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Bursa Uludağ Üniversitesi Tıp Fakültesi,
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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Clinical Presentations of IgE-Mediated Cow's Milk Allergy in Children and Factors Affecting the Development of Tolerance

Çocuklarda IgE Aracılı İnek Sütü Alerjisinin Klinik Presentasyonları ve Tolerans Gelişimini Etkileyen Faktörlerin İncelenmesi

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Abstract

Introduction: Food allergies are responses triggered by various immunological mechanisms to foods or additives, manifesting through IgE, non-IgE or mixed-type reactions. In IgE-mediated food allergies, skin findings (such as urticaria, angioedema, skin redness) are common, along with cough, rhinorrhea, shortness of breath, and symptoms extending to anaphylaxis. This study aims to evaluate the clinical presentations and factors playing a role in tolerance development in patients diagnosed with IgE-mediated cow's milk allergy (CMA).

Materials and Methods: Our study encompasses a retrospective evaluation of the files of patients who were diagnosed with IgE-mediated CMA and developed tolerance, and who presented to the Prof. Dr. Cemil Taşçıoğlu City Hospital Pediatric Allergy and Immunology clinic between the years 2018-2022.

Results: The average age of the 75 patients was 14.4 months, with 65.3% being males. Tolerance development was observed in 56% of patients before reaching 24 months of age. In the group with tolerance development age ≥ 24 months, statistically significantly higher rates of positive food challenge tests and inhalant allergen sensitivity were noted. In patients who developed tolerance after 24 months, family history of atopy, additional allergic diseases, and inhalant allergen sensitivity were more frequent. In this group, the mean skin prick induration diameter, serum-specific IgE levels for milk and casein, and total serum IgE levels were significantly higher. ROC analysis evaluated a cut-off point of 1.30 for casein-specific IgE with a sensitivity of 93% and a specificity of 67%.

Conclusion: CMA is commonly observed in children, yet research on tolerance development is quite limited. However, our study, contrary to existing literature, suggests that tolerance can develop in a shorter period. Furthermore, we found that tolerance developed later in children with a family history of atopy, accompanying additional allergic diseases, and a history of anaphylaxis, as well as those with a larger skin prick induration diameter at the time of diagnosis, and higher levels of total serum IgE, milk-specific IgE, and casein-specific IgE.

Keywords
Food Allergy, IgE-mediated hypersensitivity, milk hypersensitivity

Anahtar kelimeler
Gıda Alerjisi, IgE-aracılı hipersensitivite, süt hipersensitivitesi

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Öz

Giriş: Besin alerjileri, farklı immünolojik mekanizmalar tarafından tetiklenen ve besin veya katkı maddelerine karşı IgE ve non-IgE veya miks tipli reaksiyonlarla ortaya çıkan yanıtlardır. IgE-aracılı besin alerjilerinde, deri bulguları (ürütiker, anjiyoödem, ciltte kızarıklık) başta olmak üzere, öksürük, rinore, nefes darlığı ve anafilaksiye kadar varan semptomlar gözlemlenebilir. Bu çalışma, IgE-aracılı inek

sütü alerjisi (İSA) tanısı alan hastaların klinik prezentasyonlarını ve tolerans gelişiminde rol oynayan faktörleri değerlendirmeyi amaçlamaktadır.
Gereç ve Yöntem: Çalışmamız, 2018-2022 yılları arasında Sağlık Bilimleri Üniversitesi, Prof. Dr. Cemil Taşcıoğlu Şehir Hastanesi Çocuk Alerji ve İmmunoloji polikliniğine başvuran ve IgE-aracılı İSA tanısı alıp tolerans geliştirdiği belirlenen hastaların dosyalarının retrospektif olarak değerlendirilmesini içermektedir.
Bulgular: 75 hastanın yaş ortalaması 14.4 aydı ve %65.3'ü erkeklerden oluşmaktaydı. Hastaların %56'sında 24 aylıktan önce tolerans geliştiği görüldü. Tolerans gelişme yaşı ≥ 24 ay olan grupta, besin yükleme testi ve inhaler alerjen duyarlılığı pozitifliği istatistiksel olarak anlamlı düzeyde daha yüksekti. 24 aydan sonra tolerans gelişen hastalarda ailesel atopi, ek alerjik hastalık ve inhaler alerjen duyarlılığı daha sık görüldü. Bu grupta ortalama deri prik endurasyon çapı, serum süt ve kazein spesifik IgE değeri ile serum total IgE değeri anlamlı düzeyde daha yüksekti. ROC analizi, kazein spesifik IgE için cut off noktası 1.30 alındığında sensitivite %93 ve spesifite %67 olarak değerlendirildi.
Sonuç: İnek Sütü Alerjisi (İSA), çocuklarda yaygın olarak görülmesine karşın, tolerans gelişimi üzerine yapılan çalışmalar oldukça sınırlıdır. Fakat çalışmamız, mevcut literatürün aksine, toleransın daha kısa sürede gelişebileceğini göstermektedir. Ayrıca, ailesinde atopi öyküsü bulunan, eşlik eden ek alerjik hastalıkları ve anafilaksi tablosu ile başvuran, tanı anındaki deri prik testi endurasyon çapı büyük olan ve serum total IgE, süt ve kazein spesifik IgE düzeyleri yüksek olan çocuklarda, toleransın daha geç geliştiği tespit edilmiştir.

Introduction

Food allergy is a reaction that occurs as a result of the triggering of an immune response following exposure to a specific food. Food allergies are responses triggered by different immunological mechanisms, manifesting through IgE, non-IgE, or mixed-type reactions to foods or additives (1). In IgE-mediated food allergies, repeated exposure in food-sensitive children leads to the interaction of allergen epitopes with IgEs bound to FcεRI receptors on the surfaces of cells such as basophils and mast cells. This interaction triggers the release of many inflammatory mediators, primarily histamine, and creates allergic reactions. The most commonly encountered skin symptoms include urticaria, angioedema, and erythema, while it can present as coughing, rhinorrhea, shortness of breath, and even anaphylaxis (2). Worldwide, the prevalence of CMA has been reported as 1-3% in children, making it the most common food allergy. While there hasn't been a nationwide study concerning CMA in our country, two studies at 20-year intervals have determined the prevalence of CMA as 1.55% and 1.45%, respectively. The frequency of CMA decreases with age and drops below 1% around the age of 6 (3). Regarding tolerance development, although there are studies on the role of the microbiota, a clear consensus has not yet been reached on this matter (4,5).

The aim of this study is to evaluate the clinical presentations of patients diagnosed with IgE-mediated CMA and the factors playing a role in tolerance development.

Materials and Methods

This study encompasses a retrospective evaluation of the files of patients who presented to the University of Health Sciences Turkey, Prof. Dr. Cemil Taşçıoğlu City Hospital Pediatric Allergy and Immunology Clinic between 2018 and 2022, and were diagnosed with IgE-mediated CMA and determined to have developed tolerance. The approval for this study was obtained from the İstanbul University of Health Sciences Turkey, Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Research Ethics Committee (date: 23.01.2023, approval number: E-48670771-514.99-207865291).

Research sample

The research included patients whose information was recorded in the patient system, manifested early-type symptoms following contact with cow's milk protein, and were diagnosed through diagnostic tests (skin prick test induration diameter >3mm and/or milk-specific IgE >0.35 ku/L). Tolerance development was determined by the food challenge test. Patients with primary immunodeficiency, chronic diseases like celiac, and missing information in their files were excluded from the study.

Information such as gender, age at presentation, age at first symptom, symptoms at presentation, duration of breastfeeding, age of introduction to complementary foods, SPT and/or milk-specific IgE values at the time of presentation, casein-specific IgE value if avalable, family history of atopy, accompanying additional food allergies, presence of additional allergic diseases,

sensitivity to inhalant allergens, food challenge test result, and age of tolerance development were examined from the clinic files of the included patients. The serum total IgE, eosinophil count, percentage of eosinophils, and reactions developed during the food challenge test were recorded for the patients.

Laboratory methods

Skin Prick test (SPT): SPTs were conducted using standard Lofarma brand allergen extracts and Allertech brand single-use, 8-pronged test applicators in the pediatric allergy clinic. After applying allergen extracts to the test applicator, they were punctured into the skin of both forearms. For the positive control, 0.1% histamine (1 mg/mL) was used, and for the negative control, 0.9% sodium chloride was used. SPT results were evaluated by the same individual 15 minutes later, and the development of a wheal with a diameter 3 mm or larger compared to the negative control was considered a positive test result.

Total IgE: Serum total IgE measurements were performed using the nephelometric method in our laboratory, and the results were expressed in IU/ml (International Units per milliliter).

Cow's milk specific IgE measurement: Cow's milk and casein specific IgE levels were measured in the biochemistry laboratory using the ImmunoCAP (Pharmacia) device, and the results were reported in kU/L. Samples with cow's milk specific IgE values ≥ 0.35 kU/L were considered positive.

Food Challenge Test: To conduct a food challenge test, at least one of the following criteria was required in patients for whom the test was planned:

1. Cow's milk specific IgE ≥ 0.35 kU/L,
2. Positive skin prick test,
3. Onset of symptoms within 2 hours after consuming cow's milk,
4. Improvement or resolution of symptoms suggestive of CMA with an elimination diet.

Before the oral food challenge, possible risks were explained to the families, and informed consent was obtained. Patients scheduled for the food challenge were advised not to use antihistamines and steroid-containing medications for 15 days prior to the test. Patients were thoroughly examined before the test. For patients deemed suitable for the food challenge test, increasing amounts of cow's milk were administered at 15-20 minute intervals. If an objective reaction occurred, the test was terminated. After the test, patients were observed for at least two hours for potential early reactions, and families were informed about the possibility of post-test reactions.

Statistical Analysis

After encoding the data obtained from the study, it was transferred to a computer and analyzed using the SPSS (Statistical Package for Social Sciences) software (Version 22 for Windows, SPSS Inc, Chicago, IL, USA). The normality of all continuous variables in the statistical analysis was assessed using the Shapiro-Wilk test. Continuous variables found to be normally distributed were expressed as Mean ± Standard Deviation, while those that did not follow normal distribution were expressed as median (minimum and maximum values). Categorical data was presented as numbers and percentages (%). For normally distributed data, the parametric T-test was used for group comparisons and for data that did not follow a normal distribution, the non-parametric Mann-Whitney U Test was employed. Categorical data was compared using the Pearson chi-squared test or Fisher's exact test. The linear relationships between continuous variables were assessed using the Spearman correlation test. The strength of the relationship was categorized based on the correlation coefficient (r) value: r = 0.00-0.24 was considered "weak," r = 0.25-0.49 was "moderate," r = 0.50-0.74 was "strong," and r = 0.75-1.00 was considered "very strong." The prognostic diagnostic value of certain variables was analyzed using receiver operating curves (ROC). The optimal cutoff value for each variable was determined by calculating the Youden index. In all statistical comparisons, a significance level of $p < 0.05$ was considered.

Results

General Assessment of Patients

The average age of the 75 patients included in the study was 14.4 ± 11.7 months (min: 3- max:72), with 65.3% of the patients being male (n=49). 32.0% of the patients were in the age group of 6-11 months, while 33.3% were in the age group of 12-23 months. The average age at first reaction was 5.8 ± 4.1 months, with erythema (58.7%) being the most common presenting symptom, followed by urticaria (38.7%).

Concomitant food allergy was found in 46 patients (61.3%), while a family history of atopy was detected in 24 patients (32%).

The distribution of demographic, clinical, and laboratory characteristics of the patients is shown in Table 1.

Table 1. Distribution of demographic, clinical and laboratory data of patients

Variables		**n (%)**
Age group (months)	1-5	13 (17.3)
	6-11	24 (32.0)
	12-23	25 (33.3)
	≥24	13 (17.3)
Presenting symptom	Erythema	44 (58.7)
	Urticaria	29 (38.7)
	Anaphylaxis	2 (2.7)
Food challenge test result	Negative	35 (46.7)
	Positive	40 (53.3)
Coexisting allergic disease	None	41 (54)
	Asthma	15 (20.0)
	Rhinitis	8 (10.7)
	Atopic Dermatitis	11 (14.6)
Inhalant allergen sensitivity	None	57 (76.0)
	Present	18 (24.0)
	Mean ± SD*	**Median (min-max)****
Age of introduction foods (months)	5.7 ±0.9	4 (1-12)
Age of onset of first reaction (months)	5.8 ±4.1	6 (1-24)
Age of tolerance development (months)	24.9 ±13.7	20 (8-78)
Duration of breastfeeding (months)	20.0 ±6.5	22 (1-36)
Skin prick test (mm) (n:18)	8.2 ±2.8	7 (4-14)
Eosinophil count	2.4 ±16.6	0.3 (0.1-144.0)
Eosinophil percentage	5.1 ±5.8	3.7 (0.2- 41.0)
Serum total IgE	226.5 ±311.1	120.0 (2.9-1700.0)
Milk-specific IgE	4.6 ±9.3	0.8 (0.5-42.4)
Casein-specific IgE (n:17)	8.0 ±9.6	3.0 (0.4-30.0)

*Standard Deviation, **min-max (minimum-maximum): Smallest and largest values

Assessment based on the patients' age of onset of first reaction

Among the 75 patients included in our study, 59 (78.7%) experienced their first reaction at the age of ≤ 6 months, while 16 (21.3%) had their first reaction at an age >6 months. It was determined that the most common presenting symptom in both age groups was erythema, with frequencies of 55.9% and 68.8%, respectively.

The median duration of breastfeeding in the group with a first reaction age of ≤ 6 months was statistically significantly lower compared to the other group (21 months (1-36) and 24 months (12-30), respectively) (p=0.05). A comparison of some clinical and laboratory data of patients based on the age groups of their first reaction is presented in Table 2.

*Based on the patients' presenting symptom (n: 73)**

During the evaluation according to the presenting symptoms of the patients, 2 patients who presented with symptoms of anaphylaxis were excluded from the assessment. Upon comparing the remaining patients based on the types of reactions, it was found that the food challenge test positivity (63.6%) in patients presenting with erythema symptoms was statistically significantly higher than in patients presenting with urticaria symptoms (34.5%) (p=0.01). A statistical comparison of patients' gender and some clinical characteristics based on the types of reactions (urticaria and erythema) is presented in Table 3.

