648).

Maintenance metabolic pharmacotherapy was given to 28 patients and a total of five different drugs were used. 71.42% of the patients were given sodium benzoate and 64.28% were given sodium phenylbutyrate (in combination with sodium benzoate), 71.42% were treated with L-arginine, 21.42% with L-citrulline, and 25% with carbamylglutamate. There was no difference in neurocognitive outcomes among ammonia scavengers (p>0.32). Intestinal bacterial decontamination with metronidazole or colistin was not used. Carbamylglutamate was used for all NAGSD and CPS1D patients (off-license approval). At the time of the first HE, P26 needed CVVHDF three times, and their ammonia levels could be controlled after carbamylglutamate. We switched to carbamylglutamate because of metabolic acidosis associated with oral sodium benzoate for P28 and their ammonia level was controlled.

Extracorporeal detoxification was initiated in neonates and children with ammonia levels >500 µmol/L. Eight patients received CVVHDF in the initial period. Eight patients (27.58%) with ammonia levels >500 µmol/L during their first metabolic crisis received dialysis, and seven of them had encephalopathy before dialysis was initiated. The mean duration of CVVHDF was 11.2±17.4 hours (min.: 4 h, max.: 72 h). The mean pre-dialysis ammonia was 1,410.5±648.42 µmol/L (min.: 640, max.: 2,045). P26 required intermittent hemodialysis to keep their ammonia levels <200 µmol/L. Intermittent hemodialysis was applied for 7 days due to rebound hyperammonemia. After carbamylglutamate, hyperammonemia was under-control. No significant relationship was found between the length of stay in dialysis and pre-dialysis ammonia levels (p=0.98). No significant difference was found between the mean duration of dialysis and the disease groups [mitochondrial UCDs (n=3, the mean duration CVVHDF: 74.66±44.04 hour) and cytosolic UCDs (n=5, the mean duration of CVVHDF: 14.0±5.65 hour)] (p=0.139).

ASSD patients P9 and P10 received CVVHDF combination with therapeutic hypothermia (TH) for their first HE. P9 presented with encephalopathy and seizure. There were cerebral edema signs on transfontanelle ultrasound. The initial ammonia level was 2,430 µmol/L. Since the ammonia (pre-dialysis ammonia: 2,180 µmol/L) level did not decrease adequately with pharmacotherapy, CVVHDF and TH was started. At the 4th hour of CVHDF, ammonia decreased to 740. When ammonia was 380 µmol/L, seizure stopped. P10 presented with encephalopathy and their initial ammonia was 2,045 µmol/L. CVVHDF and TH were initiated in the second hour of pharmacotherapy because there were cerebral edema signs on transfontanelle ultrasound and pre-dialysis ammonia was 1,800 µmol/L. Ammonia decreased to 230 µmol/L at the 12th hour of CVVHDF and TH. CVVHDF was stopped at the 12th hour and TH was stopped at the 24th hour. Significant regression of cerebral edema was observed 4 hours after onset. There was no significant relationship between those who underwent TH during dialysis and those who did not in terms of neurological parameters (p=0.51).

Molecular Analyses

Five different variants were detected in the *ASS1* gene, p.Gly362Val (61.5%), p.Gly390Arg (15.3%), p.Arg363Trp (15.3%), p.Arg272Cys (3.8%) and c.970+5G>A (3.8%). Eight patients from seven different families had the p.Gly362Val variant. Three (37.5%) patients with the p.Gly362Val homozygous mutation were EO and five (62.5%) patients were LO. All patients with the p.Gly362Val variant had peak ammonia <1.000 µmol/L and initial citrulline <1.000 µmol/L. One of the three EO patients underwent extracorporeal detoxification for hyperammonemic encephalopathy. All EO patients with p.Gly362Val had normal neurodevelopmental status, however two LO patients had mild ID and one LO patient had profunda ID. In our cohort, P8 and P9 with the p.Gly390Arg variant had EO and peak ammonia >1.000 µmol/L, HEs per year of 4.5 and 3.1, initial peak citrulline levels of 2,700 µmol/L and 1,804 µmol/L, and dialysis durations of 22 and 18 hours. The patients had global developmental delay and bedridden status despite treatment.

The p.Trp484Arg variant was detected in all patients from five different families in the *NAGS* gene. All these patients were the EO phenotype. There was no significant relationship between their peak ammonia levels, dialysis, and neurological parameters. Two variants were detected in the *OTC* gene, the p.Trp58Arg (previously not reported) and p.Asn258Lys from two different families. Two siblings (P19, P20) and their asymptomatic mother (P21) had the p.Trp58Arg variant. In the *CPS1* gene, four different variants (p.Val1013del, p.Gly893Gly, p.Arg1001Leu, and p.Leu1134Phe), two of which were novel, from three different families were detected. While two patients were the EO phenotype, one patient was LO. Two different variants, c.130+1G>A and p.Gly235Arg, were detected in the *ARG1* gene. The c.918+5G>A variant was detected in the *ASL* gene.

Discussion

UCDs still have poor neurological condition despite early diagnosis and treatment (3). The main aim of this study was to address the poor neurodevelopmental outcomes in UCD patients and to investigate contributing factors. The estimated incidence of UCDs ranges from 1/8,000 to 1/35,000 births (2). Although the incidence of UCD in our country is not known exactly, the prevalence was found to be 1:839 in a Turkish pilot study evaluating the general frequency of metabolic disorders (9). Although OTC deficiency is the most common UCD in the world, ASSD is more common in our country (10,11). Consistent with the literature, ASSD (44.82%) was observed most frequently in our cohort.

Patients present with a wide spectrum of clinical severity and findings. The most severe and critical presentation is hyperammonemic encephalopathy resulting in death or severe neurological impairment. EO patients mainly present as hyperammonemic encephalopathy, while LO patients show variable manifestations (3,10,12). In our cohort, 55.17% of the patients were EO and 41.37% were LO. Consistent with the literature, the EO patients presented most frequently with hyperammonemic encephalopathy, and the LO patients with different neurological symptoms in our cohort (1,3). Early diagnosis and treatment are recommended to improve neurodevelopmental outcomes (6). However, despite increasing newborn screenings, improved treatment methods and hemodialysis technology, poor neurological outcomes continue in UCD patients (13). For most EO patients, a positive screening result is not available before the first symptom onset. While neonatal screening for UCD is available in a few regions in Europe, there is still uncertainty about the benefits of NBS for individuals with ASSD and ASLD included in newborn screening programs in the United States (14). Posset et al. (15) reported that the mean ages of initial symptoms of the NBS group and EO group were similar and even earlier in the EO group (3). There is no newborn screening for UCD in our country. Patients were diagnosed during their symptomatic period and by family screening. The mean age at initial symptom was 9.8 days and the mean age at diagnosis was 11.6 days in the EO group. This showed us that the clinical findings in EO patients started early. When the neurological parameters were evaluated according to disease onset (EO/LO), no significant difference was found in our study. There was no significant difference between the peak ammonia levels and the mean number of HEs per year in the EO and LO groups. It remains unclear whether NBS has a beneficial effect on neurocognitive outcomes, as IQ data have only been reported for a small number of patients in the NBS group in the literature. In some studies, it has been reported that ASSD and ASLD patients diagnosed with NBS have better cognitive outcomes (15). LO UCD carries a significant risk of poor neurologic outcome if not recognized and treated early (16). Due to exposure to neurotoxic metabolites, a long follow-up period is required to determine whether NBS has a lifelong positive effect on neurocognitive outcomes.

Waisbren et al. (3) reported that cytosolic UCDs had poorer neurocognitive outcomes than mitochondrial UCDs. Similarly, Nettesheim et al. (17) and Burgard et al. (18) reported worse neurocognitive outcomes in symptomatic individuals with cytosolic UCDs compared to mitochondrial UCDs, although there were fewer episodes of hyperammonemia, particularly in ASSD and ASLD patients. CPS1D and OTCD tend to have the highest risk of acute neurological damage due to HEs (16,19-21). In our study, no significant difference was found between cytosolic and mitochondrial UCDs in terms of their neurological outcomes.

In previous studies, duration of coma, peak ammonia levels and increased intracranial pressure were accepted as poor prognostic factors (19,22,23). Metabolic elimination of ammonia occurs by conversion to urea in the liver and glutamine by brain and skeletal muscle resulting in swelling of the astrocytes and cytotoxic cerebral edema. Involvement of N-methyl-D-aspartate receptors and neuronal degeneration are seen with hyperammonemia. According to research, mechanisms other than ammonia, such as impaired NO metabolism resulting in oxidative stress in the brain, are thought to contribute to neurological deterioration (3,15). Acute hyperammonemia without cerebral edema is the main pathophysiology which triggers neuronal disinhibition and seizures due to the Na+ -K+-2Cl- cotransporter isoform1 in the neuron. However, the brain damage mechanisms in UCDs are still not fully elucidated. Peak ammonia levels have been associated with poor neurocognitive outcomes in most studies (3,13,15,19,23). In the study of Uchino et al. (23) with 216 UCD patients, the 5-year survival rate was reported as 22% for EO UCD and 41% for LO UCDs. A significant relationship was found between the peak ammonia level during the first HE and neurocognitive outcomes. When peak ammonia levels are <306 µg/dL, there is no serious neurological deficit. However, when this value is over 596 µg/dL, patients either die or have severe neurological defects (23). Bachman reported that none of their patients had normal neurological outcomes when their peak ammonia levels were ≥817 µg/dL (19).

In the Posset et al. (15) study, the baseline ammonia level was associated with impaired neurocognitive outcome in those with mitochondrial UCDs. Waisbren et al. (3) reported a significant relationship between exposure to disease-specific neurotoxic biomarkers such as ammonia, citrulline, and neurocognitive outcomes. Also, there is a relationship between the mean number of HEs per year and poor neurological outcomes (3). Inconsistent with the literature, Msall et al. (20) reported no significant relationship between peak ammonia levels and IQ in 26 patients with neonatal UCDs. However, a significant negative linear correlation was found between stage III or IV hyperammonemia coma duration and 1-year-old IQ levels (20). Consistent with the literature, a significant relationship was found between abnormal neurological parameters and the peak ammonia level and the mean number of HEs per year in our study. The effect sizes of the groups were large. All these studies emphasize the importance of the timely diagnosis and rapid reduction of ammonia levels.

Enzyme activity in ASSD patients correlates with peak ammonia and initial citrulline levels (5). Peak citrulline levels were significantly higher in ASSD patients who had abnormal neurological parameters in our study group. Although we could not find a significant relationship between the citrulline subgroups and delayed milestones in ASSD patients, delayed milestones occurred in all subjects with a citrulline level over 500 µmol/L. Abnormal motor skills and tone abnormalities were observed in all cases with a citrulline level over 1.000 µmol/L. We think that the reason for not detecting a statistically significant difference is related to the small sizes of the subgroups. Consistent with the literature, we observed a relationship between high citrulline levels and poor neurological outcomes in our study. However, there were patients in our study group who had high citrulline levels but were mentally normal. Plasma citrulline levels can only partially help predict prognosis and neurological outcome, and so it is difficult to give an accurate prognosis. We could not study enzyme levels due to technical limitations. Plasma glutamine is widely used as a biomarker of control in UCD patients (24,25). In addition, plasma glutamine concentrations can be viewed as a predictor of the organism's total nitrogen load. Although diurnal variability is less, it varies with nutritional status and is highest after fasting (26). Levels exceeding 900-1,000 µmol/L are thought to be associated with HEs. Lee et al. (25) reported that glutamine was a weaker predictor than ammonia for HE and had a predictive value when evaluated with concomitant ammonia. However, in our cohort, there was no significant relationship between peak plasma glutamine levels and peak ammonia, the mean number of HEs per year or neurological parameters. Plasma glutamine levels do not accurately reflect brain levels.

A study in OTCD patients showed that IQ is a valid marker for defining neurocognitive outcomes (27). We used the WISC-R test to determine the neurocognitive status in our patients older than 6 years of age. We evaluated them according to their total IQ scores. However, we could not detect a significant correlation between any clinical or laboratory parameter and their WISC-R scores.

ASS1 deficiency is a urea cycle disorder with an estimated incidence of one in 44,300-250,000 based on the literature (28). ASS1D shows heterogeneous clinical manifestations, such as the EO form with HE, seizures, coma and cerebral edema, the LO form with varying degrees of neurological impairment, and the asymptomatic form. To date, more than a hundred variants in the *ASS1* gene located on chromosome 9q43.11 (#215700) have been reported. In our cohort, five different variants were detected in the *ASS1* gene, p.Gly362Val, p.Gly390Arg, p.Arg363Trp, p.Arg272Cys and c.970+5G>A. The second most common p.Gly362Val variant in Turkey was observed most frequently in our study group. This variant has previously been associated with highly conserved residual enzyme activity and mild or asymptomatic citrullinemia (29,30). In our cohort, five patients had normal neurodevelopmental status and two patients had mild ID. Inconsistent with the literature, P5 with LO had profunda ID and was bedridden. The mean number of HEs of P5 was 3.2 per year and peak ammonia levels were similar to mentally normal ASSD patients. P7 with EO and the p.Gly362Val variant presented with hyperammonemic encephalopathy and underwent CVVHDF and had normal WISC-R scores at the age of eight. The most common variant in the world and in our country is p.Gly390Arg (27). It has been associated with severe clinical form in previous studies. However, Daou et al. (29) reported different clinical presentations with this variant. In our cohort, both patients with this variant had EO, peak ammonia >1.000 µmol/L, mean numbers of HEs per year of 4.5 and 3.1, initial citrulline levels of over 1.000 µmol/L, and dialysis durations of 22 and 18 hours. Consistent with the literature, both patients had poor neurodevelopmental outcomes (Denver II test <-2 SDS). The p.Arg363Trp variant is associated with EO (28). In our cohort, these patients also had EO, global developmental delay and were bedridden despite early treatment and dialysis. Hypercitrullinemia is used as a biochemical marker for ASS1D, but the genotype-phenotype correlation is not very strong (5).

In our cohort, two siblings had identical genotypes for OTCD. P19 had profunda ID, whereas P20 had normal neurological parameters. Klaus et al. (30) reported two siblings with identical genotypes for CPS1D, one died as a newborn while the other had normal functions at the age of 45 with dietary therapy. While an EO patient who needed CVVHDF had normal development, there was also an adult patient with ID (31). It indicates the absence of a genotype/phenotype relationship in UCD. At present, a precise prediction of neurological outcome is not easy as there is no correlation between genotype, age of onset, and/or phenotype. The reason for this significant difference in neurological presentation is unknown.

A combination of a low protein diet with or without essential amino acid supplements, citrulline and/or arginine, and nitrogen scavengers (sodium benzoate, and sodium glycerol phenylbutyrate) has been suggested for maintenance therapy (6). Lee et al. (25) evaluated the efficacy of therapeutic modalities in UCD patients with the stable isotope protocol. However, sufficient information was not obtained about the prognostic process. The nitrogen removal efficiency of sodium phenylbutyrate is biochemically double compared to sodium benzoate. However, the superiority of each other in terms of disease severity or neurocognitive outcome is not clear in studies (32). Carbamyl acid is licensed for NAGS deficiency. Also, we used it for CPS1D patients off-license. It provided good metabolic control in CPS1D patients who needed recurrent CVVHDF due to resistant hyperammonemia. In our study group, there was no difference in neurocognitive outcome among ammonia-scavengers. These disappointing pharmacotherapeutic data, together with the poor cognitive outcomes in symptomatic UCD patients, suggest an urgent need for new treatments. In some studies, the use of multiple ammonia scavengers resulted in better cognitive outcomes (3). This has been associated with better metabolic control.

CVVHDF is currently the most effective way of ammonia detoxification. In cases of ammonia >500 µmol/L and insufficient reductions in ammonia levels after 4 hours medical treatment, hemodiafiltration is recommended according to the UCD guideline. Based on this, eight patients with ammonia levels >500 µmol/L during their first metabolic crisis received dialysis. Coma is a known predictor of poor outcome and patients with baseline ammonia >360 µmol/L are reported to have a poor prognosis. Kido et al. (33) recommended that patients with a baseline ammonia level above 180 µmol/L should receive hemodialysis. Enns et al. (34) recommended aggressive treatment including dialysis in neonatal hyperammonemia exceeding 300 µmol/L (34). Spinale et al. (35) recommended extracorporeal detoxification for ammonia level >400 µmol/L in newborns. In our study, we found a significant relationship between poor neurocognitive outcome and initial peak ammonia levels and the mean number of HEs per year. There were still poor neurocognitive outcomes despite extracorporeal detoxification. This highlights the need to reassess current treatment strategies, including the threshold for starting extracorporeal detoxification if ammonia levels exceed 500 µmol/L.

It has been shown that TH significantly reduces both morbidity and mortality in perinatal encephalopathy (36-38). Moderate hypothermia has been previously used in adult acute liver failure, and decreases in blood ammonia levels and intracranial pressure have been observed (37). In some studies, TH was used in combination with extracorporeal detoxification in UCD and organic acidemia patients, it was hypothesized that hypothermia may have reduced enzymatic ammonia production and reduced brain edema (38-40). Based on the literature, we also used TH in combination with CVVHDF in two patients with ASSD. When the neurological parameters of CVVHDF and the TH combination with CVVHDF groups were compared, no significant difference was found. However, a significant regression was observed in brain edema four hours after onset in the TH group. The use of TH by experienced teams seems promising due to its neuroprotective effects, ammonia-reducing effects, anti-inflammatory effects, and brain edema-reducing effects. Further studies with large numbers of patients are needed.

Study Limitations

This is a retrospective study and we could not perform enzyme analyses due to technical limitations. Lack of enzyme analysis made it difficult to evaluate the effects of genotype on phenotype. The negative impact of the number of subgroups and the low number of cases on the statistical results cannot be excluded.

Conclusion

There are several reasons why UCDs can have poor neurological outcomes despite early diagnosis. Based on our patient group, which were mostly diagnosed in the symptomatic period, we aimed to examine the parameters which can be easily used by clinicians in estimating disease severity and neurocognitive outcomes. Our study emphasizes the effect of peak ammonia levels and the frequency of HEs on neurological outcomes. This does

not necessarily mean that other interventions are not effective. There were still poor neurocognitive outcomes despite extracorporeal detoxification. This highlights the need to reassess current treatment strategies, including the threshold for starting extracorporeal detoxification if ammonia levels exceed 500 µmol/L. The use of TH by experienced teams seems promising due to its brain edema-reducing effects. Further studies with larger numbers of patients are needed. Close monitoring of ammonia levels, rapid and effective intervention, and the prevention of hyperammonemia crises are essential for good neurological outcomes.

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Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Diyarbakır Gaziyaşargil Training and Research Hospital (approval no: 928, date: 05.11.2021).

Informed Consent: The families of the patients' who participated were informed about this research and signed consent forms.

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Authorship Contributions

Surgical and Medical Practices: A.E.B, E.G., Concept: A.E.B., Design: A.E.B, Data Collection or Processing: A.E.B., E.G., A.T.Ü., H.M,A., İ.T. B.K., M.N.Ö., Analysis or Interpretation: A.E.B., M.K., Literature Search: A.E.B., A.T.Ü., Writing: A.E.B., M.K.

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References

1. Brusilow S, Horwich A. Urea cycle enzymes. In The metabolic & molecular bases of inherited disease. 8th edition. Edited by Scriver C, Beaudet A, Sly W, Valle D. New York: McGraw-Hill 2001.
2. Summar ML, Koelker S, Freedenberg D, et al. The incidence of urea cycle disorders. Mol Genet Metab 2013; 110:179-80.
3. Waisbren SE, Cuthbertson D, Burgard P, Holbert A, McCarter R, Cederbaum S. Members of the Urea Cycle Disorders Consortium. Biochemical markers and neuropsychological functioning in distal urea cycle disorders. J Inherit Metab Dis 2018; 41:657-67.
4. Posset R, Garcia-Cazorla A, Valayannopoulos V, et al. Age at disease onset and peak ammonium level rather than interventional variables predict the neurological outcome in urea cycle disorders. J Inherit Metab Dis 2016; 39:661-72.
5. Zielonka M, Kölker S, Gleich F, et al. Early prediction of phenotypic severity in Citrullinemia Type 1. Ann Clin Transl Neurol 2019; 6:1858-71.
6. Häberle J, Burlina A, Chakrapani A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision. J Inherit Metab Dis 2019; 42:1192-230.
7. Eratay E, Bayoğlu B, Anlar B. Preschool developmental screening with denver II test in semi-urban areas. J Pediatr Child Care 2015; 1:2-4.
8. Savaşır I, Şahin N. Wechsler çocuklar için zekâ ölçeğinin Türk kültürüne uyarlanması ve standardizasyonu. 1982; Proje no: TAG-385.
9. Demirkol M, Çelik S, Gökgay G, et al. Expanded newborn screening experience in Istanbul. J Inherit Metab Dis 2007; 30:3.
10. Seminara J, Tuchman M, Krivitzky L, et al. Establishing a consortium for the study of rare diseases: The Urea Cycle Disorders Consortium. Mol Genet Metab 2010; 100:97-105.
11. Saritaş Nakip Ö, Yıldız Y, Tokatlı A. Retrospective evaluation of 85 patients with urea cycle disorders: one center experience, three new mutations. J Pediatr Endocrinol Metab 2020; 33:721-8.
12. Dorum S, Havalı C. Urea cycle disorders clinical, laboratory and genetic features: Single center experience. MKÜ Tıp Dergisi 2022; 13:74-9.
13. Gropman AL, Summar M, Leonard JV. Neurological implications of urea cycle disorders. J Inherit Metab Dis 2007; 30:865-79.
14. Loeber JG, Burgard P, Cornel MC, et al. Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 1. From blood spot to screening result. J Inherit Metab Dis 2012; 35:603-11.
15. Posset R, Gropman AL, Nagamani SCS et al. Impact of Diagnosis and Therapy on Cognitive Function in Urea Cycle Disorders. Ann Neurol 2019; 86:116-28.
16. Smith W, Kishnani PS, Lee B, et al. Urea cycle disorders: clinical presentation outside the newborn period. Crit Care Clin 2005; 21:9-17.
17. Nettesheim S, Kolker S, Karall D, et al. Incidence, disease onset and short-term outcome in urea cycle disorders -cross-border surveillance in Germany, Austria and Switzerland. Orphanet J Rare Dis 2017; 12:111.
18. Burgard P, Kolker S, Haege G, Lindner M, Hoffmann GF. Neonatal mortality and outcome at the end of the first year of life in early onset urea cycle disorders--review and meta-analysis of observational studies published over more than 35 years. J Inherit Metab Dis 2016; 39:219-29.
19. Bachmann C. Outcome and survival of 88 patients with urea cycle disorders: a retrospective evaluation. Eur J Pediatr 2003; 162:410-6.
20. Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. N Engl J Med 1984; 310:1500-5.

21. Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. Mol Genet Metab 2004; 81:58-62.

22. Picca S, Dionisi-Vici C, Abeni D, et al. Extracorporeal dialysis in neonatal hyperammonemia: modalities and prognostic indicators. Pediatr Nephrol 2001; 16:862-7.

23. Uchino T, Endo F, Matsuda I. Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. J Inherit Metab Dis 1998; 21:151-9.

24. Batshaw ML, Tuchman M, Summar M, Seminara J. A longitudinal study of urea cycle disorders. Mol Genet Metab 2014; 113:127-130.

25. Lee B, Diaz GA, Rhead W, et al. Blood ammonia and glutamine as predictors of hyperammonemic crises in patients with urea cycle disorder. Genet Med 2015; 17:561-8.

26. Brusilow SW. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. Ped. Res 1991; 29:147-50.

27. Buerger C, Garbade SF, Dietrich Alber F, et al. Impairment of cognitive function in ornithine transcarbamylase deficiency is global rather than domain-specific and is associated with disease onset, sex, maximum ammonium, and number of hyperammonemic events. J Inherit Metab Dis 2019; 42:243-53.

28. Diez-Fernandez C, Rüfenacht V, Häberle J. Mutations in the Human Argininosuccinate Synthetase (ASS1) Gene, Impact on Patients, Common Changes, and Structural Considerations. Hum Mutat 2017; 38:471-84.

29. Daou M, Souaid M, Yammine T, et al. Analysis of ASS1 gene in ten unrelated middle eastern families with citrullinemia type 1 identifies rare and novel variants. Mol Genet Genomic Med 2023; 11:e2058.

30. Klaus V, Vermeulen T, Minassian B, et al. Highly variable clinical phenotype of carbamylphosphate synthetase 1 deficiency in one family: an effect of allelic variation in gene expression? Clin Genet 2009; 76:263-9.

31. Ono H, Suto T, Kinoshita Y, Sakano T, Furue T, Ohta T. A case of carbamoyl phosphate synthetase 1 deficiency presenting symptoms at one month of age. Brain Dev 2009; 31:779-81.

32. Nagamani SCS, Agarwal U, Tam A, et al. A randomized trial to study the comparative efficacy of phenylbutyrate and benzoate on nitrogen excretion and ureagenesis in healthy volunteers. Genet Med 2018; 20:708-16.

33. Kido J, Nakamura K, Mitsubuchi H, et al. Long-term outcome and intervention of urea cycle disorders in Japan. J Inherit Metab Dis 2012; 35:777-85.

34. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med 2007; 356:2282-92.

35. Spinale JM, Laskin BL, Sondheimer N, Swartz SJ, Goldstein SL. High-dose continuous renal replacement therapy for neonatal hyperammonemia. Pediatr Nephrol 2013; 28:983-6.

36. lzke SM, Rao S, Patole SK. A systematic review of cooling for neuroprotection with hypoxic-ischemic encephalopathy – are we there yet? BMC Pediatr 2007; 7:1-30.

37. Jalan R, Damink S. Hypothermia for the management of intracranial hypertension in acute liver failure. Curr Opin Crit Care 2001; 7:257-62.

38. Whitelaw A, Bridges S, Leaf A, Evans D. Emergency treatment of neonatal hyperammonaemic coma with mild systemic hypothermia. Lancet 2001; 358:36-8.

39. Lichter-Konecki U, Nadkarni V, Moudgil A, et al. Feasibility of adjunct therapeutic hypothermia treatment for hyperammonemia and encephalopathy due to urea cycle disorders and organic acidemias. Mol Genet Metab 2013; 109:354-9.

40. Ninković D, Mustapić Ž, Bartoniček D, et al. The Therapeutic Hypothermia in Treatment of Hyperammonemic Encephalopathy due to Urea Cycle Disorders and Organic Acidemias Klin Padiatr. 2019; 231:74-9.

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Original Article

Evaluation of the Association Between Sleeping Arrangements and Breastfeeding in Infants: A Cross-Sectional, Single Unit Study

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ABSTRACT

Aim: The bed-sharing option has been reported to positively encourage breastfeeding frequency in the literature. However, an association between sudden infant death syndrome (SIDS) and infant bed-sharing has also been identified. The present study aimed to determine the effects of mother-infant bed-sharing and room-sharing on breastfeeding and sleep patterns and whether there is any increased risk of SIDS.

Materials and Methods: We conducted a cross-sectional study among 507 mother-infant dyads between August, 2017 and August, 2018. Bed-sharing was defined as sharing a bed or mattress; room-sharing was defined as sharing the same room for any part of the night. The validated Sleep Questionnaire form was used to assess infant sleep characteristics. Potential predictors of bed-sharing were evaluated via logistic regression models (age, education, etc.).

Results: In the study period, 507 mother-infant dyads were included. The rate of room-sharing was 78.1%, and the rate of bed-sharing was 12.4%. Additionally, the rate of sleeping in a different room was 9.5%. All infants aged 1-5 months who shared a bed with their mother were breastfed, while 94% of those who did not share a bed were breastfed. For 6-12-month-old babies, the breastfeeding frequency was 86% for those who shared a bed, whereas 77% of those who did not share a bed were breastfed. However, the bed-sharing modality was not statistically shown to increase the frequency of breastfeeding [odds ratio (95% confidence interval); 0.362 (0.130-1.01)] (p=0.052).

Conclusion: Sharing a bed remains popular for infants' sleep arrangements. In our study, the majority of infants slept in separate cribs. This study revealed that mother-infant bed sharing increased breastfeeding frequency. Moreover, mother-infant bed sharing delayed the age at which babies with sleep disorders stopped breastfeeding.

Keywords: Infant, sleep, breastfeeding, bed-sharing, room-sharing, SIDS

Introduction

Sleeping is an active neurophysiological process associated with good cognitive, psychomotor, physical, and socioemotional development, mood, and behavior in infancy and childhood. Biological, socio-cultural, and environmental factors influence sleep characteristics and sleep-related behaviors, particularly in the first months after birth (1-4). In addition to sleep quality, safe infant sleep should not be ignored because 3,500 infants die tragically

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of sudden infant death syndrome (SIDS) or other sleep-related mortalities annually in the USA. A technical report was published in 2016 which aimed to decrease mortalities caused by SIDS and other sleep-related infant deaths, including many recommendations for wrong sleep positions or behaviors. One of the most prominent suggestions was a safe sleeping location where by infants should sleep in the parents' room, close to the parent's bed, but on a separate surface (5).

Systematic reviews and studies have focused on the harms and benefits of bed-sharing, which is defined as the parent/parents and the infant sleeping together on the same surface (bed, couch, chair) for many years (6,7). Although epidemiologic studies have declared that bed-sharing might increase the risk of overheating, rebreathing, airway obstruction, and head covering and thus ultimately increase the risk of developing SIDS in infants, the bed-sharing practice remains a popular option in several cultures. Data from the Centers for Disease Control, Pregnancy Risk Assessment Monitoring System, and the National Infant Sleep Position have shown that the practice increased by approximately 6.5% to 13.5% between 1993 and 2010. These studies reported that bed-sharing increases the connection between the mother and infant and supports the socio-emotional development of infants (8-10). It is also noted that an increase in the frequency and quality of breastfeeding is associated with bed-sharing (11). The possible benefits of bed-sharing include an increase in the incidence of successful breastfeeds, the encouragement of breastfeeding for mothers, and the total duration of breastfeeding (12). Nevertheless, a perplexing question has arisen regarding the relationship between bed-sharing and breastfeeding: does bed-sharing facilitate the continuation of breastfeeding, or does breastfeeding encourage the adoption of bed-sharing? Can variabilities in bed-sharing behaviors be used to predict a distinction between low-risk and high-risk infants in a dynamic environment?

The purpose of this study was to detect the prevalence of sleep-sharing practices, to assess the effects of mother-infant bed-sharing and room-sharing on the breastfeeding and sleep patterns of infants, to determine whether there is any increased risky behavior regarding SIDS, and to identify those factors which influence the preference of infant sleep location.

Materials and Methods

Study Design and Dyads

This study was designed as a single-center, cross-sectional study. It was conducted in a children's hospital among mother-infant dyads who were followed up by a social pediatrics outpatient clinic between August, 2017 and August, 2018. Approval for this study was given by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no: **17-12/35**, date: 12.12.2017). A total of 507 mothers and 507 infants under 12 months old (507 mother-infant dyads) were enrolled in this study. Oral consent from the mothers was obtained before they participated in this survey after informing them about the study's purpose and expectations.

Sleep Practices and Demographic Questionnaire

The validated Sleep Questionnaire form (adapted and modified from the Sleep Practice Questionnaire) (13) was used to assess infant sleep location, before-bedtime activities, sleep patterns, sleep duration, night waking, night feedings, bedtime routine components and times, infant sleep behaviors and environment, and parenting responses to night wakings. The participants also responded to questions relating to sleeping problems, pillow use, and breastfeeding frequency through the sleep questionnaire form.

The babies were accepted as being breastfed if breastmilk constituted >80% of their daily nutrition. The mothers were interviewed and a questionnaire regarding demographic, socio-economic, behavioral, and biological characteristics, maternal education, sleep location choices, breastfeeding features, and frequencies, etc., was given to those who agreed to be and were accepted as participants. Infants with a birth weight of less than 2,000 grams, acute infections, diseases requiring a particular nutrition program, or a chronic illness requiring hospitalization were excluded from this study. The inclusion criteria for mothers were full-term pregnancies (37 weeks) and being able to speak Turkish. The families' monthly household income levels were evaluated according to the results of the Türk-İş survey of August, 2017 (14).

Two distinct categories were identified: dyads belonging to the bed-sharing group and dyads belonging to the room-sharing group. The groups were compared to assess disparities in terms of their demographic characteristics, sleeping patterns, frequency of breastfeeding, and other socio-economic determinants.

Sleep-Sharing Definitions

Bed-sharing was defined as the infant using the same surface (bed, sofa, or chair) of sleeping location as the mother or parents. Room-sharing was defined as the parent/parents and infant sleeping in the same room on

separate surfaces. Co-sleeping is generally defined as a child sleeping close to the parent/parents on the same sleeping surface (bed-sharing) or in the same room but on a separate sleeping surface (room-sharing). Co-sleeping was not used in this study as it is a broader concept.

Statistical Analysis

Statistical analyses were performed using the IBM SPSS version 21.0 for personal computers (Chicago, IL, USA). The Shapiro-Wilk test was used to check the normality assumption of continuous variables. Fisher's exact and Pearson's chi-squared tests were used for categorical data. In cases of non-normally distributed data, the Mann-Whitney U test was utilized, and the t-test was used in cases of normally distributed data in order to determine whether the difference between the two groups was statistically significant. Pearson or Spearman correlation analyses were used in order to investigate the relationships between numerical variables. Post-hoc analysis was performed for cross-tables larger than 2x2. Logistic regression analysis was used to investigate the effects of independent variables on the dependent binary categorical variable. In univariate analysis, a $p<0.25$ value was used as statistical significance in logistic regression analyses, and then multivariate logistic regression analysis was evaluated. The independent variables were age, sex, educational status, etc., and the dependent variables were defined. It was assumed that the independent variable at the beginning of the process reveals the dependent variable of the process. A two-tailed probability value of $p<0.05$ was considered statistically significant.

Results

Participants and Baseline Characteristics

The participants' characteristics are presented in Table I. The infants were divided into four groups according to age: Group 1 (1-2 months, 39.4%), Group 2 (3-5 months, 26.1%), Group 3 (6-8 months, 20.3%), and Group 4 (9-12 months, 14.2%) (Figure 1). Table I also summarizes the infants'maternal and child characteristics and sleep

Table I. Demographic, socioeconomic, and sleep characteristics of the participants

Infant characteristics			
Age (mean ± SD), months	4.4±1.2		
		n	**%**
Gender	Male	243	47.9
	Female	264	52.1
Age groups, months	1-2	200	39.4
	3-5	132	26
	6-8	103	20.3
	9-12	72	14.2
Delivery form	Vaginal delivery	170	33.5
	Cesarean-section delivery	337	66.5
Birth weight, gr	2,000-2,500	19	3.7
	2,501-4,500	481	95
	>4,500	7	1.3
Infant sleep characteristics	Bed-sharing	63	12.4
	Room-sharing	396	78.1
	Different rooms	48	9.5
Sleep position	Supine	242	47.8
	Lateral	233	45.9
	Non-supine (prone)	32	6.3
Pillow usage	Yes	275	54.4
	No	232	45.6
Sleep surface	Soft	310	61.2
	Hard	197	38.8

Table I. Continued			
Infant characteristics			
Infant's own room	Yes	259	51.1
	No	248	48.9
The number of nights mother and child shared a bed in the last week	Never	315	62.1
	1-6 times	133	26.3
	7 times	59	11.6
Sleeping-waking features (Infants)			
	Duration of sleep in the night (mean ± SD), hours	9±1.2	
	Time to fall asleep (mean ± SD), mins	18.8±17.1	
	Frequency of night wakings (mean ± SD), times	2.7±1.3	
	Duration of sleep in the day (mean ± SD), hours	2.9±2.5	
	Frequency of sleep in the daytime (mean ± SD), times	3±1.5	
Family and maternal demographics			
Maternal age, (mean ± SD) **Maternal age at delivery, (mean ± SD), years**		30.2±5.0 29±5.6	
		n	**%**
Maternal age groups, years	18-24	71	14
	25-34	325	64
	≥35	111	22
Maternal education	Illiterate	9	1.8
	Primary school	61	12
	Secondary school	59	11.6
	High school	150	29.6
	University	228	45
Current breastfeeding status	Yes	454	89.5
	No	53	10.5
Working status of the mothers	Housewife	256	50.5
	Working	65	12.8
	Maternity leave	186	36.7
Home characteristics	Single-family home	466	91.9
	Extended family or others living in the home	39	7.7
	Divorced	2	0.4
Household income, TL	>4,500	166	32.8
	1,500-4,500	311	61.3
	<1,500	30	5.9
Care provider for the infant	Mother	357	70.4
	Babysitter	12	2.3
	Grandmother	138	27.3
Current maternal smoking status	Yes	61	12.1
	No	446	87.9
Current maternal alcohol usage	Yes	14	3
	No	493	97

SD: Standard deviation, TL: Turkish liras

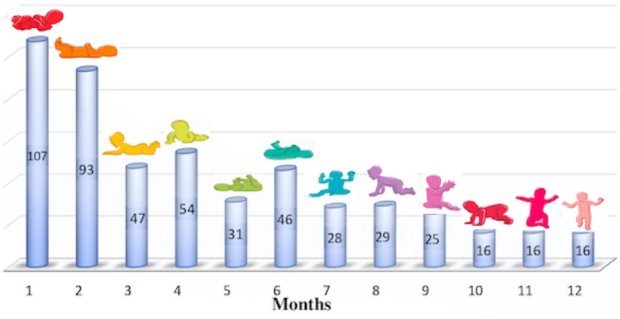

Figure 1. Distribution of the number of babies by month

features. The infants consisted of 243 (47.9%) males and 264 (52.1%) females, with a mean age of 4.4±1.2 months. The mean age of the mothers was 30.2±5.0 years, 14% were 18-24 years old, 64% were 25-34 years old, and 22% were 35 years old or older. 1.8% of the mothers were illiterate; 12% had graduated from primary school; 11.6% from secondary school; and 29.6% had graduated from high school. The rate of room-sharing in the study group was 78.1%, and the rate of bed-sharing was 12.4%. Only 9.5% of the infants slept in a room other than their mother's. It was found that 47.8% of the 507 infants slept on their backs (supine position:

Table II. Comparison of the demographic and sleeping characteristics between room-sharing and bed-sharing sleepers

	Bed-sharing sleepers (n=63)	Room-sharing sleepers (n=396)	p-value
Frequency of breastfeeding at night, n (%)			0.361
0-3 times	34 (54)	266 (67.2)	
4-6 times	29 (46)	130 (32.8)	
Sleep position, n (%)			**0.003**
Back	19 (30.1)	223 (56.4)	
Lateral/Prone	44 (69.9)	173 (43.6)	
Duration of falling sleep, n (%)			**0.006**
5-10 min	30 (47.6)	95 (24.1)	
11-30 min	22 (34.9)	271 (68.4)	
>30 min	11 (17.5)	30 (7.5)	
Frequency of night wakening, n (%)			0.539
Never	2 (3.2)	25 (6.3)	
1-3 times	39 (61.9)	288 (72.8)	
4-6 times	22 (34.9)	83 (20.9)	
Home characteristics, n (%)			0.560
Single-family home	57 (90.4)	363 (91.6)	
Extended family	6 (9.6)	33 (8.4)	
Maternal age, n (%)			0.617
19-25	10 (15.8)	82 (20.7)	
>25	53 (84.2)	314 (79.3)	
Maternal education, n (%)			0.778
Primary school	10 (15.9)	60 (15.2)	
Secondary school	5 (7.9)	54 (13.6)	
High school	29 (46)	131 (33)	
University	19 (30.2)	151 (38.2)	
Maternal working, n (%)			0.655
Working	10 (15.9)	55 (14)	
Housewife	29 (46)	267 (67.4)	
Maternity leave	24 (38.1)	74 (18.6)	
Maternal smoking and/or alcohol use, n (%)			0.341
Yes	11 (17.4)	58 (14.7)	
No	52 (82.6)	338 (85.3)	

n=242), 45.9% slept in a lateral position (n=233), and 6.3% (n=32) slept in a prone position. It was determined that the mean frequency of night waking in infants was 2.7±1.3 times. The mean nighttime sleep duration among mothers was 5.2±1.68 hours; 65.1% of mothers (n=330) described themselves as happy, while 34.9% were unhappy. According to the mothers'perceptions, the prevalence of infant sleep problems was as follows: 24 (4.7%) had severe problems, 151 (29.8%) had moderate problems, and 332 (65.5%) had no sleep problems.