Evaluation of patients according to the age of tolerance development

Out of the patients, 42 (56%) developed tolerance before the age of 24 months, while 33 (44%) developed tolerance at or after 24 months of age. Both patients who encountered anaphylaxis were in the group where tolerance development occurred at 24 months of age or older; however, no statistically significant difference was observed concerning reaction type among different tolerance development age groups (p=0.145). The occurrence of positive results in food challenge tests and sensitivity to inhalant allergens were significantly higher in the group with a tolerance development age of 24 months and above (78.8% and 42.4%, respectively) as compared to the other group (33.3% and 9.5%, respectively) (Table 4). The study results have revealed a statistically significant moderate

Table 2. Comparison of some clinical and laboratory data of patients based on the age groups of their first reaction			
Variables	**Age of onset of first reaction ≤ 6 months (n:59) n (%)**	**Age of onset of first reaction >6 months (n:16) n (%)**	**p-value**
Age at presentation (months)	12.7±11.6	20.5±10.5	**0.017[a]**
Age of introduction of foods (months)	5.7 (4-12)	6.0 (5-7)	**0.02[b]**
Age of tolerance development (months)	23.8±14.3	28.7±10.6	0.20 [a]
Duration of breastfeeding (months)	21 (1-36)	24 (12-30)	**0.05[b]**
Skin prick test (mm)	8.4±3.1	7.8±2.3	0.81 [a]
Eosinophil count	0.3 (0.01-144)	0.4 (0.1-1.46)	0.67 [b]
Eosinophil percentage	5.1±6.3	5.1±3.9	0.52 [a]
Serum total Ig E	107.0 (2.9-1700)	220.0 (29-630)	**0.03[b]**
Milk-specific IgE	0.7 (0.5-42.4)	0.8 (0.5-38.0)	0.97 [b]
Casein-specific IgE (n:17)	9.4±10.1	1.7±1.3	**0.04[a]**
[a] T test, [b]Mann Whitney U test			

Table 3. Comparison of demographic, clinical and laboratory characteristics of patients based on reaction type (n: 73)*				
Variables		**Urticaria (n:29) n (%)****	**Erythema (n:44) n (%)****	**p-value[a,b]**
Gender	Male	15 (51.7)	32 (72.7)	0.06[a]
	Female	14 (48.3)	12 (27.3)	
Family history of atopy	None	24 (82.8)	38 (86.4)	0.74[b]
	Present	5 (17.2)	6 (13.6)	
Coexisting food allergy	None	10 (34.5)	19 (43.2)	0.47[b]
	Present	19 (65.5)	25 (56.8)	
Food challenge test	Negative	19 (65.5)	16 (36.4)	**0.01[a]**
	Positive	10 (34.5)	28 (63.6)	
Coexisting allergic condition	None	23 (79.3)	28 (63.6)	0.15[a]
	Present	6 (20.7)	16 (36.4)	
Types of coexisting allergic conditions (n:22)	Asthma	5 (83.3)	8 (50.0)	**0.03[a]**
	Rhinitis	0 (0.0)	8 (50.0)	
	Atopic Dermatitis	1 (16.7)	0 (0.0)	
Inhalant allergen sensitivity	None	25 (86.2)	32 (72.7)	0.17[a]
	Present	4 (13.8)	12 (27.3)	
Age at presentation (months)	11.0 (3-51)		11.5 (4-72)	0.36[c]
Age at first reaction (months)	5.1±2.8		5.9±3.9	0.34[c]
Age at starting solid foods (months)	5.5±0.6		5.8±0.6	0.12[c]
Age of tolerance development (months)	23.6±13.1		25.6±14.4	0.54[c]
Duration of breastfeeding (months)	19.6±6.7		20.4±6.4	0.58[c]
Skin prick test (mm)	7.8± 3.4		8.3±2.6	0.70[c]
Eosinophil count	0.2 (0.01-144.0)		0.3 (0.08-1.93)	0.19[d]
Eosinophil percentage	3.3 (0.2-41.0)		3.8 (0.2-15.8)	0.87[d]
Serum total Ig E	117.5 (3.3-1700.0)		130.0 (2.9-1001.0)	0.56[d]
Milk-specific IgE	0.5 (0.5-38.0)		1.0 (0.5-42.4)	0.24[d]
Casein-specific IgE (n:17)	6.9 (1.5-30.0)		2.6 (0.4-29.8)	0.36[d]
*Statistical comparisons did not include patients who presented with anaphylaxis. **Column percentages,[a] Pearson Chi-square test [b]Fisher's Exact test [c] T-test, [d]Mann-Whitney U test				

Table 4. Comparison of tolerance development age groups by gender and some clinical features				
Variables		**Tolerance development age < 24 months (n:) n (%)***	**Tolerance development age ≥ 24 months (n:) n (%)***	**p-value**
Gender	Male	27 (64.3)	22 (66.7)	0.83[a]
	Female	15 (35.7)	11 (33.3)	
Age at first reaction (months)	≤ 6	36 (85.7)	23 (69.7)	0.09[a]
	> 6	6 (14.3)	10 (30.3)	
Reaction type	Erythema	23 (54.8)	21 (63.6)	0.145[a]
	Urticaria	19 (45.9)	10 (30.3)	
	Anaphylaxis	0 (0.0)	2 (6.1)	
Family history of atopy	None	39 (92.9)	24 (72.7)	**0.018[a]**
	Present	3 (7.1)	9 (27.3)	
Coexisting food allergy	None	19 (45.2)	10 (30.3)	0.18[a]
	Present	23 (54.8)	23 (69.7)	
Coexisting allergic condition	None	35(83.3)	16 (48.5)	**0.001[a]**
	Present	7 (16.7)	17 (51.5)	
Types of coexisting allergic conditions (n:22)	Asthma	3 (42.9)	12 (70.6)	0.25[a]
	Rhinitis	4 (57.1)	4 (23.5)	
	Atopic dermatitis	0 (0.0)	1 (5.9)	
Inhalant allergen sensitivity	None	38 (90.5)	19 (57.6)	**0.001[a]**
	Present	4 (9.5)	14 (42.4)	
Age at presentation (months)		9.6 ±5.1	20.5±14.7	0.56[b]
Age at first reaction (months)		5.5±3.3	6.1±5.0	**<0.001[b]**
Age at starting solid foods (months)		6.0 (4-8)	6.0 (5-12)	0.07[c]
Duration of breastfeeding (months)		18.9±5.9	21.5±7.0	0.09[b]
Skin prick test (mm)		6.0±1.4	8.8±2.8	**0.019[b]**
Eosinophil count		0.3 (0.01-1.17)	0.4(0.08-144.0)	0.30[c]
Eosinophil percentage		3.8±2.6	6.8±8.0	**0.042[b]**
Serum total Ig E		87.5 (3.3-1390.0)	180(2.9-1700.0)	**0.001[c]**
Milk-specific IgE		0.5 (0.5-15.7)	3.1 (0.5-42.4)	**0.002[c]**
Casein-specific IgE (n:17)		1.1 (0.4-2.5)	3.8 (0.4-30)	**0.044[c]**

*Column percentages;[a] Pearson Chi-square test [b] T-test, [c] Mann-Whitney U test

positive linear relationship between the age of first reaction and the duration of breastfeeding (r=0.28, p=0.01). Additionally, moderate statistically significant linear relationships were identified between the level of milk-specific IgE and serum total IgE, eosinophil count, percentage of eosinophils, casein-specific IgE level, and the age of tolerance development. The statistical values of these relationships are as follows: (respectively r=0.42 p<0.001; r=0.50 p<0.001; r=0.44 p<0.001; r=0.50 p=0.04; r=0.41 p<0.001). These linear relationships and statistical values are presented in Table 5 of the study.

ROC (Receiver Operating Characteristic) analyses were performed to assess the predictive value of certain parameters SPT (mm), Eosinophil Percentage (%), total IgE, Milk-Specific IgE, and Casein-Specific IgE in forecasting the development of tolerance at the age of ≥ 24 months and to determine a cutoff value. When an ideal cutoff value of 1.30 was chosen for Casein-Specific IgE, it was evaluated with a sensitivity of 93%

Table 5. Correlation analysis results of quantitative data related to patients

Variables	Age at first reaction	Skin prick test	Milk-specific IgE	Total Ig E	Eosinophil count	Eosinophil percentage	Casein-specific IgE	Duration of breastfeeding	Age at starting solid foods	Tolerance development age
Age at first reaction	1	-0.03* 0.89**	-0.02 0.81	0.18 0.11	-0.16 0.17	-0.01 0.99	-0.15 0.54	0.28 **0.01**	0.21 0.06	0.11 0.33
Skin PRICK TEST	-0.03 0.89	1	0.42 0.10	-0.04 0.87	-0.03 0.89	-0.17 0.48	-0.03 0.95	0.46 0.052	-0.11 0.64	0.36 0.14
Milk-specific IgE	-0.02 0.81	0.42 0.10	1	0.42 **<0.001**	0.50 **<0.001**	0.44 **<0.001**	0.50 **0.04**	0.18 0.10	0.11 0.35	0,41 **<0.001**
Serum total Ig E	0.18 0.11	-0.04 0.87	0.42 **<0.001**	1	0.35 **<0.01**	0.40 **<0.001**	0.18 0.47	0.04 0.74	0.39 **0.001**	0.52 **<0.001**
Eosinophil count	-0.16 0.17	-0.03 0.89	0.50 **<0.001**	0.35 **<0.01**	1	0.86 **<0.001**	0.23 0.36	-0.01 0.89	0.21 0.07	0.20 0.07
Eosinophil percentage	-0.01 0.99	-0.17 0.48	0.44 **<0.001**	0.40 **<0.001**	0.86 **<0.001**	1	0.19 0.45	-0.06 0.95	0.24 **0.03**	0.23 **0.04**
Casein-specific IgE	-0.15 0.54	-0.03 0.95	0.50 **0.04**	0.18 0.47	0.23 0.36	0.19 0.45	1	0.16 0.52	0.04 0.85	0.82 **<0.001**
Duration of breastfeeding	0.28 **0.01**	0.46 0.052	0.18 0.10	0.04 0.74	-0.01 0.89	-0.06 0.95	0.16 0,.52	1	0.04 0.73	0.24 **0.03**
Age at starting solid foods	0.21 0.06	-0.11 0.64	0.11 0.35	0.39 **0.001**	0.21 0.07	0.24 **0.03**	0.04 0.85	0.04 0.73	1	0.20 0.07
Tolerance development age	0.11 0.33	0.36 0.14	0.41 **<0.001**	0.52 **<0.001**	0.20 0.07	0.23 **0.04**	0.82 **<0.001**	0.24 **0.03**	0.20 0.07	1

and specificity of 67% (AUC value = 0.88; p = 0.044; 95% CI = 0.69-1.0). The results of the ROC analyses are presented below in Tables 6 and 7.

Discussion

CMA is common in children, yet studies on tolerance development are quite limited (3,6-8). In our study, within a 5-year period, tolerance development before 24 months of age was observed in 56% of patients diagnosed with IgE-mediated CMA, indicating a shorter duration for tolerance development compared to many previous studies. In the study by Bishop et al. (9), it was reported that 56% of children developed tolerance by the age of 4, with only 28% showing tolerance at age 2. In the study by Santos et al. (6), tolerance within the first 2 years was seen in only 5% of patients. Another long-term study reported 19% tolerance at age 4 (7). In another study conducted in Korea, it was observed that about half of the children with CMA developed tolerance by age 8 (8). However, in the EuroPrevall study conducted with the participation of 9 countries from Europe, it was recommended that the double-blind placebo-controlled Food Challenge Test (DBPCFC) be necessarily performed one year after diagnosis, and tolerance at age 2 was determined as 69% in this study (10).

In our study, 65% of the examined patients were found to be male. Literature also states that male gender is a risk factor for CMA in childhood, and allergic diseases are more common in male children (11). This situation can be attributed to the higher frequency of allergic diseases in male children until the pubertal period.

The prevalence of family atopy history in children with CMA varies in different studies. In the study by

Table 6. Prediction of tolerance development age ≥ 24 months by some parameters				
	Area under the curve (AUC) (%)	**Standard error**	**p-value**	**%95 Confidence ınterval**
Skin prick test (mm)	0.77	0.115	0.10	0.55-1.0
Eosinophil percentage (%)	0.61	0.06	0.10	0.47-0,73
Total Ig E	0.74	0.05	0.001	0.62-0.85
Milk-specific IgE	0.70	0.06	0.002	0.57-0.83
Casein-specific IgE	0.88	0.09	0.044	0.69-1.0

Table 7. Cut-off values for some parameters to predict tolerance development age ≥ 24 months			
Parameters	**cut-off point**	**Sensitivity (%)**	**Specificity (%)**
Skin prick test (mm)	6.50	71	51
Eosinophil percentage (%)	3.65	61	58
Total Ig E	113.5	73	64
Milk-specific IgE	0.57	75	54
Casein-specific IgE	1.30	93	67

Santos et al. (6), the family atopy history was 35% in children with CMA, while in the study by Dias et al. (12), it was 53% in children with persistent CMA over two years of age. In our study, the rate of patients with a family atopy history was found to be a lower percentage of 24%. Comorbid allergic diseases and inhalant allergen sensitivity are other predictive factors in our study. In a study conducted by Santos and colleagues (6), 139 children diagnosed with CMA were examined, and 32% of the patients had asthma, while 73% had inhalant allergen sensitivity. In another study with a prospective design, 118 children selected from 6209 term newborns diagnosed with CMA were followed up until 8.6 years of age; 76.7% had atopic dermatitis, 59.5% had inhalant allergen sensitivity, and 25.8% had asthma (7). It is thought that food allergy is the onset of the atopic march and the different follow-up periods in the studies reveal these differences.

The most common presenting symptoms in the patients included in our study were erythema (58.7%) and urticaria (38.7%), and two of our patients presented with anaphylaxis. Similar studies have also reported skin findings as the most common presenting symptom in patients with CMA (6,7,12). Among the factors affecting tolerance development, laboratory parameters are as important as clinical and demographic characteristics. In our study, we found that the average skin prick induration diameter was higher in the group with tolerance development age ≥ 24 months. Many studies in the literature have revealed similar results and reported different induration estimation values related to tolerance development (6). Therefore, the width of the induration diameter formed during the prick test is considered as a parameter that can be used in predicting the prognosis of CMA.

The evaluation of serum milk Sp IgE level is also an important laboratory parameter as much as the SPT (6,7). In our study, this value was significantly higher in the group with tolerance development age 24 months (p=0.001). Different estimation values have been reported in the literature regarding tolerance development; Santos et al. (6) stated that tolerance was corrected later in those with milk-specific IgE level over 20 kU/L, while Suh et al. (13) stated that the rate of tolerance development in 33 children up to the age of 5 was 19.1% in patients with milk-specific IgE level >15 kU/L. These data show that serum milk Sp IgE level can be a determinant factor on tolerance development.

Another laboratory parameter evaluated for tolerance development is casein sp IgE. In our study, the casein level was found to be significantly higher in the group with tolerance development age ≥ 24 months (p=0.044). Chatchatee et al. (14) stated in their studies, that high casein-specific IgE level is a risk factor for persistent CMA, regardless of age. Similarly, in our study, it was seen that high casein-specific IgE levels are associated with late tolerance development. When the ideal cut off point was taken as 1.30 for Casein Sp. IgE, the sensitivity was evaluated as 93% and

specificity as 67% (AUC value=0.88; p=0.044; 95% CI=0.69-1.0).

Additionally, studies in the literature have attempted to establish specific cut-off points using ROC analyses to predict the risk of anaphylaxis and positive food challenge tests in children with CMA. When two different studies are considered, the first one demonstrated that serum sIgE levels were significantly higher in patients who developed anaphylaxis to raw cow's milk allergen, and ROC analysis indicated that raw cow's milk ImmunoCAP had good sensitivity (86.7%). However, ROC analysis for molecular components was not found satisfactory in terms of sensitivity and specificity (15). In the second study, the ratios of specific IgEs for cow's milk and its components to total IgE, and the wheal size on the SPT were examined to determine the predictive value for a positive FCT. The wheal size on SPT and all specific IgEs along with the ratios of specific/total IgE, yielded significantly different results between patients with positive and negative FCTs ($p < 0.001$). The variable with the largest area under the ROC curve was identified as casein-specific IgE. It was indicated that at casein-specific IgE >0.95 kU/L, food challenge tests would be unnecessary for the diagnosis of IgE-mediated CMA in patients with an appropriate history (16).

Study Limitations

This study has several limitations that should be considered when interpreting the results. Firstly, the study's retrospective design may introduce potential biases, as it relies on previously recorded patient data, which could have inaccuracies or missing information. Secondly, the sample size of 75 patients, although providing valuable insights, may limit the generalizability of the findings to larger populations. Additionally, all the patients were seen in a single tertiary care center, which may not reflect the broader pediatric population. Lastly, the study did not account for environmental factors such as diet, microbiota variations, or exposure to other allergens that could influence tolerance development. Future prospective studies with larger and more diverse cohorts are needed to validate these findings and explore other potential contributing factors.

Conclusion

We believe that the cut-off point for casein-specific IgE obtained in our study will offer a practical approach in predicting tolerance development and preventing unnecessary food challenge tests. This cut-off point could be a significant tool in predicting specific reactions in children sensitive to cow's milk and optimizing the clinical decision-making process by avoiding unnecessary tests.

We believe our findings provide significant insights on the prediction of food allergy tolerance development, management and treatment of concurrent allergic diseases. Particularly, early diagnosis and management have the potential to improve the quality of life of children and optimize long-term health outcomes. Furthermore, understanding the factors affecting tolerance development could aid in the creation of personalized treatment plans and the prevention of allergic diseases at an early age.

Future research should include larger sample groups and long-term follow-ups in different ethnic and geographic groups. This will help us better understand the factors affecting the clinical course and tolerance development of IgE-mediated CMA, and improve general allergy management strategies. Moreover, acquiring more information about immunological markers and other potential predictive factors will inform clinical practice and provide better outcomes for patients.

Ethics

Ethics Committee Approval: The approval for this study was obtained from the İstanbul University of Health Sciences Turkey, Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Research Ethics Committee (date: 23.01.2023, approval number: E-48670771-514.99-207865291).

Footnotes

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

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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Understanding Ectopic Kidneys: Insights from a Single-Center Study

Ektopik Böbreklerin İncelenmesi: Tek Merkezli Bir Çalışmadan İzlenimler

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Abstract

Introduction: The failure of the normal ascent of the kidney can result in ectopic kidneys (EK) and fusion anomalies. EKs are often accompanied by urological and extrarenal abnormalities. This study aims to provide a comprehensive overview of EKs, associated renal and extrarenal anomalies, and kidney functions among patients with simple and cross ectopic kidneys.

Materials and Methods: Clinical records of patients diagnosed with EK admitted to the pediatric nephrology unit between June 2017 and June 2022 were retrospectively evaluated.

Results: In this study, 41.20% (n: 61) of patients had crossed ectopic (CE) kidneys. The most common type of crossed ectopia was inferior CE (n:33 56.9%). The most frequent presenting features were an empty renal fossa (7.40%, n: 11). During the first evaluation, 18.91% (n:28) of patients had hydronephrosis, most of which were mild (SFU 1-2). Vesicoureteric reflux (VUR) was evident in 7.4% of patients. The mean DMSA (dimercaptosuccinic acid) uptake was lower in EK (40.37±7.31) compared to orthotopic kidneys. Comparison of simple and CE kidneys showed similar results regarding the presence of hydronephrosis, vesicoureteral reflux (VUR), and differential function of EKs. In both groups, serum creatinine levels and estimated glomerular filtration rate (eGFR) were preserved.

Conclusion: Patients with ectopic kidneys often present with renal and extrarenal anomalies. Although hydronephrosis is a common occurrence, it is usually mild and transient, and incidence of vesicoureteral reflux is low. Considering the preservation of renal function in mid-term period, it may be more appropriate to evaluate each patient's need for a complete urological examination on a case-by-case basis.

Keywords
Ectopic kidney, fusion anomalies, VUR, DMSA

Anahtar kelimeler
Ektopik böbrek, füzyon anomalileri, VUR, DMSA

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Öz

Giriş: Böbreğin normal yerleşimindeki başarısızlığı, ektopik böbreklerin (EB) ve füzyon anomalilerinin ortaya çıkmasına neden olabilir. EB'ler genellikle ürolojik ve ekstrarenal anomalilerle birlikte görülür. Bu çalışmanın amacı, basit ve çapraz ektopik böbrekleri olan hastalardaki EB'leri, ilişkili renal ve ekstrarenal anomalileri ve böbrek fonksiyonlarını kapsamlı bir şekilde incelemektir.

Gereç ve Yöntem: Haziran 2017 ile Haziran 2022 tarihleri arasında pediatrik nefroloji ünitesine başvuran EB tanısı konmuş hastaların klinik kayıtları geriye dönük olarak değerlendirildi.

Bulgular: Bu çalışmada, hastaların %41,20'sinde (n: 61) çapraz ektopik (ÇE) böbrekler bulunmaktaydı. En yaygın çapraz ektopi türü inferior ÇE idi (n: 33, %56,9). En sık görülen bulgu boş renal fossa idi (%7,40, n: 11). İlk değerlendirme

sırasında hastaların %18,91'inde (n: 28) hidronefroz mevcuttu, çoğunluğu hafif (SFU 1-2) idi. Üriner vezikoüreteral reflü (VUR), hastaların %7,4'ünde mevcuttu. Ortalama DMSA (dimercaptosuccinic acid) alımı, EB'lerde (40,37±7,31) ortotopik böbreklere göre daha düşüktü. Basit ve ÇE böbreklerin hidronefroz, VUR ve EB'lerin diferansiyel fonksiyonu açısından karşılaştırılması benzer sonuçlar gösterdi. Her iki grupta da serum kreatinin düzeyleri ve tahmini glomerüler filtrasyon hızı (eGFR) korunduğu görüldü.
Sonuç: Ektopik böbreklere sahip hastalar genellikle renal ve ekstrarenal anomalilerle birlikte gelirler. Hidronefroz sık görülen bir durum olmasına rağmen, genellikle hafif ve geçicidir ve vezikoüreteral reflü insidansı düşüktür. Orta vadede renal fonksiyonun korunması göz önüne alındığında, her bir hastanın tam bir ürolojik muayene için ihtiyacının vaka bazında değerlendirilmesi daha uygun olabilir.

Introduction

The human kidney goes through different stages during embryonic development. Normally, the development of the kidney and urinary tract begins with the formation of a nephric duct (ND) from the intermediate mesoderm. Formation of a permanent mature kidney requires complex interactions between different cell lineages consisting of epithelial cells of the ureteric bud, mesenchymal cells of nephric blastema, and endothelial cells of capillaries (1). As morphogenesis progresses, the kidney simultaneously undergoes an ascent from its lower pelvic position to its typical intraabdominal location. The formation of the kidney and outflow tracts necessitates a complex interplay of various factors, including genetic, epigenetic, and environmental influences from both the maternal and fetal aspects of organogenesis (2-4). Distruption of convergence between genetic and environmental factors can lead to congenital anomalies of kidney (CAKUT).

CAKUT is the leading cause of chronic kidney disease in children. It presents with diverse phenotypes based on the timing of disrupted embryonic development and the type of affected segment. CAKUT can be classified according to abnormalities in kidney number (renal agenesis, aplasia), size, and morphology (hypoplasia, multicystic dysplastic kidney - MCDK, dysplasia), outflow tract abnormalities (ureteropelvic junction obstruction - UPJ, vesicoureteric reflux - VUR, duplex collecting system, ureterovesical stricture, posterior urethral valve), as well as abnormalities in kidney rotation and position (horseshoe kidneys, ectopic kidney, and fusion anomalies) (2-5).

The failure of the normal ascent of the kidney can result in an ectopic kidney located in the pelvis, lower abdomen, or even rarely in the thoracic cavity. When a kidney is situated on the opposite side of its ureteric implantation in the urinary bladder, it is termed 'crossed ectopia.' Occasionally, abnormally ascending kidneys may partially fuse to create 'crossed fused ectopia' or form a 'horseshoe kidney' by complete fusion, sometimes referred to as 'pancake pelvic kidneys (6). Regardless of the phenotype, ectopic kidneys may be associated with conditions such as UPJ (ureteropelvic junction) obstruction, VUR (vesicoureteral reflux), MCDK (multicystic dysplastic kidney), renal stone formation, and other related anomalies (7).

Previous studies on renal ectopia and kidney fusion anomalies have been limited by a small number of patients. This study aims to assess the clinical profiles, associated anomalies, and renal outcomes in children with ectopic kidneys at a referral center in the eastern part of Turkey.

Materials and Methods

We conducted a retrospective evaluation of patients admitted to the pediatric nephrology unit at Van Regional Training and Research Hospital between June 2017 and June 2022. We searched the hospital database system and polyclinic records using ICD codes Q63.2. Duplicate records, patients with inconclusive ultrasound or DMSA scan results, and patients horseshoe kidneys connected by a thin isthmus were excluded. We gathered information from the medical records, including the chief complaint at the time of diagnosis, age, sex, weight, height, body mass index, and corresponding standard deviation scores (SDSs) of the patients (8). Additionally, we recorded creatinine levels, and estimated glomerular filtration rate (eGFR) for patients older than 24 months. Ethical approval was obtained from the Van Training and Research Hospital Clinical Research Ethics Committee (date: 01.11.2014 approval number: VEAH KAEK).

The type of ectopia, whether simple or crossed, and the location of EKs (lower abdominal, pelvic,

or thoracic) were evaluated through ultrasonography and DMSA scans (Figure 1). Additionally, renal and extrarenal anomalies beyond ectopia, such as hydronephrosis (HN) and vesicoureteral reflux (VUR), as well as other system involvement, were recorded.

A standardized nuclear DMSA study protocol was already conducted for all patients and those DMSA results were categorised as 'no pathological uptake,' 'photopenic region,' 'multiple photopenic region,' ' scarring,' 'multiple scars,' or 'globally diffuse decreased uptake.'

In cases of hydronephrosis, we reported its severity as mild (SFU 1-2) or moderate to severe (3-4) using the SFU grading system and measured the anteroposterior diameter of the renal pelvis. VUR was graded according to the International Reflux Study in Children (9,10).

Statistical Analysis

Data analysis was performed using IBM SPSS version 21. Categorical data were presented as numbers and percentages, and continuous variables were presented as means and standard deviations. The chi-squared test was used to compare categorical data. The distribution of continuous data was evaluated using tests and graphs. The Mann-Whitney U test was used to compare data that did not have a normal distribution. The Wilcoxon test was used to compare dependent variables. $p<0.05$ was considered statistically significant.

Results

Among the 148 patients diagnosed with EKs in this study, 58.80% (n:87) had simple ectopic (SE) kidneys, while the remaining 41.20% (n:61) had CE kidneys. Among the CE kidneys, 95% (n:58) were cross-fused, while three of them were non-fused CE. The most common subgroup among CE fused kidneys was the inferior type, accounting for 56.9% (n:33), followed by L-shaped (17.20% - n:10), disc (12.10% - n:7), sigmoid (8.6% - n:5), and lump (5.2% - n:3) types, respectively.

Most of the ectopic kidneys were localized in the pelvic region, representing 60.10% (n:89), while the remaining 39.20% (n:58) were localized in the lower abdomen. Only one patient (0.7%) had a kidney localized in the thoracic cavity. Upon admission, all patients had serum creatinine levels within normal limits (0.40±0.15). Demographic data is provided in Table 1.

The most common identifiable presenting feature was empty renal fossa 7.40 % (n:11) and suspected renal agenesis 7.40 % (n:11). 10 % (n:15) of patients suffered from abdominal pain as a presenting symptom, but most patients with ectopic kidneys were detected incidentally while being evaluated for another reason (n:75 50.8%). Presenting features and complaints were given in Table 2.

Table 3 provides details on urological abnormalities. Out of the 148 patients, 18.91% (n:28) had hydronephrosis during their initial evaluation. Among these patients, 22 had mild hydronephrosis (SFU 1-2), with 77.2% (n:17) detected on the EK and 22.8% (n:5) on the contralateral (orthotopic) side. Additionally, there were six patients with moderate to severe hydronephrosis (SFU 3-4), with four on the ectopic side and two on the orthotopic kidney. Most cases of hydronephrosis (n:15, 68%) showed improvement during follow-up. At the last visit, six patients had mild hydronephrosis, and one patient had moderate to severe hydronephrosis. Surgical intervention was required in only one patient who underwent

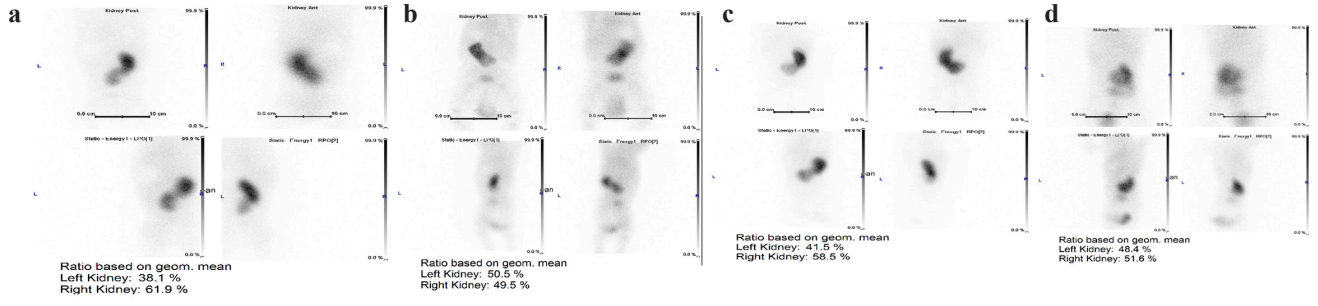

Figure 1. Examples of crossed ectopic kidney types (a-d)

temporary double J catheter placement due to UPJ obstruction. Voiding cystourethrogram (VCUG) results were available for 34 patients, revealing VUR in 11 patients (32.3%). Among these patients, nine had low-grade VUR, and two had high-grade VUR. A comparison of ectopic and orthotopic kidneys showed that eight patients had VUR in the EK, two in the orthotopic kidney, and one had bilateral VUR. Among the patients with VUR, six were managed conservatively and showed spontaneous recovery, 2 underwent endoscopic treatment, one received ureteroneocystostomy, and two were lost to follow-up. Other abnormalities included hypospadias (n:2, 1.3%), calyceal diverticula (n:1, 0.7%), undescended testis (n:4, 2.7%), neurogenic bladder (n:1, 0.7%), and UPJ obstruction (n:1, 0.7%).

In the entire group, the mean DMSA uptake was significantly lower in EKs (40.37±7.31) compared to orthotopic kidneys (59.53±7.40) ($p<0.001$). However, the differential functions were similar between simple EKs (40.44±6.12) and cross-fused EKs (40.27±8.90) ($p<0.447$). Among EKs, more than half (n:84, 57.5%) exhibited some degree of pathological DMSA uptake patterns, including hypoactive areas/scarring (n:36, 24.65%) or globally diffuse decreased uptake (n:48, 32.87%). However, there was no significant difference between simple and cross-fused ectopic kidneys in terms of DMSA uptake patterns (see Table 4).

Comparison of simple and cross-fused ectopic kidneys revealed similar results regarding the presence of any degree of hydronephrosis, VUR, and the differential function of ectopic kidneys (see Table 5).

Extrarenal malformations were identified in 14.9% (n:22) of patients. The most common abnormalities had cardiac origins, affecting 5.4% of patients (n:8). These cardiac abnormalities included atrial septal defect (ASD) (n:1), coarcation of aorta (CoA) and (atrioventricular septal defect) AVSD (n:1), Tetralogy of Fallot (n:1), ventricular septal defect (VSD) (n:1), CoA (n:1), and bicuspid aortic valve (n:1). Additionally, four patients (2.7%) exhibited VATER association (Table 6).