Bed-Sharing vs. Room-Sharing

Table II presents the participant characteristics for each group. No differences existed between groups for breastfeeding frequency at night (p=0.361). Family type, maternal age, maternal education, maternal working status, and maternal smoking or alcohol use also did not show a significant difference between the groups. However, there was a significant difference between the two groups in terms of sleep position and duration of falling asleep (p<0.05). Lateral/prone position patterns were more common in the bed-sharing group than in the room-sharing group, and bed-sharing infants fell asleep in a shorter time than room-sharing infants (p=0.003 and p=0.006, respectively).

There was no significant effect of the independent variables, including the mother's age, education, household income, the sex of the infant, or the number of siblings on bed-sharing preferences. Those infants who were breastfed did not have a statistically significant increase in bed-sharing [odds ratio (OR) (95% confidence interval (CI)); 0.362 (0.130-1.01)] (p=0.052). However, logistic regression analysis showed that the bed-sharing practice increased 1.2 times with increasing age [OR (95% CI); 1.224 (1.126-1.331)] (p<0.001) (Table III) (Figure 2).

Table III. The results of effective factors on choosing a bed-sharing modality via logistic regression analysis

Independent variables	B	S.E	Wald	df	OR (95% CI)	p-value
Breastfeeding	-1.017	0.524	3.767	1	0.362 (0.130-1.01)	0.052
Age	0.202	0.043	22.59	1	1.224 (1.126-1.331)	**<0.001**
Sex (F)	-0.160	0.280	0.328	1	0.852 (0.493-1.474)	0.567
Number of siblings	-0.274	0.210	1.707	1	0.760 (0.505-1.147)	0.191
Mother age	0.032	0.030	1.127	1	1.033 (0.973-1.096)	0.288
Household income	0.000	0.000	0.060	1	1.000 (1.000-1.000)	0.807
Unhappy mother	0.122	0.298	0.167	1	1.129 (0.630-2.026)	0.683
Sleep problems	0.100	0.299	0.111	1	1.105 (0.615-1.986)	0.739
Maternal education (Illiterate)						
Secondary/high school	-0.259	0.448	0.334	1	0.772 (0.321-1.856)	0.563
University	-0.554	0.477	1.346	1	0.575 (0.226-1.465)	0.473

OR: Odds ratio, CI: Confidence interval

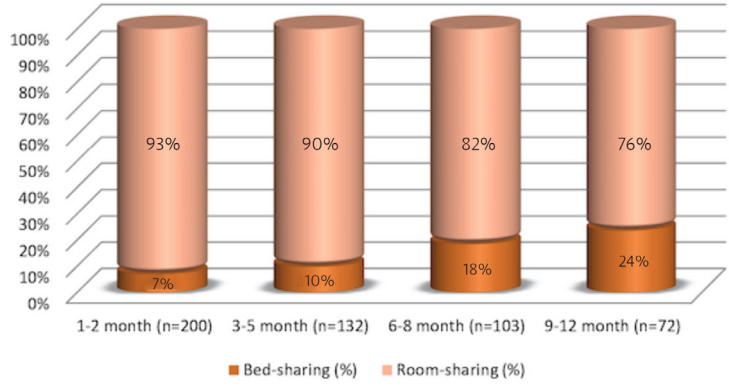

Figure 2. Bed-sharing and room-sharing rates by month

The mothers of the 507 babies who participated in this study reported that 18.8% of their infants were demanding. The age group with the most significant ratio of restless to peaceful infants was 1-2 months, while the age group with the lowest ratio was 9-12 months (Table IV). The logistic regression method was used to identify and assess the independent variables (baby age, unhappy mother, maternal age, pacifier use, breastfeeding, and/or bed sharing) which may have impacted the newborns' sleep issues. It was also found that infants had a 2.2-fold increased risk of sleep problems with unhappy mothers [OR (95% CI); 2.26 (1.53-3.33)] (p=0.006). There was no statistically significant association with the other independent variables on infant sleep problems (Table IV).

Table IV. The results of effective factors on the sleeping problems in infants via logistic regression analysis

Independent variables	B	S.E	Wald	df	OR (95% CI)	p-value
Age, months (1-2 month)						
3-5	-0.007	0.242	0.001	1	0.99 (0.61-1.59)	0.976
6-8	0.322	0.26	1.47	1	1.37 (0.82-2.32)	0.225
9-12	-0.30	0.32	0.832	1	0.74 (0.38-1.41)	0.362
Unhappy mother	0.815	0.19	16.8	1	2.26 (1.53-3.33)	**0.006**
Mother age >25	0.398	0.25	2.51	1	0.67 (0.41-1.10)	0.113
Bed-sharing (Last night)	0.065	0.294	0.049	1	1.067 (0.060-1.89)	0.824
Breastfeeding (No)	0.690	0.36	3.49	1	0.50 (0.24-0.060)	0.061

OR: Odds ratio, CI: Confidence interval

Discussion

This current article mainly examines the preference status for the practice of bed-sharing or room-sharing among mother-infant pairs. It evaluates whether there is a relationship between sleeping place choices and breastfeeding and also whether the determined factors cause sleep problems in infants. We conducted this study to determine the effects of mother-baby bed sharing and room sharing on breastfeeding and sleep patterns and our results show that bed-sharing has no advantage over room sharing in terms of breastfeeding, breastfeeding frequency, sleep patterns, and comfortable sleep during infancy.

Although it has been recommended that infants sleep in the parents' room on a separate surface (room-sharing) for the first year of life (5), most parents prefer bed-sharing situations to accommodate their infants' needs (feeding, crying, etc.). The bed-sharing practice may also provide social-emotional and psychological positive effects within the mother-child dyadic relationship for their infants' development. As is well known, it has been reported that the bed-sharing modality is associated with physical touch (e.g., skin-to-skin contact), emotional security, and physical comfort, which decrease infant distress (13). The bed-sharing modality also comforts breastfeeding mothers in terms of night-sleeping and sustainable sleep. As it may also promote an increased frequency of breastfeeding and its sustainability, infant sleep location choices still tend to maternal-infant bed-sharing in several societies (8).

An international study conducted by Nelson and Taylor (15) from China in 2001 indicated that sleep location choices may vary between countries and cultures and that numerous ethnic and religious beliefs may influence the sleeping location. In that study, a wide range of bed-sharing ratios (2% to 88%) was reported from 21 centers in 17 countries, including Turkey (15). The National Infant Sleep Position Study (9) conducted in the USA with 18,986 participants stated that bed-sharing practices increased between 1993 (6.0%) and 2010 (13.5%), with an 11% prevalence. In a pilot study (16) carried out in the Netherlands among 55 Turkish, 54 Moroccan, and 210 Dutch families, the bed-sharing ratio was reported as being 37% in Turkish families, 40.4% in Dutch, and 38.9% in Moroccan families. Yıkılkan et al. (17) showed that the prevalence of the bed-sharing practice was 16%, and their study noted that the bed-sharing option was more significantly frequent among mothers younger than 20. In this current study, the rate of bed-sharing was 12.4%. Thus, the bed-sharing modality is used in Turkey, just as in many countries which showed low or high rates of SIDS worldwide. This study reported that the risk of SIDS is more likely to occur when the mother is older, has sleep problems, or is unhappy.

Although studies have stated in the literature that bed-sharing is a positive behavior for infant development,

the strong relationship between SIDS and the bed-sharing modality should not be ignored. Sudden infant death syndrome, also known as crib or cot death, is defined as the sudden death of an infant younger than one year of age during sleep, unexplained by complete autopsy and a review of the clinical history of the case (5). The actual incidence of SIDS has not been clearly defined all over the world; however, it has been put forward that there are about 3,500 sudden unexpected infant deaths (SUID), and rates of SIDS, which is a classified subgroup of SUID, declined considerably from 130.3 deaths per 100,000 live births in 1990 to 35.2 deaths per 100,000 live births in 2018 in the United States (approximately 2,500 deaths) (18,19). The incidence of SIDS may indicate disparities based on different cultural practices, races, and ethnicities. Non-Hispanic white infants are determined to have a lower risk for SIDS than Native American, Alaskan Native, and black infants (20).

Several observational and case-control studies have specified many independent epidemiologic risk factors for SIDS. While younger maternal age, inadequate prenatal care, and maternal smoking have been identified as maternal risk factors, soft bedding or bedding accessories such as loose blankets and pillows, head coverings, preterm birth or low birth weight, prone sleeping positions, bed-sharing, and overheating have been proposed as an infant and environmental factor. Lower socioeconomic status has also been associated with an increased risk of SIDS. Gessner et al. (21) reported in their research which included 115 infants who died from SIDS between 1992 and 1997 that 113 (98%) of these 115 infants had slept in a prone position or were bed-sharing. According to our results, the education level of the mothers, household income, infant sex, or the mother's age did not affect bed-sharing choices. However, those mothers who preferred the bed-sharing modality were more likely to show several risks for SIDS, such as lateral sleeping positions. In the National Infant Sleep status study conducted in the United States, it was shown that low household income increased 2-fold the frequency of bed-sharing, high maternal education decreased sleeping with the infants on the same bed, and a negative correlation between maternal age and bed-sharing was shown (22).

Breastfeeding is a critical nutrition form recommended by the World Health Organization, and they recommend "exclusively breastfeeding for the first six months of life, after that for at least two years with the addition of appropriate complementary feedings" for infants. It plays an essential role in lifetime health. The evidence of the strong association between increased breastfeeding frequencies and bed-sharing practices has presented a confusing combination due to a clear dose-response relationship in a similar fashion to the "chicken and egg" conundrum. In a large longitudinal UK cohort study, there was seen to be a positive correlation between the two entities; namely, that breastfeeding rates increased in bed-sharing infants and decreased among those who rarely or never experienced the bed-sharing practice (23). Unfortunately, the bed-sharing practice should not only be associated with several benefits, such as improving breastfeeding rates, increasing breastfeeding duration, encouraging more optimal infant and mother sleep patterns, mother-baby interactions, etc., but also with an obvious proven risk of SIDS. A meta-analysis study showed that bed-sharing infants had a 2.89-fold higher risk of SIDS than non-bed-sharing infants (24). Several studies have also reported showing evidence of death hazards by overlaying/smothering associated with bed-sharing practices (25). As expected, the bed-sharing method is likely related to the other risk factors (such as suffocation, asphyxia, entrapment, falls, and strangulation) which could be lead to the infant's injury or death. This means that infants, particularly those under six months, should sleep through the night alone. Due to a having lower risk for SIDS (as much as by 50%) and being safer than bed-sharing as an infant's sleep location, the room-sharing sleep practice has been recommended as there seems to be a contradiction about the benefits and hazards of bed-sharing such as improved breastfeeding versus the possible risk of death from SIDS (5). Although the rate of bed-sharing practices was found to be low (12.4%) in this study, the mothers or parents had a risky attitude and behavior in deciding on their infants' sleep locations, surfaces, and positions regarding SIDS.

Study Limitations

Our analyses had several limitations. This study was conducted at a University Hospital and in families with relatively high socioeconomic levels, so our results can only be generalized for some Turkish families. Further research is need to reveal more universal parental practices. As the mothers in our cohort had a high level of education, they may not be representative of the general community, and our bed-sharing rates may be lower than those seen in other studies. The other limitation was that we used a subjective question about sleep. The mothers were not asked about their reasons for their bed-sharing choices, which could help establish a cause-and-effect relationship in some variables. Also, this study did not assess whether the ages of other

children in the home had any effect; for instance, whether experienced parents behave differently. Lastly, the infants' sleep problems and their mothers' mode were evaluated subjectively, without using any scale.

Conclusion

The results of this paper do not support that bed-sharing results in an increased breastfeeding frequency. Also, it was found that those infants who slept with a bed-sharing practice showed no differences regarding their sleeping/waking features during their sleep time. Conversely, the lateral sleeping position was more likely to be implemented in bed-sharing dyads than in room-sharing dyads. Consequently, bed-sharing has no advantage over room-sharing during infancy regarding breastfeeding, frequency of lactation nutrition, sleep patterns, or comfortable sleep. Considering the potential risk of death, it might be advocated that an infant's most preferable sleeping location/practice is "room-sharing," especially in those infants younger than six months.

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Ethics

Ethics Committee Approval: Approval for this study was given by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no: 17-12/35, date: 12.12.2017).

Informed Consent: All patient's parents received verbal and printed information, and all provided written consent before participation in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.B.Ö., Ş.G., M.T., F.U.K., Design: T.B.Ö., Ş.G., M.T., F.U.K., Data Collection or Processing: Ş.G., Analysis or Interpretation: T.B.Ö., Ş.G., Literature Search: T.B.Ö., Ş.G., Writing: Ş.G.

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References

1. Sivertsen B, Harvey AG, Reichborn- Kjennerud T, Torgersen L, Ystrom E, Hysing M. Later emotional and behavioral problems associated with sleep problems in toddlers: a longitudinal study. JAMA Pediatr 2015; 169:575-82.
2. Ross CN, Karraker KH. Effects of fatigue on infant emotional reactivity and regulation. Infant Ment Health J 1999; 20:410-28.
3. Spruyt K, Aitken RJ, So K, Charlton M, Adamson TM, Horne SCR. Relationship between sleep/wake patterns, temperament and overall development in term infants over the first year of life. Early Hum Dev 2008; 84:289-96.
4. Moore M, Mindell JA. The impact of behavioral interventions for sleep problems on secondary outcomes in young children and their families. In: Wolfson A, Montgomery-Downs H eds. Oxford Handbook of Infant, Child, and Adolescent Sleepand Behavior. New York, NY: Oxford Press; 2013; 547-58. Available from https://psycnet.apa.org/record/2013-34445-037
5. Moon RY. Task Force on Sudden Infant Death Syndrome. SIDS and other sleep-related infant deaths: Evidence base for 2016 updated recommendations for a safe infant sleeping environment. Pediatrics 2016; 138:e20162940.
6. Horsley T, Clifford T, Barrowman N, et al. Benefits and harms associated with the practice of bed-sharing: a systematic review. Arch Pedia Adolesc Med 2007; 161:237-45.
7. Das RR, Sankar MJ, Agarwal R, Paul VK. Is "Bed Sharing" beneficial and safe during infancy? A systematic review. International Journal of Pediatrics 2014; 2014:468538.
8. Ward TCS. Reasons for mother–Infant bed-sharing: A systematic narrative synthesis of the literature and implications for future research. Maternal and Child Health Journal 2015; 19:675-90.
9. Colson ER, Willinger M, Rybin D, et al. Trends and factors associated with infant bed sharing, 1993-2010: The national infant sleep position study. JAMA Pediatrics 2013; 167:1032-7.
10. Gettler LT, McKenna JJ. Evolutionary perspectives on mother-infant sleep proximity and breastfeeding in a laboratory setting. Am J Phys Anthropol 2011; 144:454-62.
11. Baddock SA, Galland BC, Bolton DP, Williams SM, Taylor BJ. Differences in infant and parent behaviors during routine bed sharing compared with cot sleeping in the home setting. Pediatrics 2006; 117:1599-607.
12. Ball HL, Howel D, Bryant A, Best E, Russell CM. Ward-Platt Bed-sharing by breastfeeding mothers: who bed-shares and what is the relationship with breastfeeding duration? Acta Paediatr 2016; 105:628-34.
13. Germo GR, Chang ES, Keller MA, Goldberg WA. Child sleep arrangements and family life: Perspectives from mothers and fathers. Infant and Child Development 2007; 16:433-56.
14. http://www.turkis.org.tr/dosya/pBDme2chn8Tg.pdf
15. Nelson EA, Taylor BJ. International Child Care Practices Study: infant sleep position and parental smoking. Early Hum Dev 2001; 64:7-20.
16. Van Sleuwen B, L'hoir M, Engelberts A, Westers P, Schulpen TW. Infant care practices related to cot death in Turkish and Moroccan families in the Netherlands. Archives of Disease in Childhood 2003; 88:784-88.
17. Yıkılkan H, Unalan PC, Cakır E, et al. Sudden infant death syndrome: how much mothers and health professionals know. Pediatr Int 2011; 53:24-8.

18. Krous HF, Beckwith JB, Byard RW, et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. Pediatrics 2004; 114:234-8.

19. Barfield W, D'Angelo D, Moon R, Lu M, Wong B, Iskander J. CDC grand rounds: public health approaches to reducing U.S. infant mortality. MMWR Morb Mortal Wkly Rep 2013; 62:625-8.

20. Matthews TJ, MacDorman MF. Infant mortality statistics from the 2010 period linked birth/infant death data set. Natl Vital Stat Rep 2013; 62:1-26.

21. Gessner BD, Ives GC, Hester KA. Association between sudden infant death syndrome and prone sleep position, bed-sharing, and sleeping outside an infant crib in Alaska. Pediatrics 2001; 108:923-7.

22. Willinger M, Ko CW, Hoffman HJ, et al. Trends in infant bed-sharing in the United States, 1993-2000: The National Infant Sleep Position Study. Arch Pediatr Adolesc Med 2003; 157:43-52.

23. Blair PS, Heron J, Fleming PJ. Relationship between bedsharing and breastfeeding: longitudinal, population-based analysis. Pediatrics 2010; 126:1119-26.

24. Vennemann MM, Hence HW, Bajanowski T, et al. Bed-sharing and the risk of sudden infant death syndrome: can we resolve the debate? J Pediatr 2012; 60:44-52.

25. Nakamura S, Wind M, Danello M. Review of hazards associated with children placed in adult beds. Arch Pediatr Adolesc Med 1999; 153:1019-42.

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Original Article

Is Dietary Macronutrient Distribution Related to Serum Lipid Profiles in Children and Adolescents with Type 1 Diabetes?

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ABSTRACT

Aim: The aim of this study was to evaluate the dietary macronutrient distribution affects on serum lipid profiles in type 1 diabetes mellitus (T1D).

Materials and Methods: This cross-sectional study included 82 children and adolescents between the ages of 2 and 18 years with a diabetes age of ≥1 year. Dietary intake was evaluated by 3-day food diaries, including three consecutive days (two weekdays and one weekend day).

Results: The mean age of the 82 patients with diabetes was 11.6±4.3 years (range: 2-18 years) (45.1% female), the median diabetes duration was 3.4 (2.9) years, the mean HbA1c level was 7.0±1.4%, and mean body mass index standard deviation score was 0.2±1.1. The median distribution of energy from carbohydrates, protein and fat in the total energy intake was 50.0% (6.2), 17.4% (2.7) and 32.5% (5.1), respectively. Dietary fiber intake was inadequate in 64 (77.9%) participants, while for 77 participants (93.9%), saturated fatty acid intake was above the recommended intake. For children and adolescents with T1D, mean serum cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein levels were 162.9±33.4 mg/dL, 86.2±49 mg/dL, 87.9±29.2 mg/dL, and 60.7±17.9 mg/dL, respectively.

Conclusion: To maintain healthy eating, consuming foods high in saturated fat should be limited, and children and adolescents with T1D should be supported by their family and healthcare professionals in the consumption of diets high in fiber.

Keywords: Type 1 diabetes, dietary macronutrients intake, children, adolescents

Introduction

Nutritional management is one of the essential components of type 1 diabetes mellitus (T1D) management, diabetes care and education. It aims to ensure the continuation of normal growth and development, to develop lifelong healthy eating habits, to provide optimal glycemic control, to prevent/delay complications which may develop due to diabetes, to reduce cardiovascular disease (CVD) risk factors, and to maintain psycho-social well-being. Current dietary recommendations for children and adolescents with T1D reflect guidelines for healthy eating developed for the general population. Implementation of an individualized meal plan with prandial insulin adjustments improves glycemic outcomes (1,2). However, previous studies have reported that children and adolescents with T1D eat more atherosclerosis-prone diets than healthy control subjects and greater dietary quality is associated with more optimal glycemic control (3-7). This study aimed to evaluate how dietary macronutrient distribution affects the serum lipid profiles.

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Materials and Methods

Study Design and Participants

This cross-sectional study included 82 children and adolescents with T1D between the ages of 2 and 18 years, with a diabetes age ≥1 year, followed up in Ege University between April and December, 2022, and whose serum lipid profiles were routinely evaluated at a diabetes outpatient clinic. T1D patients with co-morbidities (celiac disease, cystic fibrosis, etc.) affecting food consumption and nutrient intake were excluded from this study.

Anthropometric Evaluation and Metabolic Control

Height was measured to the nearest millimeter using a Seca 264® stadiometer. Weight was measured unclothed using an electronic scale to the nearest 100 grams (Desis Model KW®). Body mass index (BMI) was calculated by the formula; weight (kg)/height (m^2). Standard deviation scores (SDS) for weight, height, and BMI were calculated according to age and gender using reference values for Turkish children and adolescents (8,9). HbA1c <7% was defined as good glycemic control (10).