Table 1. Demoghraphic data and patient characteristics

Variables	n (%) or Mean±SD
Sex	
Male	83 (56.10)
Female	65 (43.90)
Age at diagnosis (months) [median-IQR]	42 [97.13]
Weight SDS at admission	-0.59±1.17
Height SDS at admission	-0.65±1.17
BMI SDS at admission	-0.22±1.16
Laterality of EK	
Left to right	72 (48.60)
Right to left	65 (43.90)
Bilateral	11 (7.40)
Localisation of EK	
Pelvic	89 (60.10)
Lower abdominal	58 (39.20)
Thoracic	1 (0.70)
Type of EK	
Simple	87 (58.80)
Cross unfused	3 (2.00)
Cross fused	58 (39.20)
Inferior	33 (56.90)
Sigmoid or S-shaped	5 (8.60)
Lump	3 (5.20)
Disc	7 (12.10)
L-shaped	10 (17.20)
Superior	0 (0.00)

SDS: Standard deviation score, BMI: Body mass index, EK: Ectopic kidney

Table 2. Clinical presentations and complaints

Chief complaint	n (%)
Suspected renal agenesis	11 (7.43)
Family history	3 (2.02)
Antenatal empty renal fossa	11 (7.43)
Congenital HN	2 (1.35)
Growth retardation	5 (3.37)
Enuresis	5 (3.37)
Dysuria	1 (0.67)
Hematuria	1 (0.67)
Hypospadias	2 (1.35)
Urinary tract infection	6 (4.05)
Abdominal pain	15 (10.13)
Syndromic appearance	10 (6.75)
Spina bifida	1 (0.67)
Tuberose sclerosis	1 (0.67)
Incidental finding	75 (50.67)

Table 3. Urological abnormalities accompanying ectopic kidneys					
			Ectopic kidney n (%)	**Contralateral kidney n (%)**	**Bilateral n (%)**
VUR(+)		Low grade (GI-III)	6 (17.6)	2 (5.8)	1(2.9)
		High grade (GIV-V)	2 (5.8)	-	-
Hydronephrosis	**Admission**	SFU I-II	17 (11.50)	5 (3.40)	-
		SFU III-IV	4 (2.70)	2 (1.40)	-
		SFU 0	127 (85.80)	141 (95.20)	-
	Last visit	SFU I-III	6 (4.1)	-	-
		SFU III-IV	1 (0.7)	-	-
		SFU 0	141 (95.2)	148 (100)	-
VUR: Vesicoureteral reflux, SFU: Society of Fetal Urology					

Table 4. DMSA uptake patterns of ectopic kidneys with respect to type of ectopia				
DMSA*		**Simple EK n (%)**	**Cross EK n (%)**	**p-value****
DMSA Normal		35 (23.97)	27 (18.49)	0.507
DMSA Abnormal		52 (35.62)	32 (21.92)	0.098
Hypoactivity/scar		24 (16.44)	12 (8.22)	
Global diffuse decreased uptake		28 (19.18)	20 (13.70)	
VUR	present	8 (23.5)	3 (8.8)	0.067
	absent	9 (26.4)	14 (41.2)	
Hydronephrosis admission	absent	75 (62.5)	45 (37.5)	0.057
	present	12 (42.9)	16 (57.1)	
*2 patients'DMSA were inconclusive due to disc-shaped kidney, **Chi-square test				

Table 5. Comparison of simple and cross ectopic kidneys			
Variables	**Simple ectopia**	**Cross ectopia**	
	Mean±SD	**Mean±SD**	**p-value**
Diagnosis time (month)	56.46±59.68	58.88±55.19	0.675
Follow-up time (month)	66.76±61.39	68.25±65.64	0.623
Cre admission	0.41±0.15	0.39±0.15	0,569*
Cre last admission	0.43±0.14	0.43±0.17	0,630*
GFR admission	135.63±28.19	152.00±35.02	0,169*
GFR last visit	159.68±35.52	161.52±30.52	0.929*
DMSA fxn EK	40.44±6.12	40.27±8.90	0.447
Cre: Creatinine, *Mann Whitney U test			

Table 6. Extrarenal anomalies accompanying ectopic kidneys	
	N (%)
Cardiac	8 (5.4)
VATER	4 (2.7)
GIS	2 (1.3)
GUS	2 (1.3)
Orthopedic	1 (0.6)
CNS	1 (0.6)
Myelomeningocele	1 (0.6)
External ear anomaly	2 (1.3)
Rhabdomyoma	1 (0.6)

GIS: Gastro intestinal system, VATER: Vertebral defects, anal atresia, cardiac, tracheoesophageal fistula, renal vertebral anorectal GUS: Genito urinary system CNS: Central nervous system

Discussion

This study aimed to provide a comprehensive overview of ectopic kidneys and associated renal and extrarenal anomalies. In our study group, simple ectopia was more common than crossed ectopia, with the pelvic region being the most common location. Although left-to-right ectopia (48.60%) appeared slightly more frequent than right-to-left (43.90%), there was no significant difference in terms of laterality. These findings align with previous reports by Arena et al. (11).

In our cohort, the most common type of cross-fused ectopic kidney was the inferior type, representing 56.90%. This corresponds to the fusion of the upper pole of the ectopic kidney to the inferior pole of the orthotopic kidney, consistent with findings in the literature (12). Cross-fused ectopic kidney has been classified by McDonald and McClellan (13) into six different types. The type of fusion anomaly and anatomical details may be important, especially in cases requiring surgical intervention. Glodny et al. (14) reported that CE kidneys may exhibit variations in vasculature (15). Identifying these complex anomalies through appropriate radiological investigations can assist in devising treatment strategies.

In this study, the most common urological abnormalities observed were hydronephrosis and VUR, with hydronephrosis evident in 23.6% of patients at admission. Current reports in the literature vary in terms of the prevalence of hydronephrosis, the existence of VUR, and the necessity for surgery. For instance, Gleason et al. (16) reported a hydronephrosis prevalence of 56% among patients with ectopic kidneys, and the need for surgical intervention was relatively frequent in their cohort, affecting 44 out of 82 ectopic kidneys (54%) .Surgical interventions included procedures such as nephrectomy, pyeloplasty, ureterocalicostomy, and ureteric reimplantation. Kramer and Kelalis (17) reported that out of 49 children with renal ectopy, 51% had hydronephrosis, and 35% of patients required surgical intervention. However, Guarino et al. (18) reported that the need for surgical intervention was approximately 1% of ectopic kidneys. Engelhardt et al. (19) reported that one-quarter of their patients required surgical intervention due to various factors such as VUR, pelvic ureteric junction obstruction, or nephrectomy. Calisti et al. (20) also reported that the need for surgical intervention in patients with CAKUT, including solitary, small, or ectopic kidneys, was less common compared to previous reports. In our study, 34 patients underwent VCUG, and reflux was detected in 11 of them (32.3%). Among these 11 patients with VUR, 2 underwent endoscopic treatment, and 1 underwent ureteroneocystostomy. Presence of VUR was similar between simple and cross-ectopic kidneys in our study, but since not all patients in our cohort underwent VCUG this comparison may not be sufficient to make definitive conclusions. In our study group, the proportion of patients requiring any surgical intervention was relatively low compared to previous reports. This might be partially explained by the limited number of patients who underwent VCUG. Fortunately, most cases of hydronephrosis resolved with close follow-up without the need for surgical intervention. Only one patient required temporary double J stent placement without further need for pyeloplasty.

One of the most striking findings in this study was that the differential functions measured by DMSA were decreased in ectopic kidneys compared to contralateral kidneys. Sarhan et al. (12) reported that impaired renal function was found in 34% of patients with ectopic kidneys, but they did not specify DMSA uptake patterns in their cohort. In our study, more than half of the ectopic kidneys exhibited some degree of pathological DMSA uptake, either in the form of

hypoactive areas/scarring or globally diffuse decreased uptake. In most cases, despite abdominal and pelvic ultrasonography being the initial diagnostic tool, it can be insufficient to fully evaluate ectopic kidneys. Therefore, complementary diagnostic tools such as DMSA scans or cross-sectional imaging modalities are often needed.

Beyond providing anatomical details, a DMSA scan can offer insights into differential functions, (the presence of renal scarring related to past pyelonephritis, and can guide the follow-up of kidney functions in affected individuals. As such, performing a DMSA scan may be a part of the evaluation for these patients. Comparing simple and cross ectopic kidneys revealed similar results regarding kidney functions. It's worth noting that the use of eGFR has limitations in patients younger than 24 months due to changing normal clearance values. Therefore, comparisons based on eGFR values were limited to patients older than 24 months.

In both groups with simple EC and crossed EC, serum creatinine levels and eGFR levels were well preserved at the last visit (156.74±28.90) compared to admission (142.80±32.37) ($p<0.05$). Throughout the entire group, after 66 months of follow-up, there was only one patient whose eGFR was <60 ml/min/1.73m² among patients who were 24 months old or older at admission.

van den Bosch et al.(21) reported that 22% of patients in their cohort exhibited a glomerular filtration rate less than 90 ml/1.73/m², although they didn't specify whether the age of the patients was taken into account. As mentioned earlier, the use of eGFR has limitations in younger patients. Based on our results, we can speculate that the overall renal prognosis was favorable in the mid-term period. In contrast to the report by van den Bosch et al.(21), where incidental diagnosis of ectopic kidneys constituted only 17% of patients, in our study, the most common presenting feature was the incidental detection of ectopic kidneys. This difference may be explained by the widespread use of ultrasonography in our center. Performing Urinary US during screening for developmental dysplasia of the hip may explain this difference. In addition to the presence of hydronephrosis and VUR, a wide range of renal and extrarenal anomalies often accompanies ectopic kidneys, either as a component of a syndrome or as isolated involvement. In this study, accompanying extrarenal anomalies were observed in 14.9% of patients, which aligns with previous reports(11). The most common extrarenal abnormality observed was of cardiac origin, seen in 5.4% of patients, and the most common association was VATER association, detected in 2.7% of patients. Other system involvements included Sprengel deformity (n:1), meningomyelocele (n:1), rhabdomyoma due to tuberous sclerosis (n:1), external ear anomalies (n:2), cerebellar dysgenesis (n:1), uterus didelphis (n:1), urachal cyst (n:1), diaphragmatic hernia (n:1), and cleft palate (n:1). In the literature, varying rates of extrarenal malformations have been reported (12,16,22).

Study Limitations

Our study has some limitations. Firstly, since this is a retrospective study, missing data may have a limiting effect. Additionally, the selection of patients for whom VCUG was performed was based on the clinician's judgment rather than a standardized protocol, which could result in the underestimation of VUR. Secondly, despite DMSA scans providing valuable information, fused ectopia can present challenges in assessment due to non-discrete renal boundaries in some cases. Therefore, anatomical variations may limit interpretations.

Conclusion

Patients with ectopic kidneys often present with renal and extrarenal anomalies. Although hydronephrosis is a common occurrence, it is usually mild and transient, and the incidence of vesicoureteral reflux is low. Considering the preservation of renal function in the mid-term period, it may be more appropriate to evaluate each patient's need for a complete urological examination on a case-by-case basis. We believe that further studies are also necessary to determine long-term risk factors in larger patient groups and to stratify patients accordingly.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Turkey, Van Training and Research Hospital Clinical Research Ethics Committee (date: 01.11.2014 approval number: VEAH KAEK).

Footnotes

Conflict of Interest: None of the authors has any financial ties that might create a conflict of interest related to the content of the manuscript.

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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

NKX2-5 Gene Variants Associated with Congenital Heart Defects in Turkish Population

Türk Popülasyonunda Konjenital Kalp Hastalıkları ile İlişkili NKX2-5 Gen Varyantları

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Abstract

Introduction: Congenital heart defects (CHDs) are the most common congenital anomaly of the newborn with high mortality and morbidity rates. Genetic and environmental risk factors have affect on cardiogenesis. NKX2-5 (NK2 homeobox 5) is a homeobox containing gene which is essential for cardiac differentiation. In this study, our aim was to detect NKX2-5 gene variants associated with CHDs in Turkish population and to better understand genotype- phenotype correlations.
Materials and Methods: In this study, we designed primers specific for NKX2-5 gene and sequenced the gene in 80 isolated CHD and 50 control group patients. Patients with chromosomal anomalies, DiGeorge syndrome and multiple congenital anomalies were not included.
Results: Most common CHDs seen in the patients were ventricular septal defects (VSD) and atrial septal defects (ASD) (20%), atrioventricular septal defects (AVSD) and tetralogy of Fallot (TOF) (8.75%). We have detected NKX2-5 gene variants in 3.75% of the patients. We found A119S, R161P and C270Y changes in TOF; PFO (patent foramen ovale) with transient supraventricular, ventricular arrhythmia; and ASD patient, respectively.
Conclusion: This study is designed to contribute to the genetic variations associated with CHD in Turkish population. NKX2-5 gene R161P variant which is on homeobox domain, was previously reported as pathogenic in an individual with thyroid ectopy and PFO. Further studies are needed to evaluate a possible role of these changes. Genetic testing is important in the follow-up and treatment of patients.

Keywords
Congenital heart defects, NKX2-5 gene, tetralogy of fallot, patent foramen ovale, atrial septal defect

Öz

Giriş: Konjenital kalp hastalıkları (KKH), yenidoğan döneminde yüksek mortalite ve morbidite oranları ile en sık görülen konjenital anomalidir. Kardiyogenezde genetik ve çevresel faktörlerin etkisi vardır. Homeobox içeren NKX2-5 (NK2 homeobox 5) geninin kardiyak farklılaşmada önemli rolü vardır. Bu çalışmamızda amaç, Türk polpulasyonunda KKH ile ilişkili NKX2-5 gen varyantlarının saptanması ve genotip-fenotip korelasyonlarına katkı sağlanmasıdır.
Gereç ve Yöntem: Bu çalışmamızda 80 izole KKH hastasında ve 50 kontrol grup hastasında NKX2-5 genine özgü primerler design edilerek gen sekanslanmıştır. Kromozomal anomalisi, DiGeorge sendromu ve multipl konjenital anomalileri olan hastalar çalışmaya alınmamıştır.

Anahtar kelimeler
Konjenital kalp hastalıkları, NKX2-5 geni, fallot tetralojisi, patent foramen ovale, atrial septal defekt

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Bulgular: Çalışmamızda hastalarda en sık ventriküler septal defekt (VSD) ve atrial septal defekt (ASD) (%20) ile atrioventriküler septal defektler (AVSD) ve Fallot tetralojisi (TOF) (8.75%) saptanmıştır. Hastalarda NKX2-5 geni varyantları %3.75 oranında görülmüştür. A119S, R161P and C270Y değişimleri sırasıyla TOF; geçici supraventriküler, ventriküler aritminin eşlik ettiği PFO (patent foramen ovale); ve ASD hastasında mevcuttu.
Sonuç: Bu çalışma, Türk populasyonunda KKH ile ilişkili varyantların saptanmasına katkı sağlanması amacıyla sunulmuştur. Tiroid ektopisi olan PFO hastasında NKX2-5 geni homeobox domainde bulunan R161P varyantı daha önceden patojenik olarak tanımlanmıştır. Varyantların olası etkilerinin değerlendirilebilmesi için daha fazla çalışmaların yapılması gerekmektedir. Genetik testlerin yapılması hastaların takibi ve tedavisi için önemlidir.

Introduction

Congenital heart defects (CHDs) are the most common congenital anomaly of the newborn with high mortality and morbidity rates even with advances in surgery (1). Genetic mechanisms involved are complex with genetic and environmental risk factors affecting cardiogenesis. A wide range of CHD spectrum includes septal defects, valve defects and lesions affecting the outflow tract (2). *NKX2-5* (NK2 homeobox 5) is a homeobox containing gene which is essential for cardiac differentiation (3). *NKX2-5* gene mutations have been found in patients with atrial septal defect (ASD) 7, with or without atrioventricular (AV) conduction defects (OMIM#108900), conotruncal heart malformations, variable (OMIM#217095), hypoplastic left heart syndrome 2 (OMIM#614435), Tetralogy of Fallot (TOF) (OMIM#187500) and ventricular septal defect (VSD) 3 (OMIM#614432).

In this study, we sequenced the *NKX2-5* gene in 80 isolated CHD patients and 50 control patients. Our aim was to evaluate the variants of the *NKX2-5* gene related to isolated CHD in the Turkish population and to better understand genotype- phenotype correlations.

Materials and Methods

In our study, 80 CHD patients and 50 control group healthy participants were included. Patients with chromosomal abnormalities, DiGeorge syndrome and multipl congenital anomalies were excluded from the study. The approval for this study was obtained from the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date: 08.01.2016, approval number: E-70737436-050.06.04). Informed consents were obtained from all the study participants.

All the participants were evaluated by a pediatric cardiologist at the Marmara University School of Medicine.This included a clinical history, physical examination, electrocardiogram, echocardiography, and catheterization. Participants were also evaluated by medical geneticist for pedigrees, family history and physical examination.

Participants genomic DNA was isolated from peripheral blood leucocytes using RINA™ M14 nucleic acid extraction kit (IVD biotechnolojy, Istanbul, Turkey) according to the manufacturers' protocols. Samples DNA quantification and qualification measurements were done by NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific, Inc., Waltham, MA, USA). For qPCR, 3 forward (F) and 3 reverse (R) primers were designed according to referential genomic DNA sequence of NKX2-5 in GenBank database (accession no. NT_023133) (Table 1). qPCR was performed with Premix Ex Taq DNA polymerase (Cat no.# RR039W) (Takara Bio Inc., Shiga, Japan) on a Biorad CFX96 Touch thermal cycler (Biorad; Berkeley, CA, USA). The PCR cycling parameters were as follows: Pre-denaturation of template and activation of the DNA polymerase at 95˚C for 10 min, followed by 45 cycles of denaturation at 95˚C for 20 sec, annealing at temperatures specific for the primers for 20 sec and extension at 72˚C for 25 sec. Amplified products were visualised on 1% agarose gels.

PCR products were sequenced with the BigDye® Terminator v1.1 Cycle Sequencing kit (Life Technologies, Carlsbad, CA, USA) with an ABI PRISM 3130XL DNA Analyzer (Applied Biosystems, Waltham, MA, USA). In order to evaluate the pathogenicity of the novel variants, we used in silico prediction tools, mutation databases (Human Gene Mutation Database and Clinvar), allele frequency in population studies (1000 Genome, Genome Aggregation Database) and the American College of Medical Genetics and Genomics (ACMG) genetic variant classification criteria (4).

Table 1. Primer pairs designed for NKX2-5				
Primer	**Forward (5'-3')**	**Reverse (5'-3')**	**Amplicon size (bp)**	**Annealing temperature (^{0}C)**
Exon 1	caaaaggagacccttccaaa	cgacaacaccaggcatcttac	844	59
Exon2a	caagcgtctctctgcctctc	gggtcccttccctaccag	685	60
Exo2b	gccgccaacaacaacttc	ggttccagcaagggttaggt	724	60

Statistical Analysis

Were done by chi-square test.

Results

Patients included in the study were aged ranging between 7 days to 17 years (median age, 13 months). Patients were 36 females (45%) and 44 males (55%). Consanguinıty of parents was seen in 12 (15%) and family history of CHD was in 10 (12.5%) patients. 16 (20%) patients had VSD and ASD, respectively. AVSD and TOF were each seen in 7 patients (8.75%) (Table 2). Healthy control group partiicipants included 30 females (60%) and 20 males (40%) aged ranging between 2 years to 45 years (median age, 24 years).

Of the 3 patients *NKX2-5* (NM_ 004387) gene variants were found in Exon 2. In a TOF female patient heterozygous c.355G>T (p.A119S) variant was found (Figure 1). Segregation analysis for this patient couldn' t have been done. In the second patient with PFO and transient supraventricular and ventricular arrhythmia in the newborn period c.482G>C (p.R161P) variant was found (Figure 2). His healthy father had this variant in a homozygous state. In an ASD male patient heterozygous c.809G>A (p.C270Y) variant was found (Figure 3). His father was found to be a heterozygous carrier (Table 3). These variants were not detected in the control group.

Discussion

NKX2-5 (NK2 homeobox5) gene which is on 5q35.1, has 2 exons and encodes a 324 aa protein. Functional domains of the NKX2-5 protein and our patients aminoasit changes are shown in Figure 4.

NKX2-5 gene variants have been found in about 3% of CHD patients, similar to our study (3.75%) (5). Previously, Akçaboy et al. (6) reported 72 conotruncal anomalies in Turkish patients and the p.R25C variant was found where together with previous studies its pathogenicity is not concluded.

Our TOF patient had A119S change that has been previously reported in patients each with adult onset cardiomyopathy and hypoplasic left heart syndrome where in functional studies a significant reduction in transcriptional activities was determined (7,8).

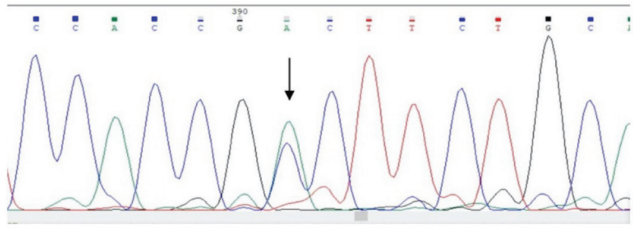

Figure 1. NKX2-5 (NM_004387) gene heterozygous c.355G>T (p.A119S) variat in TOF patient (reverse strand)

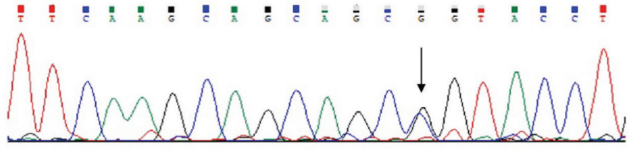

Figure 2. NKX2-5 (NM_004387) gene heterozygous c.482G> (p.R161P) variant in PFO patient

PFO: Patent foramen ovale, TOF: Tetralogy of fallot

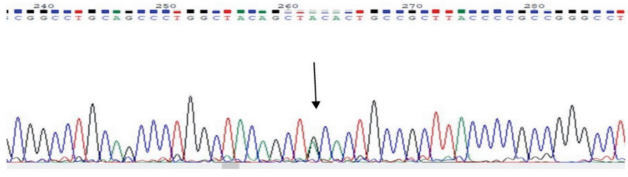

Figure 3. NKX2-5 (NM_004387) heterozygous c.809G>A (p.C207Y) variant in ASD patient

ASD: Atrial septal defect

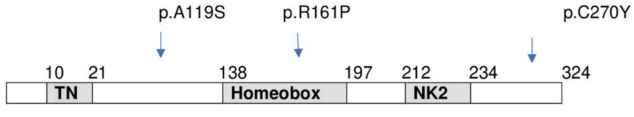

Figure 4. Functional domains of the NKX2-5 protein showing the TN, Homeobox and NK2 domains with domains with the corresponding amino acid numbers. Our three patients amino said changes are given with arrows

TN: Tinman

Table 2. Clinical characteristics of CHD patients	
Age (years)	Avr.: 3 years 9 months
Age at diagnosis (months)	Avr.: 12 months
	Number (%)
Female	36 (45%)
Consanguinity	12 (15%)
Family history	10 (12.5%)
VSD	16 (20%)
ASD	16 (20%)
AVSD	7 (8.75%)
TOF	7 (8.75%)
AQ	4 (5%)
AQ+VSD	2 (2.5%)
PDA	2 (2.5%)
PS+VSD	2 (2.5%)
VSD+PFO	2 (2.5%)
PA+VSD	2 (2.5%)
ASD+PDA	1 (1.25%)
PS	1 (1.25%)
VSD+PDA+PFO	1 (1.25%)
VSD+TA	1 (1.25%)
PS+PDA	1 (1.25%)
PA+ASD+VSD	1 (1.25%)
AQ+ASD+VSD	1 (1.25%)
TOF+ASD	1 (1.25%)
TOF+PDA+PFO	1 (1.25%)
TOF+RAA	1 (1.25%)
TGA+AVSD	1 (1.25%)
LVH+TGA	1(1.25%)
TGA+DOLV+VSD+ PS	1 (1.25%)
SV	1 (1.25%)
LVH+DORV+PA+RAA	1(1.25%)
PA, VSD, TGA, RAA	1 (1.25%)
PFO	1 (1.25%)
TrA	1 (1.25%)
Other	2 (2.5%)

Avr.: Avarage, ASD: Atrial septal defect, AQ: Aort quarctation, AVSD: Atrioventricular septal defect, DOLV: Double outlet left ventricule, DORV: Double outlet right ventricule, LVH: Left ventricular hypoplasia, PA: Pulmonary atresia, PDA: Patent ductus arteriosus, PFO: Patent foramen ovale, PS: Pulmonary stenosis, RAA: Right aortic arch, SV: Single ventricule, TA: Tricuspid atresia, TGA: Transposition of great arteries, TOF: Tetralogy of fallot, TrA: Truncus arteriosus, VSD: Ventricular septal defect

This variant has also been reported previously in an individual with thyroid ectopy, but also in relatives with normal thyroid function, which showed reduced DNA binding affinity (9). This variant is likely to be a rare, disease-modifying polymorphism (7,10). In a family with left ventricular noncompaction *MYH7, MLK2* gene variants were found and *NKX2-5* A119S change was suggested to be a modifier (11).

R161P variant found in our patient with PFO was only previously reported in an individual with thyroid ectopy. This reported patient also had PFO at birth that resolved spontaneously and had minor mitral valve insufficiency. There was minor mitral valve insufficiency in the father transmitting the R161P. This variant showed reduced DNA binding affinity (9). This variant is located on the homeobox domain (HD), and it was shown that truncation or missense mutations in the HD had severely reduced DNA binding activity and little or no transcriptional activation function (12). These variants in HD also lead to secundum ASD with AV block with a prevalence of 97.2%. Our patient also had transient supraventricular and ventricular arrhythmia in the newborn period. This variant was transmitted from a healthy father, which maybe attributed to incomplete penetrance, but we don't know if the father had PFO as a child (13).

Our patient with ASD had the C270Y variant which is on tyrosine -rich domain. This variant was previously reported as VUS (variant of unknown significance) in a patient with dilated cardiomyopathy (14). A previously reported ASD patients family members segregation analysis suggested that this variation was not correlated with CHD (13). In a RAA (right aortic arch) patient with this variant, no functional impairment in transcriptional assay was shown, still it may have caused alterations not detected by their testing system (15). In-silico analyses to examine the effects on the secondary structures of proteins showed that there was no apparent distinction between the NKX2–5 mutant protein and the wild-type protein (16). This variant is not sporodic in our patients family.

Table 3. Variants in *NKX2-5* gene and associated CHD

Patient	Exon	Nucleotid change	Amino-acid change	Domain	Familial/sporadic	CHD
1	2	c.355G>T	p.A119S	-	NA	TOF
2	2	c.482G>C	p.R161P	Homeobox	Familial	PFO
3	2	c.809G>A	p.C270Y	-	Familial	ASD

ASD: Atrial septal defect, CHD: Congenital heart defect, NA: Not available, PFO: Patent foramen ovale, TOF: Tetralogy of fallot

Conclusion

As there are few studies related with the *NKX2-5* gene in the Turkish population, we have reported our three *NKX2-5* gene variants to contribute to the genetic variations associated with CHD. We recommend genetic testing as defining a variant is important in the follow-up and treatment of patients. Further studies are needed to evaluate a possible role of these changes and to determine the genetic variations associated with CHD.

Ethics

Ethics Committee Approval: The approval for this study was obtained from the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date: 08.01.2016, approval number: E-70737436-050.06.04).

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Footnotes

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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Implementation of the Phoenix Sepsis Score in Vietnamese Pediatric Patients: Prognostic Accuracy and Comparative Analysis with Neutrophil-Lymphocyte Ratio

Phoenix Sepsis Skorunun Vietnamlı Pediatrik Hastalarda Uygulanması: Prognostik Doğruluk ve Nötrofil-Lenfosit Oranı ile Karşılaştırmalı Analizi

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Abstract

Introduction: To evaluate the implementation of the Phoenix sepsis score in the prognosis of pediatric sepsis, and to compare the prognostic accuracy of the Phoenix with that of the NLR.

Materials and Methods: A retrospective cohort analysis was conducted on pediatric patients diagnosed with sepsis. Patients were categorized using the Phoenix and the NLR.

Results: In a cohort of 63 pediatric patients diagnosed with sepsis using established criteria, the Phoenix sepsis score identified 16 as suspected sepsis and 47 as confirmed sepsis. Patients with confirmed sepsis exhibited significantly different clinical and laboratory features, such as pre-existing conditions, lower Glasgow Coma Scale scores, lymphopenia, and lactic acidosis ($p < 0.05$). The Phoenix demonstrated superior prognostic accuracy in predicting mortality compared to the NLR, with an AUC of 0.886 versus 0.649.

Conclusion: The Phoenix sepsis score is a valuable prognostic tool in pediatric sepsis, outperforming NLR in predicting mortality.

Keywords
Pediatric sepsis, classification, prognosis, clinical outcomes

Anahtar kelimeler
Pediatrik sepsis, sınıflandırma, prognoz, klinik sonuçlar

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Öz

Giriş: Phoenix sepsis skorunun pediatrik sepsisin prognozundaki uygulanmasını değerlendirmek ve Phoenix'in prognostik doğruluğunu NLR ile karşılaştırmak.

Gereç ve Yöntem: Sepsis tanısı alan pediatrik hastalar üzerinde retrospektif bir kohort analizi yapıldı. Hastalar Phoenix ve NLR kullanılarak kategorize edildi.

Bulgular: Kurulmuş kriterler kullanılarak sepsis tanısı konan 63 pediatrik hastadan oluşan bir kohortta, Phoenix sepsis skoru 16 hastayı şüpheli sepsis ve 47 hastayı doğrulanmış sepsis olarak sınıflandırdı. Doğrulanmış sepsis olan hastalar, önceden var olan durumlar, daha düşük Glasgow Koma Skalası skorları, lenfopeni ve laktik asidoz gibi önemli klinik ve laboratuvar özellikleri sergiledi ($p < 0.05$). Phoenix, NLR ile karşılaştırıldığında mortaliteyi öngörmede üstün prognostik doğruluk gösterdi ve AUC değeri 0.886 iken NLR'nin AUC değeri 0.649 olarak hesaplandı.

Bulgular: Phoenix sepsis skoru, pediatrik sepsiste değerli bir prognostik araç olup, mortaliteyi öngörmede NLR'den daha başarılıdır.

Sonuç: Phoenix sepsis skoru, pediatrik sepsiste değerli bir prognostik araç olup, mortaliteyi öngörmede NLR'den daha başarılıdır.

Introduction

Sepsis, a life-threatening condition caused by a dysregulated response to infection, poses significant challenges in pediatric populations due to atypical symptoms that complicate early diagnosis (1). Pediatric sepsis, particularly prevalent in neonates and infants with immature immune systems, is a leading cause of morbidity and mortality globally. The incidence varies widely, and early recognition is difficult due to nonspecific symptoms like fever, tachycardia, and lethargy (2). Early and accurate prognosis is crucial for optimizing care, allowing for tailored interventions, risk stratification, and resource allocation, ultimately reducing morbidity and mortality in this vulnerable group (3,4).

The Phoenix sepsis score aims to establish a standardized, evidence-based approach to defining pediatric sepsis. Recognizing the limitations of existing sepsis definitions in accurately identifying children at risk for severe illness, the Phoenix sepsis score was developed through a rigorous process involving a multidisciplinary team of experts. By incorporating a comprehensive assessment of clinical, laboratory, and organ dysfunction parameters, these criteria seek to improve early identification, risk stratification, and subsequent management of pediatric sepsis (5).