Food Diary

For each people with diabetes (PwD), dietary intake was evaluated by 3-day food diaries, consisting of three consecutive days (two weekdays and one weekend day). All food and beverages consumed (including dressings) were recorded by weighing for 3 days. The food diaries were checked by a diabetes dietician, verified for consistency and accuracy, and supplementary information was requested if needed. The analysis included dietary records of snacks and meals (732 meals, 381 snacks). Total energy intake (kcal), carbohydrate (energy %), protein (energy %), fat (energy %), saturated fatty acids (SFAs) (energy %), dietary cholesterol (mg), and dietary fiber (g/1.000 kcal) intakes were calculated using the Ebispro for Windows, Turkish Version (BeBiS 8.2) (Stuttgart, Germany).

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences version 25.0 (SPSS Inc., Chicago, IL, USA). The level of significance was defined as $p<0.05$. Categorical variables were represented as counts and percentage values. Normal distribution was tested for quantitative variables. Continuous variables with normal or skewed distributions are presented as mean (± SD) or median (interquartile range). Group differences were investigated using the independent t-test for normally distributed data and the Mann-Whitney U test for skewed data. Spearman's correlation coefficients were used to explore relationships between SFA and dietary fiber intake with serum cholesterol, triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels. Correlation values of 0.10-0.29 were interpreted as small, 0.30-0.49 as medium, and 0.50-1.0 as large (11).

Ethical Consideration

This study was approved by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval number: 22-4T/13, date: 07.04.2022). The aim of this study was explained to each participant, and written informed consent was obtained. This study's procedures followed the Declaration of Helsinki.

Results

The mean age of the 82 PwD was 11.6±4.3 years (range: 2-18 years) (45.1% female), the median diabetes duration was 3.4 (2.9) years, the mean HbA1c was 7.0±1.4%, and the mean BMI-SDS was 0.2±1.1 (Table I). Thirty-six percent of the participants were on insulin infusion pump therapy (IIPT), 63.4% were on multiple daily insulin injections (MDI) (≥4 daily injections), and all were on carbohydrate counting. There were no significant differences in age, diabetes duration, HbA1c levels, height, weight and BMI-SDS, insulin requirements, and the number of meals/snacks per day between gender and treatment models (MDI and IIPT) (Table I). Fifty-four percent of PwD met the target HbA1c for good glycemic control (HbA1c <7%).

The median distribution of energy from carbohydrates, protein and fat in total energy intake was 50.0% (6.2), 17.4% (2.7) and 32.5% (5.1), respectively, and these were in line with international recommendations (1). There were no differences in nutrient intakes between the genders (Table II). The median fiber intake [11.3 (3.6) g/1.000 kcal] was below, and SFA intake [11.3% (3.6) of energy] was above the recommendations.

In PwD, dietary cholesterol (<300 mg/day) intakes met the recommendations, and SFA intakes which should be <10% of total energy intake was above the recommendations. Seventy-eight percent of participants did not consume the recommended daily fiber intake (14 g/1.000 kcal). PwD who met the dietary fiber intake recommendations had a lower median SFA intake than those who did not have adequate intake ($p<0.001$), and a negative large correlation was found between SFA intake and dietary fiber intake ($r=-0.516$, $p<0.01$).

Mean serum cholesterol, TG, LDL and HDL levels were 162.9±33.4 mg/dL, 86.2±49 mg/dL, 87.9±29.2 mg/dL, and 60.7±17.9 mg/dL, respectively. Those with adequate dietary fiber intake (22% of the participants) had lower serum cholesterol (p=0.041) and TG (p=0.028) levels than those who did not, and there was no difference between the HDL and LDL levels of the groups (Table III). In addition, there was a negative medium correlation between serum TG levels and dietary fiber intake (r=-0.395, p<0.01).

Table I. Baseline characteristics of participants

	All (n=82)	Male (n=45)	Female (n=37)	p-value
Age (years)[a]	11.6 (4.3)	11.3 (4.0)	11.9 (4.6)	0.541[c]
Diabetes duration (years)[b]	3.4 (2.9)	2.2 (3.5)	2.8 (4.2)	0.220[d]
Diabetes onset age (years)[a]	8.3 (8.5)	8.6 (3.6)	7.9 (3.7)	0.429[c]
Weight -SDS[a]	0.3 (1.2)	0.4 (1.2)	0.23 (1.3)	0.663[c]
Height- SDS[a]	0.2 (1.2)	-0.12 (1.37)	0.1 (0.9)	0.459[c]
BMI-SDS[a]	0.2 (1.1)	0.2 (1.1)	0.2 (1.2)	0.843[c]
HbA1c (%)[b]	7.0 (1.4)	6.9 (1.2)	7.4 (1.4)	0.634[d]
Insulin (U/kg/d)	0.8 (0.3)	0.8 (0.3)	0.7 (0.2)	0.797[c]
Basal insulin (%)[a]	42.9 (13.0)	41.7 (13.0)	44.4 (13.1)	0.344[c]
Bolus insulin (%)[a]	56.9 (13.1)	58.1 (13.0)	55.6 (1.3.1)	0.386[c]
Number of meals per day[b]	3.0 (0.0)	3.0 (0.0)	3.0 (0.0)	0.026[d]
Number of snacks per day[b]	1.6 (2.0)	2.0 (2.0)	1.0 (1.0)	0.549[d]

[a]Data are mean (standard deviation), [b]Data are median (Interquartile range), [c]Independent sample t-test, [d]Mann-Whitney U test,
p-values refer to the significance of the difference between MDI users and pump therapy users
BMI: Body mass index, HbA1c: Glycated haemoglobin, SDS: Standard deviation score, MDI: Multiple daily insulin injections

Table II. Energy and nutrient intake of participants during the follow-up period

	ISPAD recommendations	All (n=82)	Male (n=45)	Female (n=37)	p-value*
Carbohydrate (energy %)	40-50	50.0 (6.2)	50.0 (4.8)	50.0 (7.5)	0.863
Protein (energy %)	15-25	17.4 (2.7)	17.0 (2.9)	17.7 (2.8)	0.692
Fat (energy %)	30-40	32.5 (5.1)	32.4 (4.9)	32.3 (5.4)	0.744
SFA (energy %)	<10	15.2 (3.5)	15.1 (3.9)	15.1 (3.7)	0.618
Dietary cholesterol (mg)	<300	300.1 (142.6)	320 (152.3)	284.0 (174.1)	0.091
Dietary fiber (g/1000 kcal)	14	11.3 (3.6)	12.2 (4.4)	11.8 (2.9)	0.150

Data as presented as median (interquartile range),[c]Mann-Whitney U test
SFA: Saturated fatty acid, ISPAD: International Society of Pediatric and Adolescent Diabetes

Table III. Comparison of serum lipid profile of children and adolescent with T1D according to dietary fiber intake

	Adequte dietary fiber intake (n=18)	Inadequate dietary fiber intake (n=64)	p-value*
Cholesterol (mg/dL)	151.7±38.2	165.2±30.8	0.124
TG (mg/dL)	102.8±51.5	82.8±51.3	0.148
LDL (mg/dlL)	82.3±34.0	89.4±27.5	0.366
HDL (mg/dL)	58.7±19.6	61.6±17.1	0.546

Data are mean (standard deviation), *Independent sample t-test
p-values refer to the significance of the difference between participants with adequate dietary fiber intake and with inadequate fiber intake
TG: Tryglycerid, LDL: Low density lipoprotein, HDL: High density lipoprotein

Discussion

This study aimed to analyze the nutrient distribution of energy intakes and investigate the effects of serum lipid profiles in children and adolescents with T1D in real-time settings.

Nutrition management recommendations for children and adolescents with diabetes reflect guidelines for healthy eating developed for the general population. The optimal macronutrient distribution varies depending on the individualized assessment and metabolic priorities of the PwD. However, the International Society of Pediatric and Adolescent Diabetes (ISPAD) gives the following thresholds as a guide: "carbohydrate intake should be 40-50% of total daily energy intake, fat intake no greater than 30-40% (SFA <10%), and protein intake of 15-25%" (1).

In our study, the participants' carbohydrate, fat, and protein intakes met the recommended levels of ISPAD nutritional management of T1D guideline (1). However, our findings regarding low fiber and high SFA in children with T1D supported previous studies (4,5,12,13). Dietary factors which raise VLDL and LDL-cholesterol increase the likelihood of the formation of atherosclerosis in teenagers and young adults, and the amount of SFA in the diet is one of the main determinants of plasma LDL cholesterol levels (14). In our study group, there was no correlation between dietary SFA intake and serum LDL levels. This can be explained by the different relationship, reabsorption in the gastrointestinal system between individuals between and genetic polymorphisms contribute to the interindividual variation between dietary fat and serum LDL cholesterol response (15).

Even though our study data showed no correlation between dietary SFA intake and serum LDL levels, dietetic interventions in the clinical setting can focus more specifically on fiber and SFA intake.

The American Heart Association suggests that children and adolescents consume a healthy diet which limits SFA and recommends replacement with polyunsaturated and monounsaturated fat in order to reduce CVD risk in later life (16). Medical nutrition therapy of T1D should be provided in order to prevent and treat co-morbidities including obesity, dyslipidemia, hypertension, and micro- and macro-vascular complications.

Study Limitations

This current analysis has strengths and limitations. This study's strength was its real-world setting and its energy and nutrient intake evaluation based on 3-day food diary records. The main limitation was its sample size. Additional evaluations with larger samples should be performed in order to confirm these results.

Conclusion

These findings associated with the participants' high SFA and low fiber intakes have potential implications for clinical practice and nutritional education. In order to maintain healthy eating, consuming foods high in SFA should be limited, and children and adolescents with T1D should be supported by their families and healthcare professionals in the consumption of legumes, fruits and vegetables, and whole grains containing fiber.

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Ethics

Ethics Committee Approval: This study was approved by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval number: 22-4T/13, date: 07.04.2022).

Informed Consent: The aim of this study was explained to each participant, and written informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.A.A., Design: Y.A.A., D.G., Data Collections or Processing: Y.A.A., Analysis or Interpretation: Y.A.A., D.G., Literature Search: Y.A.A., D.G., Writing: Y.A.A., D.G.

Conflict of Interest: None of authors have any conflicts of interest to report.

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References

1. Annan SF, Higgins LA, Jelleryd E, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Nutritional management in children and adolescents with diabetes. Pediatr Diabetes [Internet]. 2022 Dec 5; Available from: https://onlinelibrary.wiley.com/doi/10.1111/pedi.13429
2. Elsayed NA, Aleppo G, Aroda VR, et al. 14. Children and Adolescents: Standards of Care in Diabetes-2023. Diabetes Care 2023; 46:S230-53.
3. Nansel TR, Lipsky LM, Liu A. Greater diet quality is associated with more optimal glycemic control in a longitudinal study of youth with type 1 diabetes. Am J Clin Nutr 2016; 104:81-7.
4. Lipsky LM, Nansel TR, Haynie DL, Mehta SN, Laffel LMB. Associations of food preferences and household food availability

with dietary intake and quality in youth with type 1 diabetes. Appetite 2012 ;59:218-23.

5. Overby NC, Flaaten V, Veierød MB, et al. Children and adolescents with type 1 diabetes eat a more atherosclerosis-prone diet than healthy control subjects. Diabetologia 2007; 50:307-16.
6. Margeirsdottir HD, Larsen JR, Brunborg C, Øverby NC, Dahl-Jørgensen K, Norwegian Study Group for Childhood Diabetes. High prevalence of cardiovascular risk factors in children and adolescents with type 1 diabetes: A population-based study. Diabetologia 2008; 51(4):554-61.
7. Øverby NC, Margeirsdottir HD, Brunborg C, Andersen LF, Dahl-Jørgensen K. The influence of dietary intake and meal pattern on blood glucose control in children and adolescents using intensive insulin treatment. Diabetologia 2007; 50:2044-51.
8. Demir K, Özen S, Konakçı E, Aydın M, Darendeliler F. A comprehensive online calculator for pediatric endocrinologists: ÇEDD Çözüm/TPEDS metrics. J Clin Res Pediatr Endocrinol 2017; 9:182-4.
9. Neyzi O, Günöz H, Furman A, et al. Weight, height, head circumference and body mass index references for Turkish children. Journal of Child Health and Diseases 2008; 51:1-14.
10. de Bock M, Codner E, Craig ME, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Glycemic targets and glucose monitoring for children, adolescents, and young people with diabetes. Pediatr Diabetes 2022; 23:1270-6.
11. Cohen J. Statistical power analysis for the behavioral sciences [Internet]. Statistical Power Analysis for the Behavioral Sciences. 1988; 2:567. Available from: http://books.google.com/books?id=Tl0N2lRAO9oC&pgis=1
12. Gilbertson HR, Reed K, Clark S, Francis KL, Cameron FJ. An audit of the dietary intake of Australian children with type 1 diabetes. Nutr Diabetes 2018; 8:10.
13. Thomson R, Adams L, Anderson J, et al. Australian children with type 1 diabetes consume high sodium and high saturated fat diets: Comparison with national and international guidelines. J Paediatr Child Health 2019; 55:1188-93.
14. Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Dietary fat and serumlipids: an evaluation of the experimental data. Am J Clin Nutr [Internet]. 1993; 57:875-83. Available from: https://academic.oup.com/ajcn/article-abstract/57/6/875/4715761
15. Griffin BA, Mensink RP, Lovegrove JA. Does variation in serum LDL-cholesterol response to dietary fatty acids help explain the controversy over fat quality and cardiovascular disease risk? Atherosclerosis. Elsevier Ireland Ltd; 2021; 328:108-13.
16. Sacks FM, Lichtenstein AH, Wu JHY, et al. Dietary fats and cardiovascular disease: A presidential advisory from the American Heart Association 2017; 136:e1-23.

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Original Article

In vitro Antimicrobial Susceptibility of Urinary Tract Infection Pathogens in Children

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ABSTRACT

Aim: Urinary tract infection (UTI) is one of the most common bacterial infections in children. Empirical treatment is commenced according to the patient's characteristics and the antimicrobial susceptibility patterns in the region. Therefore, a determination of antimicrobial resistance patterns has a great importance in effective treatment. The aim of this study was to determine the pathogens which cause UTIs in patients admitted to a university hospital in Izmir and to determine their antimicrobial susceptibility pattern.

Materials and Methods: The files of patients aged between 0-18 years, followed up with a diagnosis of UTI, vesicoureteral reflux and neurogenic bladder in Ege University Faculty of Medicine Paediatric Nephrology Unit between February, 2013 and November, 2018 were retrospectively reviewed.

Results: A total of 1,126 positive urine cultures from 729 patients (65% female) were included in this study. Gram-negative pathogens constituted 88.2% of the cultures. *Escherichia coli* (*E. coli*) was the most commonly isolated bacteria with a prevalence of 59.1%, followed by *Klebsiella pneumonia* with 17.9%, and *Enterococcus faecalis* with 8.3% (n=93). Ampicillin, cefuroxime and trimethoprim-sulfamethoxazole with susceptibility rates of 18.6%, 39.6%, 49.0% respectively, constituted the highest resistant antimicrobials to *Enterobacteriaceae*. *Enterococcus* spp. showed the highest resistance to gentamycin with 50% resistance in tested cases. *Pseudomonas* spp. with 64.3% susceptibility showed the highest resistance to piperacillin-tazobactam.

Conclusion: This study revealed that bacterial resistance to commonly used antimicrobials in UTI is an important and challenging problem which requires planning.

Keywords: Antibiotic resistance, antimicrobial resistance, antimicrobial susceptibility, *E. coli*, paediatric, urinary tract infections, urine culture

Introduction

Urinary tract infection (UTI) is the most common bacterial infection in children under 2 years of age and also one of the most common bacterial infections in children (1). Although the numbers vary in studies conducted in different populations, UTI is common in males under one year of age, whereas it is common in girls above one year of age during their childhood (2,3). Empirical treatment is commenced according to the patient's age, antimicrobial susceptibility patterns in the region, the clinical condition of the patient

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and the presence of any underlying diseases (2,4). Therefore, the determination of antimicrobial resistance patterns by regional centre studies is of great importance in terms of effective treatment and the prevention of complications. The aim of this study was to determine the pathogens which cause UTIs in patients admitted to a university hospital in Izmir and to determine their antimicrobial susceptibility patterns.

Materials and Methods

Patients and Urine Samples

The outpatient clinic files of 729 patients aged between 0-18 years, followed up with a diagnosis of UTI, vesicoureteral reflux and neurogenic bladder in Ege University Faculty of Medicine Paediatric Nephrology Unit between February, 2013 and November, 2018 were retrospectively reviewed. The urine culture results of the patients treated with a diagnosis of UTI were evaluated. The urine cultures of those patients who were not considered to be clinically diagnosed with UTI and were not treated, were excluded from this study. The diagnosis of UTI was made with positive urine cultures accompanied by the presence of leukocytes and/or nitrite and/or leukocyte esterase in the urinalysis. The significance of the positive urine culture was also evaluated with the clinical findings in the patient's file. In those patients with no urinary control or those who were not toilet trained, urine samples were collected according to a standard hygiene protocol via urine bags. Catheterization or a clean catch method were used in cases of urine cultures which were thought to be contaminated. The urine samples were collected mid-stream in those who were toilet trained. Positive urine culture was accepted as $\geq 10^3$ CFU/mL samples taken with the catheterization method, $>10^4$ CFU/mL with the mid-stream or clean catch in patients with symptoms, or $>10^5$ in those without symptoms (1). All the information regarding the culture results and antimicrobial susceptibility for the isolated pathogens was recorded. Those with intermediate susceptibility to antimicrobials were included in the resistant group (5). Second urine cultures from the same patient within one month were not included in this study. The urine culture results which were studied in laboratories other than Ege University Faculty of Medicine Bacteriology Laboratory were not included in this study.

Ethical Consideration

Ethical approval for this study was obtained from the Medical Research Ethics Committee of Ege University Faculty of Medicine (approval no: 22-12.1T/16, date: 16.12.2022).

Antimicrobial Susceptibility Tests

The cultures of the urine samples were studied in Ege University Faculty of Medicine Bacteriology Laboratory of the Medical Microbiology Department. MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry) was used to identify the bacteria. The antimicrobial susceptibilities of these isolates were determined by VITEK 2 (bioMerieux, France) and EUCAST clinical breakpoints were used.

Statistical Analysis

For the evaluation of the study data, the SPSS (Statistical Package for the Social Sciences, version 22.0 for Windows, SPSS® Inc., Chicago, IL, USA) statistical analysis program was used. Frequencies, and means ± standard deviations of the data are provided.

Results

A total of 1,126 positive urine cultures were included in this study. Two hundred and seventy-four (24.3%) of the urine samples were taken via urine bag, 762 (67.7%) of them were obtained via mid-stream with the clean catch method and 90 (8%) with a catheter. Among the 729 cases, 64.7% of the samples were obtained from female and 35.3% from male patients. The mean infection time was 56±53 months. Most of the cultures were gram negative with a percentage of 88.2% (n=993). *Escherichia coli* (*E. coli*) was the most commonly isolated bacteria with a prevalence of 59.1% (n=666) followed by *Klebsiella pneumonia* with 17.9% (n=202), *Enterococcus faecalis* with 8.3% (n=93), *Proteus mirabilis* with 3.2% (n=37), *Enterococcus faecium* with 2.9% (n=33) and *Pseudomonas aeruginosa* with 2.5 % (n=28). The isolated bacteria and their frequencies are shown in Table I.

Enterobacteriaceae such as *Escherichia* spp., *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Morganella* spp., *Citrobacter* spp., and *Serratia* spp., were the causative pathogens in 962 cultures (85.4%) and they constitute most of the bacteria in this study, followed by *Enterococcus* spp. in 129 (11.5%), *Pseudomonas* spp. in 28 (2.5%), *Staphylococcus* spp. in 4 (0.4%) and *Acinetobacter* spp. in 3 cultures (0.3%).

There were 333 cases (29.5%) younger than one year old, among whom male gender was more prominent with a ratio of 61.6%. However, the male ratio declined to 24.2% in cases older than one year old. *Enterobacteriaceae* are the most common bacteria, followed by *Enterococcus* spp. and *Pseudomonas* spp. in both sexes.