The Phoenix sepsis score offers a significant advancement in the clinical management of pediatric sepsis. By providing a standardized, evidence-based approach to defining and identifying sepsis, clinicians can more accurately assess disease severity, initiate timely interventions, and improve patient outcomes. The criteria's emphasis on organ dysfunction and the inclusion of both clinical and laboratory parameters enhance early recognition, facilitating appropriate triage, resource allocation, and therapeutic decision-making. Ultimately, the Phoenix sepsis score have the potential to reduce morbidity and mortality associated with pediatric sepsis (5).

The neutrophil-to-lymphocyte ratio (NLR) is a readily available and inexpensive biomarker that reflects the complex interplay between inflammation and immune suppression during sepsis (6). Derived from routine complete blood count parameters, NLR quantifies the balance between neutrophils, primarily involved in the innate immune response, and lymphocytes, essential for adaptive immunity. Elevated NLR values are associated with increased severity and mortality in sepsis patients, suggesting its potential role as a prognostic indicator. However, the precise mechanisms underlying the correlation between NLR and sepsis outcome remain to be fully elucidated, necessitating further research to establish its clinical utility in different patient populations (7).

Current gaps in the literature reveal a lack of research on the newly established Phoenix sepsis score, particularly within pediatric populations in resource-limited countries. Introduced in 2024, the Phoenix sepsis score has not yet been extensively studied in these settings, where unique challenges may affect their applicability and effectiveness. This gap is especially concerning as resource-limited countries often face constraints that could influence the implementation and outcomes of such criteria. Additionally, there is a need for comparative studies that evaluate the Phoenix sepsis score alongside other established markers, such as the NLR, to better understand their utility in optimizing diagnostic and treatment strategies for pediatric patients in these contexts.

To address these gaps, our study is designed with two primary objectives: (1) to evaluate the implementation of the Phoenix sepsis score in the prognosis of pediatric sepsis, and (2) to compare the prognostic accuracy of the Phoenix sepsis score with that of the NLR.

Materials and Methods

Study Design

This study is a retrospective cohort analysis based on data derived from our previous research (8) on pediatric patients diagnosed with sepsis according to the criteria established by the International Pediatric Sepsis Consensus Conference (IPSCC) in 2005.

Study Population

The study population includes all pediatric patients from our previous research (8) conducted between 2022 and 2023 who met the necessary criteria for classification under the Phoenix framework.

Data Collection

This retrospective study includes all patients from our previous research (8) who met the full criteria for Phoenix classification.

Patients initially diagnosed with sepsis based on the IPSCC criteria were reclassified utilizing the Phoenix sepsis score. This classification system categorizes patients into three groups: sepsis suspected (0-1 points), sepsis (2 or more points), and septic shock (sepsis with at least one point in the cardiovascular criteria).

Clinical and laboratory data, including Phoenix sepsis score and NLR, were utilized to evaluate patient outcomes, specifically focusing on survival or mortality.

Phoenix Sepsis Score

The criteria for respiratory dysfunction were mechanical ventilation, the Pao2:Fio2 and Spo2:Fio2 ratios; cardiovascular dysfunction was assessed using the mean arterial pressure, lactate level, and vasoactive medications; coagulation dysfunction was assessed using the platelet count, international normalized ratio, D-dimer, and fibrinogen; and neurologic dysfunction was assessed using the Glasgow Coma Scale (GCS) and pupillary reaction (5).

The revised definition for sepsis is a Phoenix Sepsis Score of 2 or higher in patients with probable infection, and a score of 1 or more in the cardiovascular domain for sepsis meeting the requirements for septic shock.

NLR Calculation

The Neutrophil-to-Lymphocyte Ratio (NLR) was calculated using the following formula (7):

NLR = Neutrophil count (cells/μL) / Lymphocyte count (cells/μL)

Neutrophil and lymphocyte counts were obtained from complete blood count (CBC) results of the study participants.

Statistical Analysis

To evaluate the prognostic accuracy of the Phoenix sepsis score, we employed several statistical methods using SPSS version 20.0. Receiver Operating Characteristic (ROC) curves, which graph the true positive rate against the false positive rate to assess diagnostic performance, were used to measure prognostic accuracy. To measure the Phoenix sepsis score's overall discriminatory power, the area under the ROC curve (AUC) was computed. Additionally, sensitivity and specificity were computed to assess the criteria's effectiveness in correctly identifying true positives and true negatives, respectively. A comparative analysis between the Phoenix sepsis score and the NLR was conducted using these metrics to determine their relative prognostic effectiveness. Statistical significance of the comparisons was assessed using appropriate tests in SPSS 20.0 to ensure robust and reliable results.

Results

Demographic and Clinical and Laboratory Characteristics

A retrospective analysis was conducted on a cohort of 63 pediatric patients with a previous diagnosis of sepsis. Employing the Phoenix sepsis score, participants were subsequently categorized into two groups: sepsis suspected (n=16, 25.4%) and sepsis (n=47, 74.6%), with a subset of 39 cases fulfilling the criteria for septic shock.

As outlined in Table 1, demographic factors including age and sex did not exhibit significant disparities between the sepsis and sepsis suspected groups. Conversely, a statistically significant difference was observed in the prevalence of underlying diseases, with patients in the sepsis group demonstrating a higher incidence (p=0.036). Additionally, patients with sepsis were found to have significantly lower GCS (p=0.000) compared to those with suspected sepsis.

Laboratory findings revealed significant differences between the sepsis and sepsis-suspected groups. Patients with sepsis exhibited lower white blood cell and lymphocyte counts ($p<0.05$) and elevated lactate levels (p=0.013). Although a trend towards decreased neutrophil counts and increased neutrophil-to-lymphocyte ratios was observed in the sepsis group, these differences did not attain statistical significance. Platelet count, C-reactive protein, and creatinine levels did not vary significantly between the two groups. Coagulation profiles indicated higher international normalized ratios (INRs) and D-dimer levels in the sepsis group compared to the sepsis-suspected group (Table 1), while fibrinogen levels remained comparable.

Clinical outcomes demonstrated a marked disparity between the groups. Patients with sepsis had a significantly higher incidence of mechanical ventilation (66% vs. 6.2%) and mortality (61.7% vs. 6.2%, p=0.000).

Table 1. Demographic and clinical characteristics of the study population				
Parameter	**Total (n=63)**	**Sepsis suspected (n=16)**	**Sepsis (n=47)**	**p-value**
Age (month)	36 (10-96)	42 (18-108)	36 (8-96)	ns^{+}
Sex (M/F)	36/27	7/9	29/18	ns*
Underline disease (%)	47.6	25.0	55.3	0.036*
WBC (x10^3/mm^3)	13.41 (7.6-18.4)	14.25 (12.11-19.7)	12.1 (3.3-17.88)	0.042^{+}
Neutrophils (x10^3/mm^3)	9.77 (2.13-13.58)	9.99 (7.11-15.51)	8.14 (2.08-12.75)	ns^{+}
Lymphocytes (x10^3/mm^3)	1.98 (0.97-3.4)	3.19 (2.09-7.66)	1.73 (0.44-2.79)	0.001^{+}
Platelets (x10^3/mm^3)	169 (100-305)	236.5 (169-296)	148 (77-305)	ns^{+}
NLR	5.2 (2.59-7.31)	3.86 (0.92-7.05)	5.29 (2.9- 7.31)	ns^{+}
GCS	14 (12-15)	15 (15-15)	13 (10-15)	0.000^{+}
Mechanical ventilation (%)	50.8	6.2	66.0	0.000*
CRP	86.8 (34.08-84.1)	94.9 (70.62-127.2)	85.07 (21.1-161)	ns^{+}
Creatinine	47.34 (31-84.1)	42.64 (32-62.08)	49 (30.1-89.65)	ns^{+}
Lactate	3.0 (1.9-5.0)	2.05 (1.9-2.5)	3.32 (2-6.7)	0.013^{+}
INR > 1.3 (%)	47.6	18.8	57.4	0.007*
D-dimer > 2mg/L (%)	25.4	0.0	34.0	0.006$^{\#}$
Fibrinogen < 100mg/dL (%)	1.6	0.0	2.1	ns$^{\#}$
PHOENIX	4 (1-6)	0 (0-1)	5 (3-6)	0.000^{+}
Death (%)	52.4	6.2	61.7	0.000*

Data presented as median and quartile range (25% - 75%). ns: not-significant, *: Pearson chi-square test, $^{\#}$: Fisher's exact test, $^{+}$: Mann-Whitney test, WBC: White blood cells, NLR: Neutrophil to lymphocyte ratio, CRP: C reactive protein, GCS: Glasgow coma score, INR: International normalized ratio

The Phoenix sepsis score, a measure of sepsis severity, exhibited a wider range (3-6) in the sepsis group compared to the sepsis-suspected group (0-1).

Comaparative Analysis of Phoenix and NLR

Figure 1 illustrates the correlation between NLR and Phoenix sepsis score. A moderate positive correlation was observed (Pearson r = 0.3514, p = 0.047), suggesting that higher NLR values were associated with increased Phoenix sepsis score. This finding indicates a potential association between NLR and the severity of sepsis.

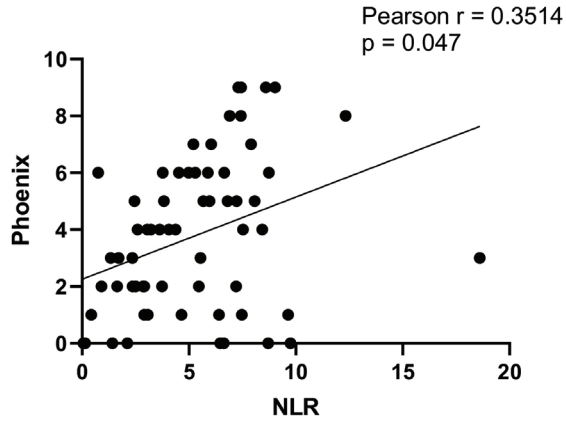

Figure 1. Correlation between Phoenix and NLR

Prognostic Performance of Phoenix Sepsis Score and NLR

Figure 2 presents the ROC curve analysis assessing the predictive performance of the Phoenix sepsis score and NLR for mortality. Both markers demonstrated acceptable discriminatory capacity in differentiating between deceased and surviving patients. Notably, the Phoenix sepsis score exhibited a marginally superior area AUC relative to NLR, implying a potentially

greater accuracy in predicting mortality within this cohort.

Table 2 presents the prognostic performance of the Phoenix sepsis score and NLR in predicting mortality. The Phoenix sepsis score demonstrated superior discriminatory capacity with an AUC of 0.886 compared to NLR with an AUC of 0.649. An optimal cut-off value of 5.5 for the Phoenix sepsis score yielded a sensitivity of 60% and a specificity of 100%, resulting in a perfect positive predictive value. In contrast, NLR, with a cut-off value of 3.7527, achieved a higher sensitivity of 80% but lower specificity of 54.5%, leading to a positive predictive value of 61.5%. Both markers exhibited comparable negative predictive values of approximately 75%.

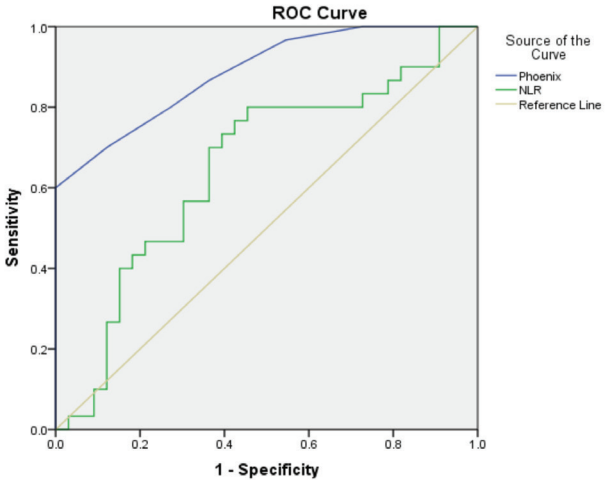

Figure 2. ROC curve analysis of the Phoenix sepsis score and NLR for predicting mortality

Subgroup Analyses

Table 3 presents the prognostic performance of the Phoenix sepsis score and NLR stratified by the presence of underlying diseases. In patients with underlying diseases, the Phoenix sepsis score demonstrated superior discriminatory capacity (AUC 0.885) compared to NLR (AUC 0.745). A cut-off value of 5.5 for Phoenix sepsis score exhibited perfect specificity and a sensitivity of 70%. NLR, with a cut-off of 6.5571, achieved moderate sensitivity and specificity.

Conversely, in patients without underlying diseases, while Phoenix sepsis score maintained a strong discriminatory ability (AUC 0.861), NLR exhibited a markedly decreased performance (AUC 0.587). A lower Phoenix sepsis score cut-off of 1.5 achieved perfect sensitivity but reduced specificity, whereas

Table 2. Prognostic performance of Phoenix sepsis score and NLR

	Phoenix	NLR
AUC	0.886	0.649
Cut off	5.5	3.7527
Sensitivity	60%	80%
Specificity	100%	54.5%
Positive predictive value	100%	61.5%
Negative predictive value	73.7%	75%

NLR: Neutrophil to lymphocyte ratio, AUC: Area under the curve

Table 3. Prognostic performance of Phoenix sepsis score and NLR in patient with and without underline diseases

	With underline disease		Without underline disease	
	Phoenix	NLR	Phoenix	NLR
AUC	0.885	0.745	0.861	0.587
Cut off	5.5	6.5571	1.5	4.0815
Sensitivity	70%	50%	100%	80%
Specificity	100%	100%	52.2%	56.5%
Positive predictive value	100%	100%	47.6%	44.4%
Negative predictive value	62.5%	50%	100%	86.7%

NLR: Neutrophil to Lymphocyte Ratio, AUC: Area Under the Curve

NLR's cut-off of 4.0815 yielded moderate sensitivity and specificity.

These findings suggest that the Phoenix sepsis score may be a more robust predictor of mortality across different patient subgroups, particularly in those with underlying diseases.

Discussion

This retrospective study aimed to evaluate the prognostic performance of the Phoenix sepsis score and NLR in a pediatric sepsis population. This study represents the pioneering application of the Phoenix sepsis score to pediatric sepsis patients at a tertiary hospital in Vietnam. Our findings indicate that patients with sepsis exhibited distinct clinical and laboratory characteristics compared to those with sepsis suspicion. Notably, underlying diseases and lower GCS were significantly associated with sepsis.

Laboratory abnormalities, including lymphopenia and lactic acidosis, were prominent in the sepsis group, aligning with previous studies (9,10). While the NLR did not reach statistical significance in this study, its correlation with the Phoenix sepsis score suggests a potential role in sepsis severity assessment. While several studies have demonstrated the potential of the NLR as a prognostic biomarker for pediatric sepsis severity, others have yielded inconclusive results. This variability may be attributed to age-related changes in lymphocyte and neutrophil counts, which can influence the NLR's predictive value (7,11). In our subgroup analysis, the NLR was found to be a prognostic indicator in pediatric patients with sepsis and underlying diseases.

The Phoenix sepsis score demonstrated superior prognostic performance in predicting mortality compared to NLR, as evidenced by the higher AUC and superior diagnostic metrics. Furthermore, the Phoenix sepsis score maintained its predictive ability across different patient subgroups, including those with and without underlying diseases, highlighting its potential as a robust prognostic tool in pediatric sepsis.

Study Limitations

The present study has several limitations that warrant consideration. Firstly, the retrospective design inherent to this research limits the ability to establish definitive causal relationships between variables. Secondly, the relatively small sample size might have influenced the statistical power to detect certain effects, potentially leading to type II errors.

Additionally, while the Phoenix sepsis score and NLR demonstrated significant prognostic value, the study did not delve into the impact of early intervention based on these markers on patient outcomes. Furthermore, the focus on pediatric patients limits the generalizability of the findings to other age groups.

Future Research Directions

Expanding on the results of this investigation, other directions for further study can be pursued. Larger sample numbers are necessary for prospective studies to verify the predictive validity of the NLR and Phoenix sepsis score, as well as to assess how they affect patient outcomes. Investigating the potential of combining the Phoenix sepsis score with other biomarkers or clinical variables to enhance predictive accuracy could be a promising direction.

Furthermore, exploring the role of the Phoenix sepsis score in guiding therapeutic decisions and its correlation with specific interventions would provide valuable insights into optimizing patient management. Expanding the study population to include different age groups would contribute to a broader understanding of the generalizability of the findings.

Finally, investigating the cost-effectiveness of implementing the Phoenix sepsis score in clinical practice would provide crucial information for resource allocation and healthcare policy decisions.

A more thorough understanding of the function of the Phoenix sepsis score and NLR in pediatric sepsis management can be attained by resolving these limitations and following the suggested research initiatives, which will ultimately improve patient outcomes.

Conclusion

This study underscores the importance of early and accurate sepsis identification in pediatric patients. The Phoenix sepsis score emerged as a valuable prognostic tool, exhibiting superior discriminatory capacity for mortality prediction compared to NLR. The consistent performance of the Phoenix sepsis score across different patient subgroups strengthens its potential clinical utility. Further prospective studies are warranted to validate these findings and explore the impact of early intervention based on the Phoenix sepsis score on

patient outcomes. By implementing the Phoenix sepsis score in clinical practice, healthcare providers may be able to identify high-risk patients earlier, facilitating timely and appropriate interventions, ultimately improving patient outcomes.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Ethics

Ethics Committee Approval: Animal and human rights statement.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Footnotes

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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Evaluation of the Children with Secondary Osteoporosis: A Single-Center Experience

Sekonder Osteoporoz Tanısı Alan Çocuk Hastaların Değerlendirilmesi: Tek Merkez Deneyimi

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Abstract

Introduction: Osteoporosis is a skeletal disease characterized by low bone mass, which increases the risk of fractures and can arise from primary or secondary causes. The aim of this study is to evaluate the frequency and causes of secondary osteoporosis diagnosis in patients presenting to our clinic, as well as to assess the clinical characteristics and treatment responses of these patients.

Materials and Methods: Seventy patients with secondary osteoporosis, who were followed and treated for at least two years due to chronic disease, were included in the study. The clinical characteristics, comorbidities, medications used, laboratory tests, Dual X-ray Absorptiometry (DXA) scans, magnetic resonance imaging results, and treatment protocols of the patients were evaluated.

Results: The mean age of the patients was 10.37±3.81 years. The mean age at diagnosis of the primary disease (chronic illness) was 4.47±3.54 years. The mean duration for the development of osteoporosis was 5.76±4.31 years. Among the cases, 21 (30%) had oncological, 15 (21.5%) had rheumatological, 11 (15.7%) had nephrological, 11 (15.7%) had hematological, 4 (5.7%) had neurological diseases, and 8 (11.4%) had other diseases. Of the patients, 35 (50%) had a history of steroid use, 16 (22.9%) used both steroids and methotrexate (MTX), 10 (14.3%) used MTX, and 9 (12.9%) used antiepileptic drugs. The mean vertebral DXA Z-score before treatment was -3.06±1.05, while the DXA Z-scores at the 1st and 2nd years of treatment were -2.51±1.09 and -2.16±1.15, respectively. A significant difference was found between the pre-treatment and 1st and 2nd year DXA Z-scores ($p<0.001$).

Conclusion: In our study, patients with secondary osteoporosis caused by various chronic diseases and their treatments were evaluated. With treatment, significant positive changes in bone mineral density and clinical findings were observed. There is a need for the development of guidelines for the diagnosis, treatment, and follow-up of secondary osteoporosis patients and for the creation of larger databases through prospective studies to guide clinical practices.

Keywords
Secondary osteoporosis, bone mineral density, Dual X-ray Absorptiometry

Anahtar kelimeler
Sekonder osteoporoz, kemik mineral yoğunluğu, Dual X-ray Absorbsiyometri

Öz

Giriş: Osteoporoz, kırık riskinde artış yapan, primer ya da sekonder nedenlerle ortaya çıkabilen, düşük kemik kitlesi ile karakterize bir iskelet hastalığıdır. Bu çalışmanın amacı, kliniğimize başvuran hastaların ne sıklıkta ve hangi nedenlerle sekonder osteoporoz tanısı aldığını, hastaların klinik özelliklerinin ve tedavi yanıtlarının değerlendirilmesidir.

Gereç ve Yöntem: Kronik hastalık nedeniyle izlenen, sekonder osteoporoz tanısı alıp en az iki yıl takip ve tedavi edilen 70 hasta çalışmaya dahil edildi. Hastaların

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klinik özellikleri, ek hastalıkları, kullandığı ilaçlar, laboratuvar testleri, Dual X-ray Absorbsiyometri (DXA) taraması ve manyetik rezonans görüntüleme sonuçları ve tedavi protokolleri değerlendirildi.
Bulgular: Hastaların ortalama yaşı 10,37±3,81 yıldı. Primer hastalığın (kronik hastalık) ortalama tanı yaşı 4,47±3,54 yıldı. Osteoporoz gelişme süresi ortalama 5,76±4,31 yıldı. Olguların 21'inde (%30) onkolojik, 15'inde (%21,5) romatolojik, 11'inde (%15,7) nefrolojik, 11'inde (%15,7) hematolojik, 4'ünde (%5,7) nörolojik hastalık ve 8'inde (%11,4) diğer hastalıklar tespit edildi. Hastaların 35'inde (%50) steroid, 16'sında (%22,9) steroid ve metotreksat (MTX), 10'unda (%14,3) MTX, 9'unda (%12,9) antiepileptik ilaç kullanım öyküsü vardı. Tedavi öncesi, ortalama vertebral DXA Z skoru -3,06±1,05 iken 1. yıl ve 2. yılda DXA z skorları sırasıyla -2,51±1,09 ve -2,16±1,15 idi. Tedavi öncesi ile 1. yıl ve 2. yıl DXA z skorları arasında anlamlı fark saptandı ($p<0,01$).
Sonuç: Çalışmamızda, çeşitli kronik hastalıkların ve tedavilerinin neden olduğu sekonder osteoporoz hastaları değerlendirildi. Tedavi ile, kemik mineral yoğunluğu ve klinik bulgularda anlamlı pozitif değişiklik saptadık. Sekonder osteoporoz hastalarının tanı, tedavi ve takip kılavuzlarının geliştirilmesi ve klinik pratikleri yönlendirmek için prospektif çalışmalarla daha geniş veri tabanlarının oluşturulmasına ihtiyaç bulunmaktadır.

Introduction

Osteoporosis is a global health issue characterized by decreased bone mineral density (BMD) and disruption of bone microarchitecture, which increases bone fragility and susceptibility to fractures. Pediatric osteoporosis can present primarily as osteogenesis imperfecta (OI) and idiopathic juvenile osteoporosis (IJO), or secondarily due to long-term treatments for various chronic diseases or as a result of immobilization (1,2). The complication of treating chronic diseases in childhood, resulting in prolonged life spans, provides sufficient time for the development of osteoporosis. Consequently, pediatric osteoporosis has become increasingly common in recent years. Fractures resulting from osteoporosis can lead to pain and reduced quality of life in pediatric patients (3). Therefore, it is crucial to diagnose the condition quickly and accurately to begin treatment as soon as possible.

The diagnosis of osteoporosis is made based on the concordance of clinical findings and Dual X-ray Absorptiometry (DXA) results. According to the 2019 report by the International Society of Clinical Densitometry (ISCD), pediatric osteoporosis is defined by the presence of a clinically significant fracture or a significant fracture history along with low BMD (4). Therefore, to diagnose a child with osteoporosis, there must be both reduced bone mass and the presence of fractures.

Deciding whether and when to start treatment in children is challenging. The clinical disease spectrum is broad, and therefore a one-size-fits-all treatment strategy does not exist. Important factors to consider include, among others, the presence of symptoms (e.g., back pain or musculoskeletal pain), the nature and severity of any underlying condition, the level of mobility, and the likelihood of spontaneous or medication-assisted recovery. For instance, the timing and recovery potential of osteoporosis related to leukemia are completely different compared to osteoporosis due to Duchenne muscular dystrophy, thus requiring different treatment approaches and durations (5).

In our study, we aimed to examine the clinical features of patients diagnosed with secondary osteoporosis, the underlying causes, pre- and post-treatment DXA results, laboratory tests, and the treatment protocols they received. By determining the relationship of this condition with fractures, we also aimed to review these causes alongside a literature review.

Materials and Methods

Study Design and Data Collection

Data of patients diagnosed with osteoporosis at the Pediatric Endocrinology Clinic of Akdeniz University between January 2019 and January 2023 were retrospectively reviewed from hospital and file records.

Patients who were followed up for at least two years after diagnosis and received treatment were included in the study. The patient data of a total of 176 patients were reviewed. Thirty-two patients were excluded from the study due to having a diagnosis of OI and three due to having a diagnosis of IJO (Idiopathic Juvenile Osteoporosis). Forty-one patients with a follow-up period of less than two years were excluded from the study. Thirty patients with missing DXA information in their files were also excluded from the study. A total of 70 patients were included in the study (Figure 1).

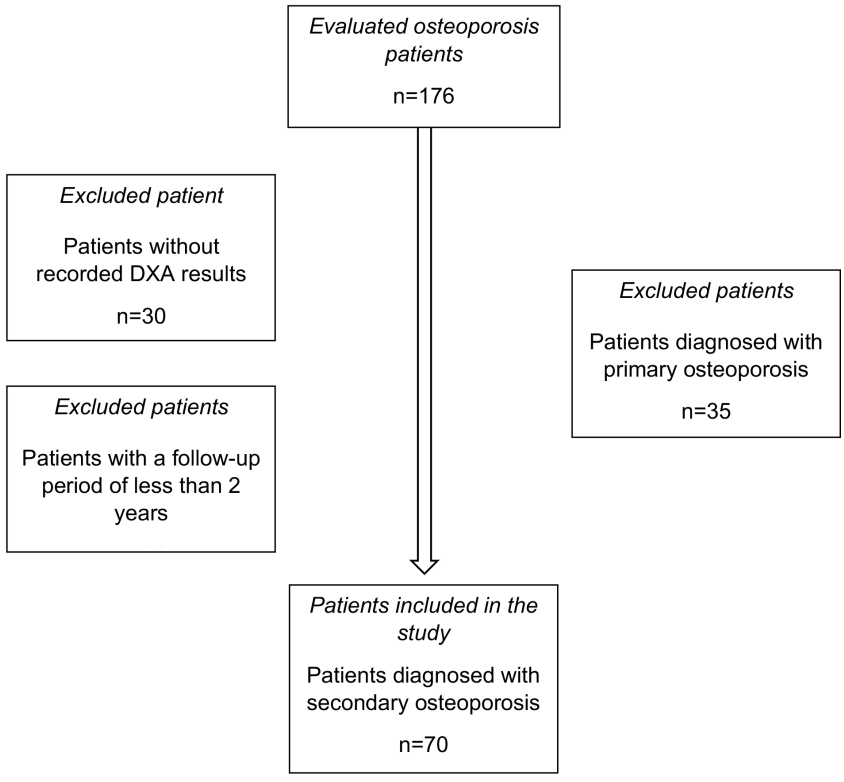

Figure 1. Working flow chart

The clinical features, underlying etiologies, laboratory tests, DXA scan results, treatment protocols, and the duration and types of previous medication treatments of the patients were evaluated. Data were recorded at the start of treatment and at the 1st and 2nd years of treatment. After the baseline DXA scan, the DXA results at the 1st and 2nd years of treatment were recorded to assess the effectiveness of the treatment. MRI data were recorded to evaluate vertebral compression fractures before treatment and at the 1st and 2nd years of treatment. Our cases were evaluated in accordance with the Helsinki Declaration. Approval for the study was obtained from the Ethics Committee of Akdeniz University (date: 25.01.2023, approval number: 70904504/64).

Identification and Diagnostic Procedure

ISCD criteria were used to define osteoporosis (6):

I. One or more vertebral compression fractures in the absence of high-energy trauma or local disease, independent of BMD z-score

II. BMD Z-score ≤-2 with a history of clinically significant fracture in patients without vertebral compression fractures

Clinically significant fractures were defined as:

a) Two or more long bone fractures by the age of 10 years

b) Three or more long bone fractures at any age up to 19 years

The anthropometric, clinical, laboratory, and radiological data of the patients were obtained from patient records. Height and body weight measurements were taken using a wall-mounted, calibrated Harpenden Stadiometer (Holtain Ltd.) and an electronic scale with 0.1 kg precision. The patients' height, height standard deviation score (SDS), body weight, body weight SDS, body mass index (BMI), and BMI SDS values were recorded. BMI was calculated as the ratio of weight in kilograms to the square of height in meters (kg/m²). Each anthropometric measurement's SDS was calculated according to Turkish children's standards (7).

Imaging

BMD measurement, the lumbar region (L1-L4) was assessed using DXA (QDR 4500, Hologic Inc., Bedford, MA, USA). The BMD results were expressed as Z-scores in SDS, based on age- and gender-matched

national reference data specific to the equipment used (8). Magnetic resonance imaging (MRI) was utilized to evaluate vertebral compression fractures.

Biochemical Analyses

Serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), parathyroid hormone (PTH), 25-hydroxy vitamin D [25(OH)D], urine Ca, and urine creatinine levels were analyzed. Serum and urine Ca, P, and ALP were measured using a colorimetric method on the Roche Cobas 8000 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). PTH was measured using the "Electrochemiluminescence Immunoassay" (ECLIA) method on the Roche Modular Analytics E170 Immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Urine creatinine was measured using the Modified Jaffe method on the Roche Cobas 8000 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). 25(OH)D was measured using a chemiluminescence immunoassay method on the Siemens Centaur XP device (Siemens Healthcare Diagnostics, Forchheim, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0. Categorical measurements were presented as numbers and percentages, while continuous measurements were presented as mean ± SD or median (interquartile range). The Shapiro-Wilk test and the Kolmogorov-Smirnov test were used to determine whether the parameters in the study followed a normal distribution. For the comparison of continuous measurements between groups, distributions were checked, and for parameters that did not show a normal distribution, a p-value of less than 0.05 obtained from the Wilcoxon test was considered significant. For repeated measures, a p-value of less than 0.016 obtained from the Friedman test was considered significant.

Results

Of the 70 cases included in the study, 36 (51.4%) were male and 34 (48.6%) were female. The mean age of the patients was 10.3±3.8 years. The mean height SDS was -3.0±2.5, the mean body weight SDS was -1.9±2.9, and the mean BMI SDS was -0.1±1.9.

In our study, the average age at diagnosis of the primary disease (chronic disease) was 4.47±3.54 years. The average time to develop osteoporosis after being diagnosed with a chronic disease was 5.76±4.31 years. Among the cases, 21 (30%) had oncological diseases, 15 (21.5%) had rheumatological diseases, 11 (15.7%) had nephrological diseases, 11 (15.7%) had hematological diseases, 4 (5.7%) had neurological diseases, and 8 (11.4%) had other diseases (Figure 2).