Ampicillin, cefuroxime and trimethoprim-sulfamethoxazole with susceptibility rates of 18.6%, 39.6%, and 49.0% respectively, saw the highest resistance with

Table I. The frequency of isolated bacteria in culture positive patients

Microorganism	**No of subtypes**	**%**		**n=1126**	**%**
E. coli	666	59.1	*Escherichia* spp.	666	59.1
Klebsiella pneumoniae	202	17.9	*Klebsiella* spp.	209	18.6
Klebsiella oxytoca	7	0.6			
Enterococcus faecalis	93	8.3	*Enterococcus* spp.	129	11.5
Enterococcus faecium	33	2.9			
Enterococcus raffinosus	2	0.2			
Enterococcus avium	1	0.1			
Proteus mirabilis	37	3.2	*Proteus* spp.	44	3.9
Proteus vulgaris	7	0.6			
Pseudomonas aeruginosa	28	2.5	*Pseudomonas* spp.	28	2.5
Enterobacter cloacae	13	1.2	*Enterobacter* spp.	18	1.6
Enterobacter aerogenes	5	0.4			
Morganella morganii	16	1.4	*Morganella* spp.	16	1.4
Citrobacter freundii	6	0.5	*Citrobacter* spp	8	0.7
Citrobacter amalonaticus	2	0.2			
Staphylococcus aureus	2	0.2	*Staphylococcus* spp.	4	0.4
Coagulase negative staphylococcus	2	0.2			
Acinetobacter baumanii complex	3	0.3	*Acinetobacter* spp.	3	0.3
Serratia marcescens	1	0.1	*Serratia* spp.	1	0.1

Enterobacteriaceae. The antimicrobials with the highest susceptibility in this group were meropenem, imipenem and ertapenem with rates of 99.2%, 97.1% and 96.1% respectively. The antimicrobial susceptibility rates of microorganisms according to extended-spectrum beta-lactamase positivity are shown in Table II.

Enterococcus spp. showed the highest resistance to gentamycin with 50% resistance in tested cases. None of the tested cases showed resistance to vancomycin or linezolid. *Pseudomonas* spp. with 64.3% susceptibility, showed the highest resistance to piperacillin-tazobactam. With a rate of 96.4%, *Pseudomonas* spp. showed the highest

Table II. *In vitro* antimicrobial resistance patterns of *Enterobacteriaceae* to common antimicrobial agents according to ESBL positivity

			% Antimicrobial susceptibility of isolated microorganisms							
			***Enterobacteriaceae* n=962**		***Escherichia* spp. n=666**		***Klebsiella* spp. n=209**		***Proteus* spp. n=44**	
Antimicrobial agent			n=340	n=622	n=246	n=420	n=85	n=124	n=2	n=42
Nitrofurantoin	Total		90.8		96.8		83.6		54.5	
	ESBL+	ESBL-	92.8	89.7	96.1	97.2	87.0	80.4	50.0	54.8
Ampicillin	Total		18.6		25.3		0.0		20.5	
	ESBL+	ESBL-	1.5	28.1	1.6	39.1	0.0	0.0	50.0	19.0
Amoxicillin-Clavulanate	Total		61.2		61.4		64.1		79.5	
	ESBL+	ESBL-	37.4	74.5	34.8	77.1	44.7	77.6	100.0	78.6
Piperacillin-Tazobactam	Total		61.4		63.7		46.9		97.7	
	ESBL+	ESBL-	40.9	72.9	44.3	75.2	29.4	59.2	100.0	97.6
Cefuroxime	Total		39.6		44.2		22.5		79.5	
	ESBL+	ESBL-	3.2	59.8	3.7	68.8	1.2	36.8	50.0	81.0

Table II. Continued

			% Antimicrobial susceptibility of isolated microorganisms							
			***Enterobacteriaceae* n=962**		***Escherichia* spp. n=666**		***Klebsiella* spp. n=209**		***Proteus* spp. n=44**	
Ceftriaxone	Total		43.2		45.1		26.3		69	
	ESBL+	ESBL-	2.9	65.4	2	70.4	4.7	41.6	50.0	90.5
Cefixime	Total		90.9		91.5		88.4		100.0	
	ESBL+	ESBL-	82.5	95.5	84.5	95.5	77.9	96.2	100.0	100.0
Amikacin	Total		85.9		86.3		82.3		93.2	
	ESBL+	ESBL-	76.2	91.1	77.5	91.4	71.8	89.7	100.0	92.9
Gentamycin	Total		68.0		67.7		65.6		70.5	
	ESBL+	ESBL-	47.6	79.0	46.3	80.0	50.6	76.9	50.0	71.4
Trimethoprim-Sulfamethoxazole	Total		49.0		50.0		42.1		36.4	
	ESBL+	ESBL-	29.7	59.5	29.9	61.7	22.3	56.2	50.0	35.7
Imipenem	Total		97.1		100.0		95.7		70.5	
	ESBL+	ESBL-	98.5	96.3	100.0	100.0	100.0	92.8	0.0	73.8
Meropenem	Total		99.2		100.0		96.6		100.0	
	ESBL+	ESBL-	99.7	98.9	100.0	100.0	100.0	94.4	100.0	100.0
Ertapenem	Total		96.1		98.5		88.9		100.0	
	ESBL+	ESBL-	95.6	96.6	97.5	99.3	91.8	87.1	100.0	100.0

ESBL: Extended-spectrum beta-lactamase

susceptibility to imipenem and meropenem. The *in vitro* antimicrobial susceptibility rates of *Pseudomonas* spp. to common antimicrobials are shown in Table III.

Table III. Antimicrobial susceptibility rates of *Pseudomonas* spp.

Antimicrobials	**% Antimicrobial susceptibility**
	***Pseudomonas* spp. n=28**
Piperacillin-Tazobactam	64.3
Amikacin	89.3
Gentamycin	89.3
Imipenem	96.4
Meropenem	96.4
Ciprofloxacin	89.3
Ceftazidime	89.3
Cefepime	89.3

Discussion

Bacterial resistance is an important challenging problem in UTI. Antimicrobial-resistant infections have been predicted to reach 10 million lives per year by 2050 (6). Especially in the paediatric population, antimicrobial prescriptions are higher, mostly in cases of over-diagnosed viral upper respiratory infections. If paediatricians have a symptomatic child with a positive urinalysis, it is inevitable to start the initial treatment of acute UTI without obtaining culture results (6-8). Certainly, early diagnosis and proper treatment is important, not only to prevent complications such as renal abscess or septicaemia, but also it is crucial to avoid renal scarring, and even renal failure (9). Additionally, the loss of schooltime as well as the loss of parental worktime are the indirect costs which cannot be underestimated (10,11).

The increment in the resistance to common antimicrobials, especially in paediatric uropathogens, has led to challenges due to the more difficult treatment of UTI over time (9). Antimicrobial resistance has variabilities such as geographic differences in bacterial patterns and local antimicrobial prescription practices (7,12-14).

The frequency of *E. coli* has been reported as having a wide range in the literature. Rai et al. (15) reported *E. coli* at a very high level of 93.3%. We also found that *E. coli* was the most common organism isolated with 59.1%, which was quite low compared to their paper. Other studies such as Akram et al. (16) reported a lower prevalence (21.4%) of

E. coli. *E. coli* is reported as the most common pathogen in those studies conducted in the adult population. While the prevalence of *E. coli* in UTI was reported as 50% in a multicentre study conducted in China, in a meta-analysis conducted in Iran, the most common pathogen causing UTI was reported to be *E. coli* with a frequency of 62% (17,18). Catal et al. (12) demonstrated an increasing rate of *Klebsiella* spp. in urine cultures between 2000 and 2006, with rates of 7.2% and 18%, respectively. Gunduz and Uludağ Altun (19) reported the *Klebsiella* spp. rate to be 14.9%. In this study, we have a higher rate (18.6%) of *Klebsiella* spp. Unfortunately, *non-E. coli* pathogens are more resistant to most antimicrobial agents. Therefore, those patients with *Klebsiella* spp. needed hospitalization and parenteral antibiotic therapy also increases the treatment costs (20).

The current American Academy of Pediatrics guideline suggests giving oral or parenteral antimicrobials for 7-14 days for the management of UTIs in febrile infants and young children. Ceftriaxone, cefotaxime, ceftazidime, gentamicin, tobramycin, and piperacillin are the drugs of choice for parenteral therapy; amoxicillin-clavulanate, sulfonamide (trimethoprim-sulfamethoxazole or sulfisoxazole), or cephalosporin (cefixime, cefpodoxime, cefprozil, cefuroxime axetil, or cephalexin) are recommended as oral agents for treating UTI (7,8). For an antimicrobial to be considered as a first-line empirical treatment for UTI, resistance should not exceed 20% (13). In a meta-analysis conducted by Bryce et al. (14), this threshold was reached for many first-line antimicrobials used for paediatric *E. coli* UTIs. In most countries, half of all isolates were resistant to ampicillin, 1/3 to co-trimoxazole, and 1/4 to trimethoprim. This supports the increased risk of *E. coli* resistance to that particular antimicrobial due to previous antimicrobial usage (14). In our study, we also had similar resistance results with the exception of increased rates to cefuroxime. Our results showed the first resistance rates to ampicillin, cefuroxime, and trimethoprim-sulfamethoxazole, which have the highest resistance for *Enterobacteriaceae,* with resistance rates of 81.4%, 58.8% and 51.0% respectively.

Gunduz and Uludağ Altun (19) reported lower resistance rates of *E. coli* to ampicillin, co-trimoxazole, ceftriaxone, amikacin, amoxicillin-clavulanate and the same resistance rates for gentamicin, nitrofurantoin, and ciprofloxacin in comparison to Bryce's meta-analysis. Al Benwan and Jamal (21) also showed that approximately 62% of *E. coli* and 77% of *non-Coliform* bacteria were resistant to ampicillin, similar to our study. Dejonckheere et al. (22) also reported that resistance to amoxicillin-clavulanic acid for *E. coli* strains had increased from 16% to 36% (2015-2019) over the last 20 years. They pointed out that the problem of increasing antimicrobial resistance to antimicrobials commonly used in UTIs is a growing health problem (22). Although it is known that *in vivo* antibiotic responses are important in the treatment of UTI, it is thought that the development of *in vitro* antimicrobial resistance may pose a risk for *in vivo* responses in the coming years.

Study Limitations

The retrospective nature of this study is its limitation.

Conclusion

This study, in line with the literature, shows that resistance to commonly used antimicrobials is still very important. This highlights the necessity of planning in this regard.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Medical Research Ethics Committee of Ege University Faculty of Medicine (approval no: 22-12.1T/16, date: 16.12.2022).

Informed Consent: Written informed consent was obtained from all the patients or their parents/guardians.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

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References

1. Okarska-Napierała M, Wasilewska A, Kuchar E. Urinary tract infection in children: Diagnosis, treatment, imaging - Comparison of current guidelines. J Pediatr Urol 2017;13:567-73.
2. Schmidt B, Copp HL. Work-up of Pediatric Urinary Tract Infection. Urol Clin North Am 2015;42:519-26.
3. Stein R, Dogan HS, Hoebeke P, et al. Urinary tract infections in children: EAU/ESPU guidelines. Eur Urol 2015;67:546-58.
4. Hodson EM, Willis NS, Craig JC. Antibiotics for acute pyelonephritis in children. Cochrane Database Syst Rev 2007;(4):CD003772.
5. Abejew AA, Denboba AA, Mekonnen AG. Prevalence and antibiotic resistance pattern of urinary tract bacterial infections in Dessie area, North-East Ethiopia. BMC Res Notes 2014;7:687.

6. Elder JS. Urologic disorders in infants and children. In: Behrman RE, Kliegman RM, Jenson HB (eds). Nelson textbook of pediatrics 18th ed. Philadelphia, WB Saunders, 2010;1829-34.

7. Alberici I, Bayazit AK, Drozdz D, et al. Pathogens causing urinary tract infections in infants: a European overview by the ESCAPE study group. Eur J Pediatr 2015;174:783-90.

8. Yolbaş I, Tekin R, Kelekci S, et al. Community-acquired urinary tract infections in children: pathogens, antibiotic susceptibility and seasonal changes. Eur Rev Med Pharmacol Sci 2013;17:971-6.

9. Ipek IO, Bozaykut A, Arman DC, Sezer RG. Antimicrobial resistance patterns of uropathogens among children in Istanbul, Turkey. Southeast Asian J Trop Med Public Health 2011;42:355-62.

10. Çoban B, Ülkü N, Kaplan H, Topal B, Erdoğan H, Baskın E. Five-year assessment of causative agents and antibiotic resistances in urinary tract infections. Turk Pediatri Ars 2014;49:124-9.

11. Senel S, Karacan C, Erkek N, Göl N. A single-center experience of antimicrobial resistance patterns in pediatric urinary tract infection. Med Princ Pract 2010;19:359-63.

12. Catal F, Bavbek N, Bayrak O, et al. Antimicrobial resistance patterns of urinary tract pathogens and rationale for empirical therapy in Turkish children for the years 2000-2006. Int Urol Nephrol 2009;41:953-7.

13. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52:103-20.

14. Bryce A, Hay AD, Lane IF, Thornton HV, Wootton M, Costelloe C. Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. BMJ 2016;352:939.

15. Rai S, Pant ND, Bhandari R, et al. AmpC and extended spectrum beta-lactamases production among urinary isolates from a tertiary care hospital in Lalitpur, Nepal. BMC Res Notes 2017;10:467.

16. Akram M, Shakil S, Khan AU. Prevalence of integrons, blaCTX-M and blaTEM resistance markers among ESBL-producing uropathogenic Escherichia coli isolates: first report of genomic blaCTX-M from India. J Chemother 2011;23:131-4.

17. Qiao LD, Chen S, Yang Y, et al. Characteristics of urinary tract infection pathogens and their in vitro susceptibility to antimicrobial agents in China: data from a multicenter study. BMJ Open 2013;3:e004152.

18. Mortazavi-Tabatabaei SAR, Ghaderkhani J, Nazari A, Sayehmiri K, Sayehmiri F, Pakzad I. Pattern of Antibacterial Resistance in Urinary Tract Infections: A Systematic Review and Meta-analysis. Int J Prev Med 2019;10:169.

19. Gunduz S, Uludağ Altun H. Antibiotic resistance patterns of urinary tract pathogens in Turkish children. Glob Health Res Policy 2018;3:10.

20. Marcus N, Ashkenazi S, Yaari A, Samra Z, Livni G. Non-Escherichia coli versus Escherichia coli community-acquired urinary tract infections in children hospitalized in a tertiary center: relative frequency, risk factors, antimicrobial resistance and outcome. Pediatr Infect Dis J 2005;24:581-5.

21. Al Benwan K, Jamal W. Etiology and Antibiotic Susceptibility Patterns of Urinary Tract Infections in Children in a General Hospital in Kuwait: A 5-Year Retrospective Study. Med Princ Pract 2022;31:562-9.

22. Dejonckheere Y, Desmet S, Knops N. A study of the 20-year evolution of antimicrobial resistance patterns of pediatric urinary tract infections in a single center. Eur J Pediatr 2022;181:3271-81.

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Original Article

Evaluation of the Etiological Factors of Black Tooth Stain in Children

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ABSTRACT

Aim: Tooth discoloration is a common clinical finding which is considered primarily as an aesthetic problem. Black stain (BS) is a specific type of extrinsic tooth discoloration mostly seen in children, but also in adults and it is not dependent on gender. The present study aimed to investigate the relationships between the presence of BS and dental caries incidence, dental plaque scores and to examine the colonization of *Streptococcus mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *Capnocytophaga* spp. in dental plaque samples with or without BS. The socioeconomic status of the family, the oral hygiene and dietary habits of the children, and the medical and dental history of the children were also compared between the two groups.

Materials and Methods: A total of 1000 children aged 3-12 years were evaluated to take part in this study. From this group, those children with BS (n=44) were selected as the study group. With the same number as the study group, and with a same age and gender profile, 44 children without BS were selected as a control group. Dental examinations including the presence of BS, dental caries incidence and dental plaque scores were performed by the same investigator. Structured questionnaires were completed by the parents. The levels of *S. mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *Capnocytophaga* spp. were determined from dental plaque samples. All data were analyzed by SPPS 25.0 using Student's t-test, the Mann-Whitney U, Fisher's exact and the chi-squared tests.

Results: BS was detected in 4.4% of the patients in the present study. DMFT and DMFS scores were significantly lower in those children with BS than in those without BS (p=0.001 and p=0.010). However, no statistically significant difference was found between dmft and dmfs scores and the presence of BS (p>0.05). Lower numbers of *S. mutans* and *Lactobacillus* spp. and greater numbers of *Actinomyces* spp. and *Capnocytophaga* spp. were found in those children with BS. There was no statistically significant relationship between *S. mutans* and *Actinomyces* spp. and the presence of BS (p>0.05). Colonizations of *Lactobacillus* spp. were statistically significantly lower, while colonizations of *Capnocytophaga* spp. were significantly higher in the BS group than in the control group (p<0.05).

Conclusion: It could be suggested that the different microbial composition of BS might be associated with lower caries experiences in affected subjects.

Keywords: Black stain, extrinsic, microbiology, dental caries

Introduction

Tooth discoloration is a common clinical finding which is considered primarily as an aesthetic problem. It can be influenced by many different factors and usually differs in etiology, appearance, composition, location and degree of adherence (1). According to its location, it is divided into three groups, namely intrinsic, extrinsic or internalized stains. Intrinsic stains occur when the pigmented material

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penetrates into the tooth structure. Extrinsic stains are inserts on the tooth surface or on the acquired pellicle. Internalized stains are formed by the inclusion of extrinsic stains on the tooth surface together with tooth development (2,3).

Black stain (BS) is a characteristic extrinsic discoloration which is defined as a dark line or incomplete coalescence on the tooth surface following the gingival margin in the cervical third of the tooth. BS is a common finding in children; however, it can also be seen in adults. Studies have shown equal prevalence in both sexes. The prevalence of BS varies between 1.6% and 21% because of the unspecified criteria used for diagnosis and the different populations included in studies (4-6).

Black tooth stains are considered as a dental plaque form which contains insoluble iron salt and high calcium and phosphate content. BS material is a ferric sulfate formed by the interaction of iron in saliva or gingival fluid with hydrogen sulfide produced by bacteria in the periodontal ligament (1,2,4).

Dietary habits may also play a role in its etiology. The consumption of vegetables, fruits, dairy products, eggs, and soy sauce promotes BS development. Drinking tap water instead of bottled mineral or natural spring water also seems to be associated with higher prevalences of BS (4).

Gram-positive rods and chromogenic bacteria have often been found to be associated with BS (1,2). In terms of microbial diversity and abundance, *Actinomyces, Cardiobacterium, Haemophilus, Corynebacterium, Capnocytophaga, Tannerella* and *Treponema* spp. levels were higher in those children with BS than in those without BS (2,4).

Most of the authors showed that the presence of BS is associated with a lower caries experience. The causative factors of BS are not fully understood. Certain types of bacteria seem to be involved in its etiology. It is not clear how the presence of BS on the tooth surface reduces susceptibility to caries. The relationship between the presence of BS in children and their experience of low dental caries has made the characterization of factors which contribute to the formation and nature of BS more important. When studies on the etiology of caries are examined independently from BS, it is noteworthy that there is an inverse correlation between cariogenic *Streptococci* and *Capnocytophaga* spp. Oral *Capnocytophaga* might prevent the proliferation of certain caries-causing organisms such as *S. mutans* (7,8).

However, despite this inhibitory activity, the relationship between the presence of *Capnocytophaga* spp. and the low caries indices of children with BS has not been investigated to date. Although BS does not cause any pathology, treatment is usually carried out for aesthetic reasons. The professional polishing process on the tooth surface eliminates the BS of the teeth, but these stains can re-occur over time.

The aim of this study was to evaluate the prevalence of black tooth stain among children aged 3-12 years who applied for routine dental examination and to determine any relationship between the presence of BS and dental caries incidence, dental plaque scores and to examine the colonization of *Streptococcus* mutans, *Lactobacillus* spp., *Actinomyces* spp. and *Capnocytophaga* spp. in dental plaque samples with or without BS. The socio-economic status of the families, the oral hygiene and dietary habits of the children, and the medical and dental history of the children were also compared between the two groups.

Materials and Methods

A total of 1000 children aged 3-12 years who applied to the Department of Pedodontics, Ege University Faculty of Dentistry, for routine dental examination were enrolled in this study. From this group, those children with BS (n=44) were selected as the study group. With the same number as the study group, and with the same age and sex profile, 44 children without BS were selected as a control group. Ethical approval was obtained from the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no: 17-4/18, date: 20.04.2017) and written informed consent was acquired from each parent. The parents were interviewed based on a structured questionnaire including questions on the socio-economic status of the family, the oral hygiene and dietary habits of the children, and the medical and dental history of the children.

Those children who had taken antibiotic therapy within the 3 months prior to the dental plaque sampling and those children with any systemic disease were not included in this study.

Dental Examination

The presence and grading of BS, dental caries incidence and dental plaque scores were recorded by the same pediatric dentist (G.İ.). The dental examinations of the children were conducted under natural light with the aid of a dental mirror and explorer. The dental caries scores were recorded according to World Health Organization criteria using DMFT/DMFS and dmft/dmfs indices (9). All of the children were classified according to their DMFT/dmft scores; Group-1: caries active (dmft+DMFT≥1), Group-2: caries-free (dmft+DMFT=0). The dental plaque scores were recorded according to the Sillness and Löe (10) index.

The BS scores were classified according to the work of Gasparetto et al. (8).

The classifications of Gasparetto et al. (8):

Score 1: Presence of pigmented dots or thin lines with incomplete coalescence parallel to the gingival margin

Score 2: Continuous pigmented lines, which are easily observed and limited to half of the cervical third of the tooth surface

Score 3: Presence of pigmented stains extending beyond half of the cervical third of the tooth surface.

Dental Plaque Sampling

Dental plaque samples from those children with BS (study group) and those without BS (control group) were taken. The sampling was performed 2 hours after breakfast between 9.00-11.00 a.m. The dental plaque samples were gently collected with a sterile dental curette and placed into Eppendorf tubes containing Stuart transport medium. All Eppendorf tubes were weighed before and after sampling (Ohaus, Adventurer, AR3130). The samples were transferred to a laboratory on ice. All microbiological procedures were performed at Ege University Faculty of Science, Department of Basic and Industrial Microbiology Department Laboratory.

Isolation and Identification of Microorganisms

The plaque samples were dispersed in a vortex mixer in order to obtain a homogeneous suspension and cultivated on selective VCAT medium (Oxoid) for *Capnocytophaga* spp., mitis salivarius agar (Difco) with 15% sucrose (Difco) and 0.2 units/mL of bacitracin (Sigma, Sigma-Aldrich Co., St Louis, MO, USA) for *S. mutans*, MRS Agar was used for the isolation of *Lactobacilli* spp. and Actinomyces Selective Agar was used for the isolation of *Actinomyces* spp.

All of the plates were incubated for 2-5 days at 37 °C in 8% CO_2. Suspected colonies were counted, and two bacterial isolates were recovered from each of the cultivation media for the identification and verification of the isolates. Suspected *Capnocytophaga* colonies were then transferred to McConkey agar plates and incubated under the same conditions. The identification of the representative isolates were performed using the VITEK II identification system (Biomerioux, France) in addition to microscopic and cultural examinations. VITEK NH card panels (Biomèrieux) and VITEK GP Card panels were used for the identification of *Capnocytophaga* spp. and *S. mutans* isolates, respectively.

Statistical Analysis

All data were analyzed by SPPS 25.0 (SPSS Statistics for Windows, Armonk, NY: IBM Corp.). In the analysis of data, the t-test and Mann-Whitney U test were used for the comparison of the two groups. Categorical data were analyzed by Fisher's exact test and the chi-squared test. For the significance level of the tests, $p<0.05$ and $p<0.01$ were accepted.

Results

In this study, 480 girls and 520 boys aged between 3-12 years who attended the clinic for routine dental examination were evaluated. From these 1000 children, BS was detected in 44 children (4.4%) and this group was categorized as the study group. The control group (n=44) was randomly selected from the same group with the same age and gender profile.

No statistically significant relationship was found between the presence of BS and gender, sex and the plaque scores ($p>0.05$).

No statistically significant relationship was found between socio-economic factors (education levels of the mother and father, family income) and the presence of BS ($p>0.05$).

No statistically significant relationship was found between medical history (systemic disease, drug usage, vitamin intake, breastfeeding and formula intake in infancy) and the presence of BS ($p>0.05$).

No statistically significant relationship was found between dental history (previous dental visits, previous dental treatment, tooth brushing habits, usage of toothpaste, fluoride content of the toothpaste, professional fluoride applications) and the presence of BS ($p>0.05$).

No statistically significant relationship was found between dietary habits (eating before sleeping, keeping food in the mouth, the number of main and intermediate meals, drinking water source, frequency of the consumption of milk, yogurt, buttermilk, probiotics, meat, chicken, fish, eggs, vegetables, fruit, bread, biscuits, wafers and chocolates) ($p>0.05$). A negative correlation was found between the presence of BS and fizzy drink consumption ($p=0.035$).

The caries scores of those children with or those without BS is presented in Table I. DMFT and DMFS scores were lower in those children with BS than in those without BS and this relation was statistically significant ($p=0.001$ and $p=0.010$). However, no statistically significant difference was found between the dmft and dmfs values and the presence of BS ($p>0.05$) (Table I).

Bacterial counts are shown in Table II. A lower number of *S. mutans* and *Lactobacillus* spp. and a greater number of *Actinomyces* and *Capnocytophaga* spp. were found in those children with BS. However, there was no statistically significant relation between *S. mutans* and *Actinomyces* spp. and the presence of BS (p>0.05). The colonizations of *Lactobacillus* spp. were statistically significantly lower while the colonizations of *Capnocytophaga* spp. were significantly higher in the BS group than in the control group (p<0.05). No significant relationship was found between the caries scores and colonizations of *S. mutans*, *Lactobacillus* spp., *Actinomyces* and *Capnocytophaga* spp. (p>0.05).

Table I. The relationship between the presence of black tooth stain and caries index scores

	BS (-)		BS (+)		p-value
	Average	**SD**	**Average**	**SD**	
DMFT	2.67	2.98	0.89	1.43	**0.001***
dmft	4.60	4.23	3.86	3.83	>0.05
DMFS	4.65	6.31	2.23	4.25	**0.01***
dmfs	10.74	9.60	7.95	8.59	>0.05

*p<0.05 Statistically significant
SD: Standard deviation, BS: Black stain

Table II. The relationship between the presence of black tooth stain and microorganism

CFU/mL	BS (-)	BS (+)	p-value
S. mutans	$6.8x10^5 \pm 1.0x10^5$	$4.3x10^5 \pm 1.8x10^5$	>0.05
Lactobacillus spp.	$1.1x10^5 \pm 8.0x10^4$	$4.0x10^4 \pm 2.0x10^4$	**0.002***
Actinomyces spp.	$2.0x10^7 \pm 1.1x10^7$	$2.4x10^7 \pm 1.7x10^7$	>0.05
Capnocytophaga spp.	$4.2x10^6 \pm 2.3x10^6$	$2.2x10^6 \pm 1.3x10^6$	**0.01***

*p<0.05 Statistically significant
BS: Black stain

Discussion

Tooth discoloration is a common dental finding and it is associated with clinical and esthetic problems. The clinical diagnosis of BS is based on the presence of pigmented dark lines parallel to the gingival margin or an incomplete coalescence of dark dots rarely extending beyond the cervical third of the crown (1-3).

The prevalence of BS ranges from 1.6% to 21% in the literature (4-6). The prevalence of BS was found to be 4.4% (n=44/1000) in the present study. The differences in the prevalences of BS between studies may be due to differences in age, diet and oral hygiene habits, microbiological differences and diagnostic criteria and also the quantitative characteristics of the groups.

In this study, no statistically significant relationship was found between gender and black tooth stain prevalence. Garcia Martin et al. (11), Akyüz et al. (12), Chen et al. (6), and França-Pinto et al. (13) also did not find any relationship between gender and black tooth stain.

In the present study, there was no statistically significant difference between the socio-demographic factors and the presence of black tooth stain, dental caries and dental plaque scores (p>0.05). Akyüz et al. (12) also did not find any relationship between the socio-demographic factors and the presence of BS. Chen et al. (6) reported a negative correlation between the education levels of the parents and the presence of BS.

No statistically significant correlation was found between the medical and dental history of the children and the presence of BS (p>0.05). The results of the study of Chen et al. (6) were also in accordance with our findings. They did not report any significant correlation between the systemic disease and drug usage and the presence of BS.

It has been reported that dietary habits may also play a role in the etiology of BS. The consumption of vegetables, fruits, dairy products, eggs, and soy sauce promotes BS development. No significant correlation was detected between dietary habits and the presence of BS in the present study (p>0.05). A positive correlation was detected between the consumption of fizzy drink and the presence of BS (p=0.035). Children who had been fed with formula during infancy tend to have higher BS occurrence (6,11). No significant correlation was found between feeding with formula during infancy and the presence of BS in the present study (p>0.05). Drinking tap water instead of bottled mineral or natural spring water also seems to be associated with a higher prevalence of BS (13). No significant correlation was found between the source of drinking water and the presence of BS in the present study (p>0.05). The content of the formula and tap water might explain the different results of these different studies.

The DMFT and DMFS scores in those children with BS were lower than in those without BS and this relationship was found to be statistically significant in the present study (p=0.001 and p=0.010). However, there was no statistically significant relationship between dmft and dmfs scores and BS (p>0.05). Most of the authors have shown that the presence of BS is associated with a lower caries experience. It has been assumed that the presence of BS is associated

with a predominance of *Actinomyces* spp.. Immunological studies and investigations on bacterial adhesion found that high levels of *Actinomyces naeslundii* in biofilms on teeth correlated with lower caries experiences and lower *Streptococcus mutans* adhesion. This may explain from another perspective why those children with BS had lower caries prevalences (3,14).

A lower number of *S. mutans* and *Lactobacilli* spp. and a greater number of *Actinomyces* spp. were found in those children with BS. There was no statistically significant relationship between *S. mutans* and *Actinomyces* spp. and the presence of BS ($p>0.05$). However, the relationship between *Lactobacilli* spp. and BS was statistically significant ($p=0.002$). No significant relationship was found between the caries scores and colonization of *S. mutans*, *Lactobacilli* spp. and *Actinomyces* spp. ($p>0.05$).

It has been reported that those children with BS have a lower caries incidence, but the cause of this phenomenon is still not fully understood. The thin black-brown lines on the teeth were observed and it has been suggested that this is a sign for a low caries index (3,8). Many other recent studies also support an inverse relationship between BS and tooth caries (5,7,8).

Capnocytophaga spp. are prevalent members of the BS microbiota but their role in the etiology of BS or their relation between the low caries frequency in children with BS has not been investigated yet. *Capnocytophaga* spp. can produce bacteriocins which can inhibit the growth of other bacteria including oral *Streptococci* which cause caries (15,16).

A lower caries index was found in those children with BS in the present study. *Capnocytophaga* spp. levels were statistically higher in those children with BS ($p=0.01$). Although *Capnocytophaga* members themselves are opportunistic pathogens, they can show an antagonist activity against persistent *S. mutans,* and thus, may reduce the caries index. Furthermore, co-aggregation studies between *Streptococcus* spp. and *Actinomyces* spp. showed that the interbacterial adhesion between these two bacteria improved earlier dental plaque biofilm formation. Earlier colonizers provide specific binding sites for other bacteria and can improve biofilm progress directly or by the saliva glycoproteins which bind to the pioneer organisms (17). *Capnocytophaga* members also exhibits a co-aggregation property, this co-aggregation may also prevent the attachment of persister *S. mutans* to the BS. Consequently, the *Capnocytophaga* abundance in BS may affect *S. mutans* cells through proposed or unknown mechanisms and prevent caries progress.

Study Limitations

In the present study based on 1000 children, BS was detected in 44 children (4.4%). The microbiological evaluation was determined in these patients alone. Further studies with a larger number of subjects may support our results.

Conclusion

Capnocytophaga spp. levels in children with BS were significantly higher when compared to the control group. Higher levels of *Capnocytophaga* directly or indirectly may prevent caries formation. This should be clarified by further studies which can reveal whether *S. mutans* in children with BS are persisters or whether *Capnocytophaga* members actually inhibit the proliferation of *S. mutans*. The identification of *Capnocytophaga* isolates at species level with metagenomic studies may also contribute to identify this link.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no: 17-4/18, date: 20.04.2017).

Informed Consent: Written informed consent was acquired from each parent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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References

1. Ronay V, Attin T. Black stain - a review. Oral Health Prev Dent. 2011; 9:37-45.
2. Li Y, Zhang Q, Zhang F, Liu R, Liu H, Chen F. Analysis of the Microbiota of Black Stain in the Primary Dentition. PLoS One 2015; 10:e0137030.
3. Żyła T, Kawala B, Antoszewska-Smith J, Kawala M. Black stain and dental caries: a review of the literature. Biomed Res Int2015; 2015:469392.
4. Prskalo K, Klarić Sever E, Alerić I, Antonić Jelić T, Žaja I. Risk Factors Associated with Black Tooth Stain. Acta Clin Croat 2017; 56:28-35.
5. de Rezende VS, Fonseca-Silva T, Drumond CL, Ramos-Jorge ML, Paiva SM, Vieira-Andrade RG. Do Patients with Extrinsic Black Tooth Stains Have a Lower Dental Caries Experience? A Systematic Review and Meta-Analysis. Caries Res 2019; 53:617-27.
6. Chen X, Zhan J, Lu HX, et al. Factors associated with black tooth stain in Chinese preschool children. Clin Oral Investig 2014; 18:2059-66.
7. Jolivet-Gougeon A, Sixou JL, Tamanai-Shacoori Z, Bonnaure-Mallet M. Antimicrobial treatment of Capnocytophaga infections. Int J Antimicrob Agents 2007; 29:367-73.
8. Gasparetto A, Conrado CA, Maciel SM, Miyamoto EY, Chicarelli M, Zanata RL. Prevalence of black tooth stains and dental caries in Brazilian schoolchildren. Braz Dent J 2003; 14:157-61.
9. World Health Organization. Oral health surveys: basic methods. 4th ed. Geneva, 1997.
10. Sillness J, Löe H. Periodontal Disease in Pregnancy II. Correlation Between Oral Hygiene and Periodontal Condition. Acta Odontol Scand 1964; 22:121-35.
11. Garcia Martin JM, Gonzalez Garcia M, Seoane Leston J, Llorente Pendas S, Diaz Martin JJ, Garcia-Pola MJ. Prevalence of black stain and associated risk factors in preschool Spanish children. Pediatr Int 2013; 55:355-9.
12. Akyüz S, Garan A, Kaya MS. Prevalence of Black Stain and Dental Caries in Children Attending a University Pediatric Dentistry Clinic in Istanbul. Marmara Clin Exp Health Sci 2015; 5:109-14.
13. França-Pinto CC, Cenci MS, Correa MB, et al. Association between black stains and dental caries in primary teeth: findings from a Brazilian population-based birth cohort. Caries Res 2012; 46:170-6.
14. Heinrich-Weltzien R, Monse B, van Palenstein Helderman W. Black stain and dental caries in Filipino schoolchildren. Community Dent Oral Epidemiol 2009; 37:182-7.
15. Ehrmann E, Jolivet-Gougeon A, Bonnaure-Mallet M, Fosse T. Antibiotic content of selective culture media for isolation of Capnocytophaga species from oral polymicrobial samples. Lett Appl Microbiol 2013; 57:303-9.
16. Nakamura T, Shibata Y, Shimura R, Fujimura S. Isolation and properties of the Capnocytophaga ochracea bacteriocin. Oral Microbiol Immunol 1992; 7:96-9.
17. Okuda T, Okuda K, Kokubu E, Kawana T, Saito A, Ishihara K. Synergistic effect on biofilm formation between Fusobacterium nucleatum and Capnocytophaga ochracea. Anaerobe 2012; 18:157-61.

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Original Article

Evaluation of Electrocardiographic Changes in Girls Receiving Gonadotropin-Releasing Hormone Analogs for Precocious Puberty

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ABSTRACT

Aim: Gonadotropin-releasing hormone analogs (GnRHa) are standard medical treatments for precocious puberty. Studies on their side effects in adults have shown that these drugs can cause changes in electrocardiography (ECG), along with some cardiovascular effects; however, the number of studies on children is limited. This study investigated the effects of these drugs on ECG parameters in children diagnosed with central precocious puberty (CPP).

Materials and Methods: This prospective study included 44 girls who were initiated GnRHa treatment and diagnosed with CPP. ECG was performed before treatment and repeated after 6 months of treatment.

Results: The mean age of the children was 9.13±1.55 years. Leuprolide acetate (3.75 mg IM) was administered to all of the patients following the standard protocol. A comparison of the pre-treatment and 6-month ECG parameters revealed a prolonged QT interval after treatment, with a statistically significant difference ($p<0.001$). There were no significant differences in the pre- and post-treatment values of PR, QRS, QT interval, QTc interval, QT dispersion, or QTc dispersion ($p>0.05$).

Conclusion: Despite a significant increase in QT interval on ECG with GnRHa compared to pre-treatment ECGs, this increase was attributed to a variability in heart rate. Even if regular ECG monitoring is considered after initiation of GnRHa treatment, they are believed to be safe drugs in children.

Keywords: ECG, GnRH agonist, precocious puberty, QT, QTc, QT dispersion, QTc dispersion

Introduction

Precocious puberty is defined as the onset of secondary sexual development before 8 years of age in girls and 9 years of age in boys, and its treatment depends on its underlying cause. Central precocious puberty (CPP) results from the premature maturation of the hypothalamic-pituitary-gonadal axis. It is characterized by sequential maturation of the breast bud and pelvic hair in girls and maturation of the testicles, penis, and pubic hair in boys. While CPP is idiopathic in 80-90% of girls, intracranial lesions are identified in 40-75% of boys with CPP (1,2). If necessary, the progression of puberty in CPP can be halted by administering gonadotropin-releasing hormone analogs (GnRHa). GnRHa acts by providing sustained stimulation to the pituitary gonadotropes rather than physiological

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pulsatile stimulation from hypothalamic GnRH. This results in the continuous stimulation of gonadotroph cells and leads to desensitization and suppression of gonadotropins. This results in a decrease in sex steroid production, which is called the suppression of the pituitary-gonadal axis (3). There are different pharmacological forms of GnRHa, including triptorelin, leuprolide, buserelin, nafarelin, and goserelin. In Turkey, only leuprolide is within the scope of reimbursement; therefore, this single agent is used for patients. There are two forms of leuprolid acetate: once every 4 weeks and once every 3 months (4).

The risk of GnRHa treatment causing a prolonged QT interval was reported during the treatment of men with prostate cancer, and it was suggested to be associated with changes in circulating testosterone concentrations (5). There have been no reports of prolonged QT intervals in women using GnRHa (6). In the current literature, the GnRHa reported to increase the risk of a prolonged QT interval are leuprolide and degarelix (7). However, it remains unclear whether this is a class effect and may indicate an increased risk for other GnRHas. Among the conditions treated with GnRHa in pediatrics, gender dysphoria in adolescent males is the clinical condition most similar to that in which the risk of prolonged QT interval with GnRHa has been reported (8). The number of pediatric studies in this field is limited.

This study aimed to evaluate the effects of GnRHa on electrocardiogram (ECG) parameters in girls diagnosed with precocious puberty.

Materials and Methods

This was a prospective study which included 44 girls who presented to the Adana City Training and Research Hospital, Clinic of Pediatric Endocrinology between April, 2020 and December, 2021, and who were initiated on the treatment of GnRH agonist with a diagnosis of CPP. Ethical approval for this study was obtained from the Clinical Research Ethics Committee of Adana City Training and Research Hospital (approval no: 1459, date: 17.06.2021). This study was initiated after obtaining written consent from all the participants and/or their families. The inclusion criteria were girls followed up with a diagnosis of idiopathic central precocious puberty and treatment with GnRHa (leuprolide acetate) 3.75 mg/28 days. In this tertiary center, 200 girls are diagnosed with CPP annually. The diagnosis was based on breast budding before 8 years of age, stimulated (LHRH test) LH level >5 IU/L, supported by bone age and pelvic ultrasonography. Serum LH and FSH concentrations were measured using an immunochemical assay, and dehydroepiandrosterone sulfate and T levels were measured using a radioimmunoassay (Ria) (Diagnostic Products Corporation, Los Angeles, CA, USA). Estradiol levels were measured using an immunoassay (Siemens Centaur XP).

The exclusion criteria were an existing congenital heart disease with hemodynamic effects, arrhythmia, the use of other drugs which can change ECG parameters, and abnormal admission ECG. A standard 12-lead ECG study was performed at 25 mm/s and 10 mm/mV, measured manually by a single pediatric cardiologist, and interpreted by a specialist. ECG was performed before treatment in patients whose diagnosis was confirmed after hormonal and biochemical evaluations. The ECG examination was repeated at 6 months of treatment in those patients who regularly used the treatment. While puberty examination and laboratory values were evaluated by the Department of Pediatric Endocrinology, the ECG parameters were determined blindly by the same pediatric cardiologist. Age at diagnosis, GnRHa (leuprolide acetate) administration, the prescribed dose, and any history of other diseases or medications were recorded on a pre-prepared form. This study was conducted in accordance with the principles of the Declaration of Helsinki.

The QT interval was measured from the beginning of the QRS complex to the end of the T wave on the ECG. QT dispersion was calculated by measuring the distance between the longest QT interval (QTmax) and the shortest QT interval (QTmin) on the ECG. The corrected QT interval as a function of heart rate was calculated using Bazett's formula ($QTc=QT/\sqrt{RR}$) and was defined as the corrected QT (QTc). QTc dispersion was measured by calculating the difference between the longest QTc (QTc max) and the shortest QTc (QTc min).

Statistical Analysis

The Statistical Package for the Social Sciences software (version 26.0) was used for the statistical analysis of the data. Categorical measurements are summarized as numbers and percentages, and continuous measurements as means and standard deviations (median, minimum-maximum, and Q1-Q3) where necessary. The Shapiro-Wilk test was used to determine whether the parameters in this study were normally distributed. A paired-sample t-test was used for normally distributed parameters and Wilcoxon signed-rank tests were used for non-normally distributed parameters. A multivariate linear regression model was used to determine the contribution of the independent variables to the variance of the dependent variable. The statistical significance level was set as 0.05.

Results

Forty-four girls with a mean age of 8.99±1.53 years were included in this study. The clinical characteristics of all the participants are summarized in Table I. All patients were initiated on an intramuscular injection of leuprolide acetate 3.75 mg/28 days as GnRHa. The mean GnRHa was 112.9±33.0 µg/kg.

The comparison of the pre-treatment and 6-month ECG parameters of the patients revealed a prolonged QT interval and a lower pulse rate (beats/minute) after treatment, with a statistically significant difference (p<0.001, p<0.001). There were no significant differences in the pre- and post-treatment values of PR, QRS, QTc interval, QT dispersion, or QTc dispersion (Table II). There was a significant decrease in the heart rate on 6 months ECG.

As shown in Table III, multivariate linear regression analysis was performed to determine the correlation between the ECG parameters and GnRHa dose (µg/kg). A significant negative correlation was observed between GnRHa dose and PR interval (p=0.049), whereas no significant correlation was found between GnRHa dose and QRS, QT mean, QT dispersion, QTc, or QTc dispersion (Table III). Boxplot diagrams of the pre- and post-GnRHa treatment OTc intervals are shown in Figure 1.

Table I. Clinical and laboratory characteristics of patients

Characteristics	Mean ± SD	Min.-Max.	Q1	Med	Q3
Age at diagnosis (years)	8.99-1.53	0.82-10.85	8.39	9.24	9.89
Weight SDS	0.96±1.18	-1.94-3.21	0.28	1.06	1.81
Height SDS	0.58±1.20	-2.11-3.69	0.10	0.59	1.11
BMI SDS	0.80-1.01	-1.20-3.21	-0.005	0.93	1.37
LH (mIU/mL)	2.04-1.85	0.0-7.61	0.63	1.54	3.04
FSH (mIU/mL)	5.11-2.05	1.0-10.0	3.23	5.18	6.21
Estrodiol (pg/ml)	30.92-19.99	0-0.97	18.5	28.0	45.5
GnRHa dose (mg)	3.80-0.60	2.50-7.50	3.75	3.75	3.75
GnRHa dose (µg/kg)	112.9-33.0	64.66-227.27	91.8	104.45	118.3

Continuous variables are expressed as mean ± standard deviations
Q1, Q3: 1st and 3rd quartile, Med: Median, SDS: Standard deviation scores, BMI: Body mass index, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, GnRH: Gonadotropin-releasing hormone, Min.-Max.: Minimum-Maximum

Table II. Comparison of pre- and post-GnRHa treatment ECG parameters

Parameters		Mean ± SD	Q1	Med	Q3	p-value
Heart rate (beats/minute)	**Pre-treatment**	95.3±16.6	83.3	94	104	**<0.001**‡,**
	Post-treatment	84.5±16.3	71.3	83	92.5	
PR (ms)	**Pre-treatment**	121.9±13.8	114	120	128	0.643‡
	Post-treatment	120.5±15.5	110.5	119	133.5	
QRS (ms)	**Pre-treatment**	74.8±7.3	70.0	74	79.5	0.246‡
	Post-treatment	76.5±12.5	70.5	75	80	
QT min (ms)	**Pre-treatment**	297.1±25.1	280	290	320	**<0.001**‡,**
	Post-treatment	311.2±27.3	296	320	325.5	
QT max (ms)	**Pre-treatment**	317.7±26.3	297	320	337.5	**<0.001**†,**
	Post-treatment	336.3±29.8	320	340	360	
QT mean (ms)	**Pre-treatment**	307.4±25.2	288.5	304.5	325	**<0.001**†,**
	Post-treatment	323.8±27.8	308.5	324	341.5	
QT dispersion (ms)	**Pre-treatment**	20.5±9.9	12	20	30	0.071‡
	Post-treatment	25.0±13.6	17	20	40	

Table II. Continued						
Parameters		**Mean ± SD**	**Q1**	**Med**	**Q3**	**p-value**
QTc min (ms)	**Pre-treatment**	374.2±22.7	358.8	374.5	387	0.074†
	Post-treatment	366.5±18.1	354	367	375.8	
QTc max (ms)	**Pre-treatment**	399.9±21.3	384.3	400.5	416	0.651†
	Post-treatment	398.3±18.8	385.5	400	413	
QTc mean (ms)	**Pre-treatment**	387.1±20.4	372.5	386.25	401.5	0.187†,**
	Post-treatment	382.38±16.54	368.5	381.3	395.5	
QTc dispersion (ms)	**Pre-treatment**	25.8±16.4	11.25	22.5	36	0.148‡
	Post-treatment	31.7±16.5	21	31.0	42	

*p<0.05, **p<0.001, †Paired-sample t-test, ‡Wilcoxon signed ranks test
Q1, Q3: 1st and 3rd quartile, Med: Median, SD: Standard deviation, GnRHa: Gonadotropin-releasing hormone analogs, ECG: Electrocardiography

Table III. Multivariate linear regression model analysis results between ECG parameters and GnRHa dose (µg/kg)			
Parameters	**β**	**(95% CI)**	**p-value**
PR (ms)	-0.690	-1.378- -0.003	0.049*
QRS (ms)	-1.217	-2.654- 0.220	0.094
QT mean (ms)	-0.222	-.0727- 0.283	0.378
QT dispersion (ms)	-0.144	-1.228- 0.940	0.789
QTc mean (ms)	-0.344	-0.873- 0.185	0.195
QTc dispersion (ms)	0.133	-0.548- 0.814	0.694

A p-value <0.05 is considered statistically significant
ms: Milliseconds, CI: Confidence interval, GnRHa: Gonadotropin-releasing hormone analogs, ECG: Electrocardiography

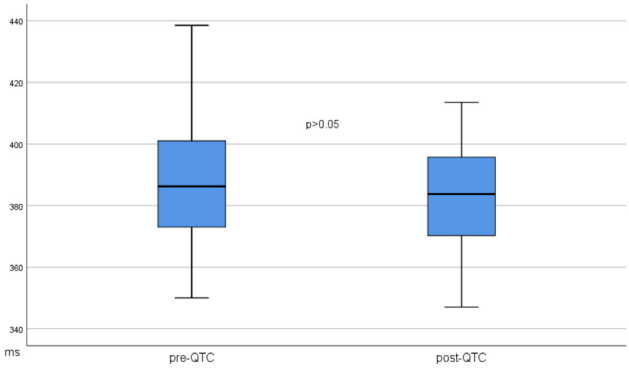

Figure 1. Boxplot diagram of pre- and post-GnRHa treatment QTc intervals
p>0.05 (paired Student's t-test). Abbreviations: ms, milliseconds; pre-QTC, pre-treatment values of QTc intervals; post-QTC, pre-treatment values of QTc intervals.

Discussion

Precocious puberty leads to premature closure of the epiphyseal disks and short stature in adulthood and therefore has important social consequences (9,10). GnRH agonists are preferred for the treatment of CPP. This study prospectively evaluated the effects of this treatment on ECG parameters in girls diagnosed with CPP who were treated with GnRHa. Administration of GnRH agonists (leuprolide acetate) had a statistically significant effect on QT intervals but had no significant effect on PR, QRS, QT, QTc intervals, QT dispersion, or QTc dispersion. However, the pre- and post-treatment values of each participant were within the normal range, according to the reference ECG parameters for age (11).

GnRH agonists and QT interval prolongation have been carefully studied in prostate cancer studies (5). There have been no reports of prolonged QT intervals in women using GnRHa. A prolonged QT interval increases the risk of developing torsades de pointes, which is a ventricular arrhythmia which can lead to sudden cardiac death (12). In addition, studies have shown that ventricular repolarization parameters QT, QTc interval, and QT dispersion can predict ventricular arrhythmia events and death. The list of drugs known to cause a prolonged QT interval is regularly updated (13,14). Currently, the GnRHas with a reported increase in the risk of prolonged QT interval are leuprolide and degarelix (15,16). A study by Gagliano-Jucá et al. (14) compared one group using leuprolide acetate treatment for androgen suppression in prostate cancer and another group not using

this treatment and they found a statistically significantly prolonged QTc interval in the group receiving treatment. In our study, all parameters indicative of ventricular repolarization were evaluated, and no difference was found in the QTc interval with leuprolide acetate administration; however, the results showed a statistically significant increase in the QT interval. This may have been caused by a statistically significant change in pre- and post-treatment heart rates.

In Smith et al.'s (16) study of the cardiovascular safety of degarelix treatment in prostate cancer, the results were similar to those of the group receiving leuprolide treatment. In contrast, another study evaluating the effect of degarelix treatment and a placebo on cardiac polarization in a prostate cancer group reported that degarelix treatment was safe even at high doses (17). In our study, linear regression was performed to evaluate the correlation between leuprolide acetate doses (μg/kg) and ECG parameters, which revealed that increasing therapeutic doses did not significantly increase PR, QRS, or QT intervals.

Among the conditions treated with GnRHa in childhood, gender dysphoria is the clinical condition most similar to those in which the risk of a prolonged QT interval has been described. The testosterone levels in these patients were suppressed by GnRHa administration. A retrospective study examining the ECG parameters of 33 adolescents (19 assigned males, 14 assigned females) who used leuprolide acetate for puberty suppression due to gender dysphoria found no QTc prolongation in any young people (18). Although the number of pediatric studies in this field is limited, the Drug and Therapeutics Committee has made the following recommendations for individuals receiving GnRHa treatment: A screening ECG is recommended if the patient is taking other drugs known to cause a prolonged QT interval; has a history of congenital heart disease, arrhythmia, or long QT syndrome; has a family history of long QT syndrome or sudden cardiac death; or has symptoms associated with long QT syndrome, including syncope. After ECG screening, the ECG should be repeated when the GnRHa dose reaches a steady state. If the patient has long QT syndrome with prolonged QTc or other ECG abnormalities, a family history of long QT syndrome, or sudden death, the patient should be referred to cardiology. Healthcare professionals should counsel patients about arrhythmia symptoms, including palpitations and syncope. Healthcare professionals should continue to receive information regarding new drugs while continuing GnRHa treatment (17). Therefore, regular ECG monitoring should be considered after treatment initiation in patients treated with GnRH agonists.

Study Limitations

The limitations of this study were its single-center design, small sample size, and the lack of a control group. In addition, only 6-month post-treatment ECG data were available.

Conclusion

The results of this study showed that there were no significant changes in the parameters studied, including QRS, QTc, QT dispersion, and QTc dispersion, after the use of GnRH agonists. Evaluation of the correlation between GnRH agonist dosege (μg/kg) and ECG parameters revealed that it did not significantly increase QRS, QT dispersion, or QTc dispersion at increasing therapeutic doses. Even if regular ECG monitoring is considered after the initiation of GnRHa treatment, they are believed to be safe drugs in children, as there is not enough evidence to contradict this yet.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Clinical Research Ethics Committee of Adana City Training and Research Hospital (approval no: 1459, date: 17.06.2021).

Informed Consent: This study was initiated after obtaining written consent from all the participants and/or their families.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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References

1. Aguirre RS, Eugster EA. Central precocious puberty: From genetics to treatment. Best Pract Res Clin Endocrinol Metab 2018;32:343-54.
2. Acinikli KY, Erbaş İM, Besci Ö, Demir K, Abacı A, Böber E. Has the Frequency of Precocious Puberty and Rapidly Progressive Early Puberty Increased in Girls During the COVID-19 Pandemic? J Clin Res Pediatr Endocrinol 2022;14:302-7.
3. Cheuiche AV, da Silveira LG, de Paula LCP, Lucena IRS, Silveiro SP. Diagnosis and management of precocious sexual maturation: an updated review. Eur J Pediatr 2021;180:3073-87.
4. Lahlou N, Carel JC, Chaussain JL, Roger M. Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics. J Pediatr Endocrinol Metab 2000;13(Suppl 1):723-37.

5. Kunath F, Borgmann H, Blümle A, et al. Gonadotropin-releasing hormone antagonists versus standard androgen suppression therapy for advanced prostate cancer A systematic review with meta-analysis. BMJ Open 2015;5:e008217.

6. Mizusawa Y, Wilde AA. QT prolongation and mortality in hospital settings: identifying patients at high risk. Mayo Clin Proc 2013;88:309-11.

7. Lopes RD, Higano CS, Slovin SF, et al. Cardiovascular Safety of Degarelix Versus Leuprolide in Patients With Prostate Cancer: The Primary Results of the PRONOUNCE Randomized Trial. Circulation 2021;144:1295-307.

8. Waldner RC, Doulla M, Atallah J, Rathwell S, Grimbly C. Leuprolide Acetate and QTc Interval in Gender-Diverse Youth. Transgend Health 2022;8:84-8.

9. Cesario SK, Hughes LA. Precocious Puberty: A Comprehensive Review of Literature. J Obstet Gynecol Neonatal Nurs 2007;36:263-74.

10. Klein KO. Precocious puberty: who has it? Who should be treated? J Clin Endocrinol Metab 1999;84:411-4.

11. Sahn DJ. Moss and Adams' Heart Disease in Infants, Children, and Adolescents, Including the Fetus and Young Adult, 6th ed. Circulation 2001;104:139-40.

12. Zhang Y, Post WS, Blasco-Colmenares E, Dalal D, Tomaselli GF, Guallar E. Electrocardiographic QT interval and mortality: a meta-analysis. Epidemiology 2011;22:660-70.

13. Woosley RL, Heise CW, Gallo T, Tate J, Woosley D and Romero KA. QTdrugs List, AZCERT, Inc. 1457 E. Desert Garden Dr., Tucson, AZ 85718. www.CredibleMeds.org

14. Gagliano-Jucá T, Travison TG, Kantoff PW, et al. Androgen Deprivation Therapy Is Associated With Prolongation of QTc Interval in Men With Prostate Cancer. J Endocr Soc 2018;2:485-96.

15. Olsson H, Petri N, Erichsen L, Malmberg A, Grundemar L. Effect of Degarelix, a Gonadotropin-Releasing Hormone Receptor Antagonist for the Treatment of Prostate Cancer, on Cardiac Repolarisation in a Randomised, Placebo and Active Comparator Controlled Thorough QT/QTc Trial in Healthy Men. Clin Drug Investig 2017;37:873-9.

16. Smith MR, Klotz L, Persson BE, Olesen TK, Wilde AA. Cardiovascular safety of degarelix: results from a 12-month, comparative, randomized, open label, parallel group phase III trial in patients with prostate cancer. J Urol 2010;184:2313-9.

17. Valaskova Z, Hulin I, Hassoun OE, Polak S, Mladosievicova B. The effect of GnRH agonists on angiogenesis and its implications for the myocardium in patients with cardiac risk. Bratisl Lek Listy 2019;120:601-3.

18. Bangalore Krishna K, Fuqua JS, Rogol AD, et al. Use of Gonadotropin-Releasing Hormone Analogs in Children: Update by an International Consortium. Horm Res Paediatr 2019;91:357-72.

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Original Article

Changes in Pediatric Trauma During the COVID-19 Pandemic; Does the Pandemic Have an Effect on the Severity of Traumas?

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ABSTRACT

Aim: Traumas are the most common cause of mortality and morbidity in children. Coronavirus disease-2019 (COVID-19) was shown to affect pediatric mental health, child neglect, and the occurrence of traumatic injuries. This study aimed to characterize pediatric trauma cases presenting to our institution before and after the pandemic began.

Materials and Methods: Patients with trauma who were admitted to our emergency department between March, 2019 and March, 2021 were included. The patients were classified into two groups as before and after the pandemic began (March, 2020). Trauma characteristics, the severity of the injury, and prognosis were assessed. The patients' injury severity scores (ISS) and pediatric trauma scores (PTS) were noted.

Results: A total of 1,718 patients were included in this study. The number of pediatric trauma admissions dropped from 1,039 to 679 after the pandemic started. There was no difference between these groups in terms of age (p=0.874) or gender (p=0.106). There was a significant decrease in the number of traumatic injuries (p<0.001) especially during the first shutdown period (April, May, and June, 2020. Additionally, there was a significant increase in terms of foreign body aspiration (p=0.001) and pedestrian injuries (p=0.016). Although a significant increase was noted in the ISS of the patients (p<0.001), no differences were found between the PTSs (p=0.075) or multi-organ injuries (p=1.000). Also, no significant differences were observed regarding mortality (p=0.650), household accidents (p=0.600), trauma type (p=0.533), the need for transfusion (p=0.166), surgery (p=0.077) or mechanical ventilation (p=0.464) between the two groups.

Conclusion: The COVID-19 pandemic altered social patterns, leading to a decrease in pediatric traumas. This decrease was most prominent during the shutdown period. The variables showing severe trauma, such as the need for surgery, did not change despite a significant increase in ISS. This was attributed to a decrease in admissions for minor trauma.

Keywords: Trauma, ISS, COVID-19, pediatrics, lockdown

Introduction

The infections of the Coronavirus disease-2019 (COVID-19) began in December, 2019 in Wuhan, China. In March, 2020, the World Health Organization declared COVID-19 a global pandemic (1). The first case in Turkey was reported on March 10th, 2020. Later, restrictions were put in place, such as "stay at home" orders and a ban on children and adolescents under the age of 20 leaving their homes after April 4th, 2020 (2).

The COVID-19 pandemic significantly impacted childhood trauma in several ways. During the early stages of the pandemic, there was a significant decrease in emergency

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department (ED) visits across all age groups due to a fear of exposure to the virus, government restrictions, and stay-at-home orders (3-5). However, it is important to note that while overall ED visits declined, the severity of medical conditions among pediatric patients who did present to the ED increased (6). Moreover, the pandemic also resulted in changes in behavior and mobility patterns, which may have affected the types of pediatric injuries. With children spending more time at home, there may be an increase in home-related injuries such as burns, falls, and choking. At the same time, there may be a decrease in injuries related to outdoor activities, such as sports-related injuries.

We, therefore, hypothesized that the rate of pediatric trauma admissions, hospitalization patterns, and the mechanisms of trauma differed before and during the pandemic. This study aimed to retrospectively evaluate the changes in mechanisms and outcomes among the pediatric trauma population during the COVID-19 pandemic.

Materials and Methods

After the ethical approval of the University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Clinical Research Ethics Committee (2021/03-09), the medical records of trauma patients were retrospectively reviewed. This study was conducted over 2 years (March, 2019 to March, 2021) at a large urban tertiary center. All children (0-18 years old) who were admitted to the ED because of trauma and who consulted with our clinic were included. Those patients with incomplete medical records were excluded.

The patients were divided into two groups. Those patients who applied before the COVID-19 pandemic formed Group A. Patients who applied during the pandemic formed Group B. The demographic data of the patients were examined. Application month, time, and type of transport to the center (direct or in an ambulance) were evaluated. The mechanism of the trauma and the affected organs were assessed. The patients' injury severity scores (ISS) and pediatric trauma scores (PTS) scores were noted. The groups were compared in terms of the following parameters: hospitalization time, mechanical ventilation needs, consultations, surgery, and prognosis.

All pre-structured forms were collected, and the data were transferred into the Excel 2010 (Microsoft, Redmond WA, USA) format. Normally distributed data were reported as means ± standard deviations. The statistical significance between normally distributed data for different groups was calculated with the independent samples t-test. Non-normally distributed data were reported as medians (quartiles and ranges). The statistical significance between non-normally distributed data for different groups was calculated with the non-parametrical Mann-Whitney U test. Pearson's chi-squared, Yates' continuity correction and Fisher's exact test were used to calculate statistical significance between frequencies. Significance was set at $p<0.05$. All statistical tests were performed using IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA).

Results

A total of 1,718 children presented to our clinic due to trauma during the study period. The 659 patients (Group B) in 2020-2021 represented a 35% decrease in overall volume compared to the 1,039 patients (Group A) in 2019-2020. The greatest decrease in patient volumes was 70.1% between April, 2020 and June, 2020 (278 versus 83 patients, respectively) ($p<0.001$) (Figure 1).

There was no significant difference in median ages and sex distribution between the groups ($p<0.106$, $p<0.106$). Most of the patients (n=506, 29.4%) were admitted to the hospital for foreign body ingestion. Fifty-nine patients (3.4%) were admitted for foreign body aspiration, 329 (19.2%) patients were admitted for corrosive ingestion, and 109 patients (6.3%) were admitted for penetrating injuries (gunshot wounds, stab wounds, or other). There was no significant difference between the groups in the terms of injury types resulting from vehicle traffic accidents, falls from height, bicycle accidents, corrosive substance intake, crushes, genital traumas, or foreign body ingestion. Although the percentage of patients with foreign body aspiration ($p=0.001$) and pedestrian trauma ($p=0.016$) increased, the percentage of patients with penetrating injuries ($p=0.014$) decreased statistically significantly during

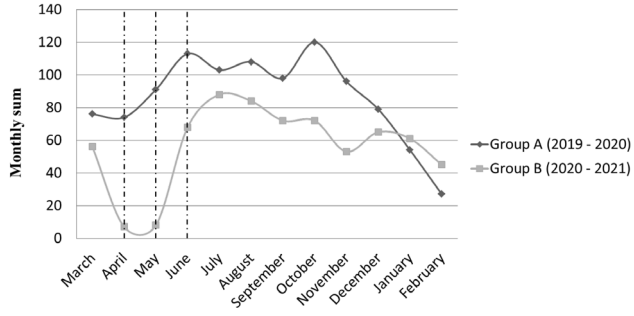

Figure 1. Volume of admissions by month. Group A comprised pre-pandemic applicants (March 2019-2020), while Group B consisted of post-pandemic applicants (March 2020-2021). Notably, the relative proportions from April to June experienced a significant decrease from 2019 to 2020 (278 versus 83 patients, respectively) ($p<0.001$)

the pandemic. In terms of transport type to the hospital, the percentage of admission by ambulance decreased significantly during the pandemic (p<0.001). The time of admission rates decreased between 8 a.m. and 4 p.m. during the pandemic period and increased between midnight and 8 a.m. (p<0.001). There was no difference between the groups in terms of the rates of patients with organ or multisystem injuries at admissions (Table I).

ISS and PTS were used to determine the trauma severity of the cases. ISS was significantly higher during the pandemic (p<0.001). When the ISS subgroups were detailed, it was found that there was a decrease in the incidence of minor traumas (ISS=0 to 8) and an increase in the incidence of serious traumas (ISS=25 to 49) during the pandemic. There was no statistically significant difference in PTS between the two groups (Table II).

Although hospitalization rates increased during the pandemic (p<0.001), hospital length of stay (LOS) was similar in the pre-pandemic and pandemic periods (p=0.665). There was no significant difference in the terms of operation rates (p=0.770) or the admission-operation time interval between the groups (p=0.904). The intensive care unit (ICU) rate (p=0.200) was similar between the two groups. Although ICU LOS was longer in the pandemic group (p=0.003), there was no significant difference in the terms of intubation rates (p=1.000), mechanical ventilation times (p=0.783), or mortality rates (p=0.650) between the groups. The outcomes of the patients are summarized in Table III.

Table I. Demographic characteristics of patients

		Group A (n=1039)	**Group B (n=679)**	**p-value**
Median age, n (%)	Year (Q1-Q3)	4 (2-9)	4 (2-9)	0.874
Sex	Male (%)	651 (62.7)	399 (58.8)	0.106
Injury subgroups, n (%)	Motor vehicle collision	23 (2.2)	12 (1.8)	0.641
	Pedestrian	77 (7.4)	73 (10.8)	**0.016**
	Bicycle accident	54 (5.2)	46 (6.8)	0.172
	Fall	220 (21.2)	131 (19.3)	0.344
	Crush	43 (4.1)	22 (3.2)	0.340
	Penetrating injury (gunshot wounds, stab wounds, or other)	78 (7.5)	31 (4.6)	**0.014**
	Genital trauma	12 (1.2)	2 (0.3)	0.058
	Foreign body ingestion	313 (30.1)	193 (28.4)	0.450
	Foreign body aspiration	23 (2.2)	36 (5.3)	**0.001**
	Corrosive ingestion	196 (18.9)	133 (19.6)	0.710
Transport type, n (%)	With ambulance	290 (27.9)	132 (19.4)	**<0.001**
	Direct admission	749 (72.1)	547 (80.6)	
Admission to E.R. time (o'clock), n (%)	0-8	87 (8.4)	146 (21.5)	**<0.001**
	8-16	310 (29.8)	108 (15.9)	
	16-24	642 (61.8)	425 (62.6)	
Injuries, n (%)	None	541 (52.1)	359 (52.9)	0.745
	Multisystem injury	21 (2)	13 (1.9)	1.000
	Other	477 (45.9)	307 (45.2)	0.777

E.R.: Emergency Room

Table II. Severity of trauma

	Group A (n=1039)	**Group B (n=679)**	**p-value**
ISS: median (Q1-Q3)	0 (0-1)	1 (0-4)	**<0.001**
Minor (ISS=0-8)	965 (92.9%)	581 (85.6%)	**<0.001**
Moderate (ISS=9-15)	51 (4.9%)	47 (6.9%)	0.079
Serious (ISS=16-24)	6 (0.6%)	32 (4.7%)	**<0.001**
Severe (ISS=25-49)	15 (1.4%)	15 (2.2%)	0.319
Critical (ISS=50-75)	2 (0.2%)	4 (0.6%)	0.221
PTS: median (Q1-Q3)	11 (10-11)	11 (10-11)	0.075

Table III. Outcome

	Group A (n=1039)	Group B (n=679)	p-value
Hospitalized Hospital, LOS, days: median (Q1-Q3)	216 (20.8%) 3 (1-4)	195 (28.7%) 3 (1-4)	**<0.001** 0.665
Operated Admission-operation time interval, hours: median (Q1-Q3)	106 (10.2%) 4 (2-10)	88 (13%) 4 (1.5-10)	0.770 0.904
ICU ICU, LOS, days: median (Q1-Q3)	17 (1.6%) 3 (2-5)	18 (2.7%) 6 (3-11)	0.200 **0.003**
Intubated Mechanical ventilation, LOS, days: median (Q1-Q3)	11 (1.1%) 1 (1-2)	8 (1.2%) 1 (1-4.5)	1.000 0.783
Mortality	2 (0.2%)	2 (0.3%)	0.650

Discussion

The COVID-19 pandemic had a profound impact on childhood trauma in various aspects (3-5). Additionally, the pandemic led to alterations in behavior and mobility patterns, potentially influencing the nature of pediatric injuries. The social transformations imposed by COVID-19 had consequences for pediatric mental health, child neglect, and the occurrence of pediatric traumatic injuries (7,8). Within our extensive study, we observed variations in pediatric trauma trends, injury patterns, and injury severities throughout the COVID-19 pandemic.

During the COVID-19 lockdowns, there was a notable reduction in ED visits and hospital admissions, as is evident from systematic reviews in the literature (3,9). In our study, we observed a decrease in the number of patients admitted due to traumatic injuries. The months of March to June, 2020, characterized by curfew periods and stringent restrictions, experienced the most significant decline in trauma cases. Even with the relaxation of restrictions during the summer, the number of applications did not return to pre-pandemic levels. Also, many individuals chose to directly seek hospital care without utilizing ambulances. These trends can be attributed to various factors, including policies aimed at ensuring hospitals were not overwhelmed and could maintain uninterrupted health services, and also by promoting social isolation to mitigate the risk of contracting COVID-19, and the implementation of stay-at-home policies. These measures, particularly in children, contributed to a substantial decrease in the incidence of traumas. In our study, no significant differences in age and gender were observed between the pre-pandemic and pandemic periods. However, it was noted that traumas were more prevalent among boys in both groups, with the median age group being 4 years old for both periods.

The existing literature indicates that approximately 91% of unintentional injuries take place within or near the child's home, and it has been determined that preschool children make up 55% of the victims of home accidents. Injuries resulting from accidents remain the leading cause of morbidity and mortality among children. Among the various types of injuries, the most common ones include ingesting foreign bodies, poisoning, falls, drowning, and burns (10). In order to gain a comprehensive understanding of the mechanisms behind trauma, we categorized injuries into specific subgroups, including motor vehicle collisions, pedestrian incidents, bicycle accidents, falls, crush injuries, penetrating injuries (such as gunshot wounds, stab wounds, or other types), foreign body ingestion, foreign body aspiration, and corrosive ingestion. The reason for these subdivisions is due to the limited number of studies available which specifically examine the impact of the COVID-19 pandemic on pediatric trauma mechanisms and outcomes. By delving into these distinct categories, we aimed to shed light on the diverse effects of the pandemic on pediatric traumas (11). Numerous studies have demonstrated elevated rates of domestic injuries, with some indicating higher occurrences during the pandemic compared to the pre-pandemic period (12,13). In our analysis of specific subgroups, we observed an increase in incidents related to pedestrians, bicycle accidents, foreign body aspiration, and corrosive ingestion. Surprisingly, contrary to expectations based on the existing literature, we found that the number of pedestrian injuries and bicycle crashes did not decrease proportionally during the pandemic, but instead showed an opposite trend (8). These findings highlight the complex and potentially unexpected effects of the pandemic on specific types of injuries within the domestic setting.

The implementation of social distancing measures and stay-at-home orders inadvertently resulted in prolonged periods spent at home where children, with or without adult supervision, may be more prone to swallowing or aspirating foreign objects. In our study, we observed an increase in cases of foreign body aspiration and corrosive ingestion.

Notably, foreign body ingestion emerged as the most common reason for hospital admission in both groups. While there was a decrease in the number of patients seeking medical attention for this reason during the pandemic period, this decline was not statistically significant. Reports indicate that these accidents predominantly occur within the home setting, regardless of family supervision (10,14). Moreover, a previous study highlighted an increase in domestic accidents, particularly in relation to foreign body ingestion (12). During the pandemic period, there was also a decrease in motor vehicle collisions, falls, crush injuries, and penetrating injuries. These types of injuries are typically more common outside the home and tend to affect children older than 6 years. Thus, it can be inferred that stay-at-home policies effectively reduced the risk of trauma exposure for children.

As our hospital does not have a burns unit, we did not encounter any patients presenting with burns during the study period. Furthermore, due to the retrospective nature of this study, we were unable to specifically investigate cases of suspected abuse. However, one study conducted in the United States reported a concerning increase in child abuse cases (15). Considering that children may experience increased loneliness and vulnerability to abuse while staying at home, exacerbated by heightened stressors and prolonged time spent indoors, healthcare professionals should maintain a high level of suspicion regarding abuse or neglect. It is imperative that public health units thoroughly evaluate the implications of school closures, and health policies should be tailored accordingly to address these concerns.

In a single-center study examining admission times for pediatric patients with trauma over a 16-year period, it was observed that the busiest admission times occurred in the afternoon, while nighttime admissions decreased (16). Similarly, prior to the pandemic, this pattern was observed in our country as well. However, during the pandemic, daytime admissions decreased as children who spent more time at home with their mothers were less likely to experience trauma. Conversely, night-time admissions increased as families preferred hours when the emergency room density was lower.

Furthermore, there was a decrease in the number of patients presenting with multiple system injuries. However, interestingly, the overall number of children presenting to the hospital did not change, despite the absence of any injury. This suggests that families' concerns about potential trauma outweighed their fear of the pandemic, prompting them to seek medical attention for their children even in the absence of actual injuries.

The ISS serves as a valuable measure to assess the severity of injuries, particularly in patients with multiple traumas (17). In 1987, Tepas et al. (18) introduced the PTS with the aim of swiftly and accurately evaluating injured children during field triages. The PTS is a scoring system specifically developed for children. Notably, the developers discovered a statistically significant linear relationship between PTS and ISS, further emphasizing the utility of the PTS in predicting injury severity.

Our study revealed no significant differences between the ISS and PTS scores among the groups. This finding aligns with those results observed in previous studies within the literature, which also did not report significant variations in ISS scores (11,19). However, upon further examination of the ISS scores, we observed a decrease in minor traumas and an increase in the rates of severe traumas. Notably, with an increasing number of patients classified as experiencing serious trauma, particularly those with ISS scores ranging from 16 to 24, a higher proportion of patients were admitted to the hospital and required intensive care when compared to the pre-pandemic group.

It was also noteworthy that during the pandemic, a significant majority of those patients who sought hospital care, regardless of the reason, presented with severe clinical conditions. Another study examining rates of appendicitis admissions in our hospital reported a significant increase in the number of patients presenting with complicated appendicitis (20). This may be attributed to the heightened fear of contracting illnesses in hospital settings, resulting in longer delays in seeking medical attention. Consequently, those cases who presented to the hospital during the pandemic tended to be in more severe conditions and thus required hospitalization.

In our study, we observed that the duration of hospital stays remained unchanged, while the hospitalization rates increased. While only one study reported reduced lengths of stay for pediatric traumas during the pandemic, suggesting efficient resource utilization, other studies indicated an increase in hospitalization rates during this period (11,21). Furthermore, no significant differences were found in the number of operations, the time interval between admission and operation, or the need for mechanical ventilation and intubation. These findings underscore the importance of maintaining adequate reserves in pediatric ICUs (PICUs) and operating theaters at level 1 trauma centers. Despite the impact of the COVID-19 pandemic on pediatric traumas, there was no observed change in mortality rates.

Study Limitations

Our study was subject to certain limitations which should be acknowledged. Firstly, being a retrospective review of electronic medical records, there was a possibility of reporting errors and inaccuracies in the recorded information. To minimize this potential bias, we conducted individual chart reviews instead of relying solely on ICD-10 codes. However, the inherent limitations of retrospective studies still apply. Secondly, we did not specifically investigate the socioeconomic status of the families or the number of individuals residing in each household, which can potentially contribute to the occurrence of home accidents. Understanding these factors could provide further insights into the rates and patterns of injuries within the home setting. Despite these limitations, our analysis is consistent with previous retrospective reports which have described changes in traumatic injuries during stay-at-home measures in Turkey. While we recognize the need for further research to address these limitations and provide a more comprehensive understanding, our study contributes to the existing body of evidence on the impact of stay-at-home precautions on traumatic injuries.

Conclusion

The COVID-19 pandemic and its related policies, including hospital management strategies, social isolation measures, and stay-at-home policies, effectively reduced pediatric trauma cases by altering social patterns. In order to promote child safety, it is crucial to secure dangerous objects, address home accident risks, and educate families regarding abuse and neglect, given the fluctuating mental states at home. Healthcare professionals should approach these issues cautiously and be vigilant in detecting potential cases. The efficient utilization of hospital resources is crucial during pandemics, considering the increase in severe clinical presentations while maintaining a steady number of surgical interventions and intensive care cases.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Clinical Research Ethics Committee (2021/03-09).

Informed Consent: The medical records of trauma patients were retrospectively reviewed.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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References

1. Paules CI, Marston HD, Fauci AS. Coronavirus Infections-More Than Just the Common Cold. JAMA 2020; 323:707-8.
2. İlhan MN, Tüzün H, Kiliç R, Yildirim N. Nonpharmaceutical interventions in Turkey and worldwide during COVID-19 pandemic. Turk J Med Sci 2021; 51:3207-14.
3. Kruizinga MD, Peeters D, van Veen M, et al. The impact of lockdown on pediatric ED visits and hospital admissions during the COVID19 pandemic: a multicenter analysis and review of the literature. Eur J Pediatr 2021; 180:2271-9.
4. Silvagni D, Baggio L, Lo Tartaro Meragliotta P, et al. Neonatal and Pediatric Emergency Room Visits in a Tertiary Center during the COVID-19 Pandemic in Italy. Pediatr Rep 2021; 13:168-76.
5. DeLaroche AM, Rodean J, Aronson PL, et al. Pediatric Emergency Department Visits at US Children's Hospitals During the COVID-19 Pandemic. Pediatrics 2021; 147:e2020039628.
6. Liang T, Chamdawala HS, Tay ET, et al. Pediatric emergency care in New York City during the COVID-19 pandemic shutdown and reopening periods. Am J Emerg Med 2022; 56:137-44.
7. Heiber M, Lou WYW. Effect of the SARS outbreak on visits to a community hospital emergency department. CJEM 2006; 8:323-8.
8. Sanford EL, Zagory J, Blackwell JM, Szmuk P, Ryan M, Ambardekar A. Changes in pediatric trauma during COVID-19 stay-at-home epoch at a tertiary pediatric hospital. J Pediatr Surg 2021; 56:918-22.
9. Pala B, Pasikowska N, Pala T, Klepinowski T, Sagan L. The global impact of COVID-19 outbreak on the pediatric trauma volume and its causes – a systematic review. Pomeranian J Life Sci 2022; 68:16-20.
10. Balcı Ö, Karaman A, Karımlı B, et al. COVID-19 pandemic and lockdown: what has changed in common home accidents such as foreign bodies and corrosive injuries? Pediatr Surg Int 2022; 38:1657-62.
11. Yeates EO, Grigorian A, Schellenberg M, et al. Effects of the COVID-19 pandemic on pediatric trauma in Southern California. Pediatr Surg Int 2022; 38:307-15.
12. Ferro V, Nacca R, Pisani M, et al. Children at risk of domestic accidents when are locked up at home: the other side of COVID-19 outbreak lockdown. Ital J Pediatr 2022; 48:129.
13. Bressan S, Gallo E, Tirelli F, Gregori D, Da Dalt L. Lockdown: more domestic accidents than COVID-19 in children. Arch Dis Child 2021; 106:e3.
14. Neal JT, Monuteaux MC, Porter JJ, Hudgins JD. The Effect of COVID-19 Stay-At-Home Orders on the Rate of Pediatric Foreign Body Ingestions. J Emerg Med 2022; 63:729-37.

15. Hairston TKS, Philpott D, Ryan LM, Nasr I, Genies M, Badaki-Makun O. A traumatic pandemic: High acuity pediatric trauma in the COVID19 era. Injury 2022; 53:3289-92.

16. Ramgopal S, Dunnick J, Siripong N, Conti KA, Gaines BA, Zuckerbraun NS. Seasonal, Weather, and Temporal Factors in the Prediction of Admission to a Pediatric Trauma Center. World J Surg 2019; 43:2211-7.

17. Baker SP, O'Neill B, Haddon W Jr, Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. J Trauma 1974; 14:187-96.

18. Tepas JJ 3rd, Mollitt DL, Talbert JL, Bryant M. The pediatric trauma score as a predictor of injury severity in the injured child. J Pediatr Surg 1987; 22:14-8.

19. Chaudhari PP, Anderson M, Ourshalimian S, et al. Epidemiology of pediatric trauma during the coronavirus disease-2019 pandemic. J Pediatr Surg 2022; 57:284-90.

20. Çığşar Kuzu EB, Öztan MO, Erincin Ö, Toker Kurtmen B, Köylüoğlu G. Effects of Covid-19 Pandemic On Pediatric Appendicitis Management. Turkish J Ped Surg 2022; 36:9-14.

21. Güleryüz O, Colak O, Güneysu ST, Fidancı İ. The Home Accident Cases Applying to The Pediatric Emergency Department during the Covid-19 Pandemic: What Did the Pandemic Change? Preprints 2021.