Among the patients, 35 (50%) had a history of steroid use, 16 (22.9%) had used both steroids and methotrexate (MTX), 10 (14.3%) had used MTX, and 9 (12.9%) had a history of using antiepileptic drugs (Figure 3). The median duration of drug use was 0.96 (2.92) years. The average dose of steroid therapy, calculated as the equivalent of hydrocortisone in mg/m²/day, was 41.81±22.72 at the time of presentation.

At the time of presentation, seven (10%) cases had fractures, while 63 (90%) cases did not. During follow-up, a fracture was observed in one (1.4%) patient, whereas 69 (98.6%) did not have any fractures. In the MRI evaluations before treatment, two patients had vertebral compression fractures, but no fractures were

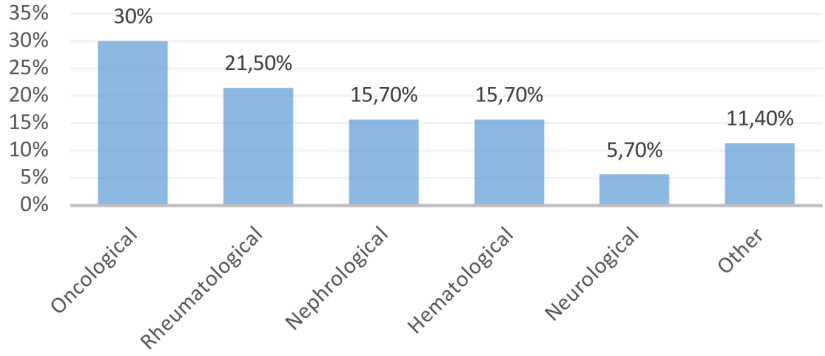

Figure 2. Classification of patients by etiology

detected in the MRIs assessed in the first and second years of treatment.

At the time of presentation, 24 (34.3%) patients had various complaints such as low back pain, widespread bone pain, and pain in the lower extremities, while 46 (65.7%) had no complaints. In treatment, 63 (90%) patients were given oral calcium and vitamin D, and 7 (10%) were given intravenous bisphosphonates and oral vitamin D. Among the patients followed, 60 (85.7%) had good treatment adherence, and no side effects related to the treatment were observed in any of the patients.

Before treatment, the average vertebral DXA z-score was -3.06±1.05. Significant improvement was observed when comparing pre-treatment with the first year of treatment, pre-treatment with the second year of treatment, and the first year of treatment with the second year of treatment ($p<0.05$). A significant increase in 25(OH)D levels was observed when comparing pre-treatment with the first year of treatment and pre-treatment with the second year of treatment ($p<0.05$). No significant changes were detected in serum Ca, P, ALP, PTH, and urine Ca/Creatinine values (Table 1).

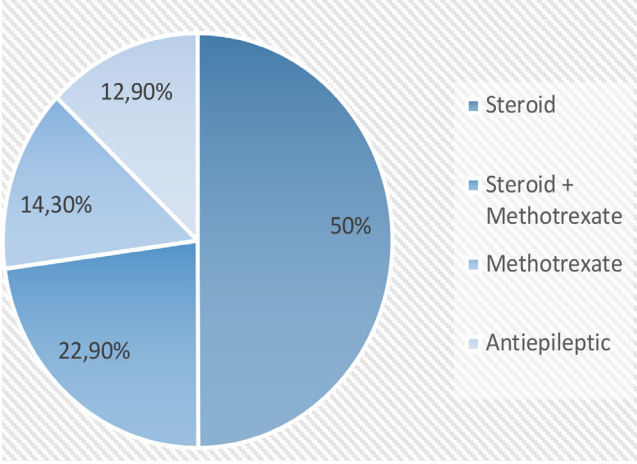

Figure 3. History of drug use among patients

Among the seven patients undergoing intravenous bisphosphonate treatment, two (28.6%) were found to have oncological diseases, two (28.6%) had rheumatological diseases, one (14.3%) had a neurological disease, and two (28.6%) had other diseases. Four patients (57.1%) had a history of steroid use, two (28.6%) had a history of antiepileptic drug use, and one (14.3%) had a history of both steroid and methotrexate use. The average duration of medication use was 3.65±1.29 years. At the time of admission, five patients (71.4%) had fractures, while two (28.6%) did not. In the pre-treatment MRI evaluations of the two patients without fractures, vertebral compression fractures were present. The average age at diagnosis of the primary disease was 4.34±3.71 years. The average time to develop osteoporosis after receiving a chronic disease diagnosis was 3.82±5.42 years. At admission, five patients (71.4%) reported various complaints such as back pain, generalized bone pain, and pain in the lower extremities, while two patients (28.6%) had no complaints. Compared to pre-treatment, there was a significant improvement in the average vertebral DXA Z score at the 1st year and 2nd year (respectively -4.19±1.15; -3.16±1.54; -2.27±1.56, $p<0.05$).

Table 1. Comparison of laboratory and imaging data before treatment, and at the 1st and 2nd years of treatment

	Before treatment	The first year of treatment	The second year of treatment	p-value
Vertebral DXA Z score	-3.06±1.05[a]	-2.51±1.09[b]	-2.16±1.15	**<0.001**
Ca (mg/dL)	9.48±0.64	9.60±0.46	9.72±0.43	0.019
P (mg/dL)	4.61±0.77	4.65±0.65	4.48±0.71	0.39
ALP (U/L)	189.3±89.88	202.1±92.52	221.1±170.11	0.322
PTH (ng/L)	53.87±33.94	46.93±20.15	38.09±19.15	0.021
25(OH)D (µg/L)	24.03±17.4[c]	42.18±130.17	27.24±9.68	**<0.001**
Urinary Ca/Kreatinine (mg/mg)	0.15±0.19	0.11±0.16	0.14±0.18	0.465

The values are presented as mean ± SD. DXA: Dual X-ray absorptiometry, Ca: Calcium, P: Phosphorus, ALP: Alkaline phosphatase, PTH: Parathyroid hormone, 25(OH)D: 25-hydroxy vitamin D, a:The difference in vertebral DXA Z score between pre-treatment and the first year of treatment and between the first and second year of treatment was $p<0.05$, b: The difference in vertebral DXA Z score between the first and second year of treatment was $p<0.05$, c: The difference in 25 (OH)D levels between pre-treatment and the first year of treatment and between the first and second year of treatment was $p<0.05$
(According to the Wilcoxon test)

Discussion

The conditions most commonly associated with secondary osteoporosis include inflammatory diseases (causing malabsorption), myopathies (e.g., Duchenne muscular dystrophy), malignancies, hemoglobinopathies (e.g., thalassemia), immobilization, and hypogonadism (6,7).

In our study, oncological diseases were found to be the most common cause of secondary osteoporosis. Secondary osteoporosis stems from direct effects of malignant cells on the skeletal system, increased inflammation-related effects, and detrimental impacts of cancer treatments on bone tissue (9). In a study examining the causes of secondary osteoporosis, the frequency of malignancy was found to be 38.4% (10). Similarly, in our study, we identified the frequency of malignancy as 30%.

The second most commonly identified chronic disease in our study was rheumatological diseases. In these patients, proinflammatory cytokines, glucocorticoid use, growth retardation, delayed puberty, inactivity, and inadequate calcium and vitamin D are among the risk factors for developing osteoporosis (1). Altaş et al. (10) found the frequency of rheumatological diseases to be 17.3% in their study. In another study, the frequency of rheumatological diseases was determined to be 9.1% (11). In our own study, we found the frequency of rheumatological diseases to be 21.5%.

In our study, nephrological and hematological diseases were equally identified as causes of secondary osteoporosis, each accounting for 15.7% of cases. In these disease groups, the high frequency of solid organ and stem cell transplantation increases the risk of osteoporosis. Altaş et al. (10) reported that 6.2% of patients had chronic kidney failure and 0.3% had undergone kidney transplantation in their study. In another study, hematological disease was found to be 18.2%, while renal diseases were detected at a rate of 9.1% (11). Additionally, Sağlam et al. (12) reported a frequency of hematological disease at 24% in their study.

In our clinic, neurological diseases were identified as a cause of secondary osteoporosis at a rate of 5.7%. Risk factors contributing to the development of osteoporosis in these patients include immobilization, reduced sunlight exposure, nutritional disorders, growth retardation, delayed puberty, and anticonvulsant therapy (1). Sağlam et al. (12) reported a frequency of neurological disease at 37% in their study. In another study, the rate of neurological disease was reported as 22.7% (11).

Among the causes of medication-induced osteoporosis, steroid use is most commonly observed (13). According to the results of our study, the medications used by patients under follow-up for secondary osteoporosis were ranked in order of frequency as steroids, steroids and MTX, MTX and antiepileptics, with steroid use being the most common, consistent with the literature.

Methods employed to prevent or treat osteoporosis in children differ from those in adults, and treatment options are limited. In our study, 63 patients (90%) received oral calcium and vitamin D, while 7 patients (10%) were treated with intravenous bisphosphonate and oral vitamin D. Bisphosphonates inhibit osteoclasts and are among the most commonly used drugs for osteoporosis treatment (14). In a study, a significant improvement was reported in bone mineral density Z scores at baseline and final evaluation after intravenous bisphosphonate treatment was administered to 34.1% of patients (-3.3±1.0 and -2.4±0.9, p=0.004, respectively) (11). Intravenous and oral bisphosphonates, as confirmed in previous studies reporting the use of bisphosphonates in secondary osteoporosis, increase bone mineral density in children (15,16).

In our study, a significant increase in 25 (OH) D levels was observed when comparing pre-treatment levels with those after the first year of treatment and also when comparing pre-treatment levels with those after the second year ($p<0.05$). However, no significant difference was found between the first and second years of treatment. It was suggested that this might be related to patients' adherence being better in the first year compared to the second year.

Study Limitations

Since our study was retrospectively planned, not all risk factors that may contribute to secondary osteoporosis could be evaluated. Additionally, being a single-center study with a limited number of patients, our results may not reflect the entire population.

Conclusion

In our study, we evaluated a heterogeneous group of various chronic systemic diseases and observed significant positive changes in BMD Z-scores and clinical findings with treatment. There is limited research specifically examining the causes of secondary osteoporosis, treatment processes, DXA monitoring, fracture development, and its relationship with bone pain in pediatric patients. There is a need for prospective studies to develop diagnostic, treatment, and follow-up guidelines for patients with secondary osteoporosis and to create larger databases to guide clinical practices.

Ethics

Ethics Committee Approval: Our cases were evaluated in accordance with the Helsinki Declaration. Approval for the study was obtained from the Ethics Committee of Akdeniz University (date: 25.01.2023, approval number: 70904504/64).

Footnotes

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

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

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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Difficulties and Advantages Experienced by Mothers of Pediatric Oncology Patients During the COVID-19 Pandemic

Pediatrik Onkoloji Hastalarının Annelerinin COVİD-19 Pandemisi Sırasında Yaşadığı Zorluklar ve Avantajlar

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Abstract

Introduction: This study addressed the difficulties and advantages experienced by the mothers of pediatric oncology patients during the COVID-19 pandemic.
Materials and Methods: Descriptive and cross-sectional study, the sample consisted of 300 mothers of children treated in the pediatric oncology and hematology clinic of a university hospital in Türkiye. The study was approved by an ethics committee. Permission was obtained from the hospital. Informed consent was obtained from participants. Data were collected using a questionnaire. The data were analyzed using descriptive statistics and the Chi-square test.
Results: Most participants regarded COVID-19 as a risk factor for their children with cancer (96.7%). More than a quarter of the participants worried that their children with cancer would not survive COVID-19 (38.7%). More than half of the participants experienced extra stress during the COVID-19 pandemic (68%). Only one in ten participants stated that they needed psychological support during the COVID-19 pandemic (12.3%). The diagnosis, gender (child), and economic status did not significantly affect the participants' extra stress during the COVID-19 pandemic ($p>0.05$). Most participants noted that the COVID-19 pandemic helped them isolate their children with cancer (84.3%), wear masks (94%), understand the importance of masks during cancer treatment (90.7%), and relieve disease management (83.0%)
Conclusion: Although the COVID-19 pandemic exacerbated the current situation for mothers of pediatric oncology patients, it contributed to the isolation process and disease management.

Keywords
Mothers, COVID-19, pandemics, Lymphoblastic Leukemia

Öz

Giriş: Bu çalışmada, pediatrik onkoloji hastalarının annelerinin COVID-19 salgını sırasında yaşadığı zorluklar ve avantajlar ele alınmıştır.
Gereç ve Yöntem: Tanımlayıcı ve kesitsel tipte olan araştırmanın örneklemini, Türkiye'de bir üniversite hastanesinin Çocuk Onkoloji/Hematoloji Kliniği'nde tedavi gören çocukların anneleri oluşturmuştur (n=300). Etik kurul, kurum ve annelerin onamı alınan çalışmada veriler, anket formu ile toplanmış, değerlendirilmesi bilgisayar ortamında tanımlayıcı istatistikler ve ki-kare testi kullanılarak yapılmıştır.
Bulgular: Araştırmaya katılan annelerin çoğunluğu (%96.7) Covid-19'u, kanser tedavisi alan çocukları için riskli bulduklarını, %38.7'si çocuğunun Covid'e yakalanması durumunda iyileşemeyeceğinden endişe ettiğini, %68.0'i pandemi sürecinde ekstra stres yaşadığını, %12.3'ü bu süreçte psikolojik destek almak

Anahtar kelimeler
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durumunda kaldıklarını belirtmişlerdir. Annelerin pandemi sürecinde ekstra stres yaşamalarında, çocuğun tanısı, cinsiyeti ve ailenin ekonomik durumunun etkili olmadığı (p>0.05) bulunmuştur. Yanısıra anneler pandemi sürecinin; kanser tedavisi alan çocuklarının izolasyon sürecine yardımcı olduğunu (%84.3), maske kullanımına kolaylık sağladığını (%94.0), kanser tedavi sürecinde 'neden maske kullanıldığının' bireyler tarafından daha rahat anlaşıldığını (%90.7) ve hastalık yönetimini rahatlattığını (%83.0) düşündüklerini ifade etmişlerdir.

Sonuç: Çalışma sonucunda pandemi sürecinin, kanser tedavisi alan çocukların annelerinin varolan zorlu ve stresli yaşam koşullarına yenilerinin eklenmesine neden olmakla birlikte, izolasyon süreci ve hastalık yönetimine de olumlu katkısının olduğu bulunmuştur.

Introduction

The novel coronavirus disease (COVID-19) shook the whole world in the first months of 2020. The rapid spread of the virus led to a global alarm (1-3). On March 11, 2020, the World Health Organization (WHO) declared the situation a pandemic (4). The COVID-19 pandemic caused financial, psychological, and sociological problems taking a toll on everybody (5-7).

Older adults, people with chronic diseases, immunocompromised patient groups, and those receiving immunosuppressive therapy were the top risk groups due to the severe course of the COVID-19 infection (8,9). Research has shown that children present with milder symptoms than adults (10,11). However, some children and their parents began the pandemic at a disadvantage and remained disadvantaged throughout the course of the process. Pediatric cancer patients constitute a high-risk group for the coronavirus for several reasons. First, they are vulnerable to infections due to reduced immunological competence. Second, they receive cancer treatment, which can cause additional physical and mental stress. Third, they visit hospitals frequently. Fourth, they are in constant contact with healthcare professionals (12,13). For all these reasons, these children have been placed at the top of the risky and disadvantaged groups during the pandemic (14,15).

Mothering a child who is being treated for cancer is one of the most challenging ordeals a mother can face. During treatment, the mother provides help and support that affects both the child's treatment outcome and the health of all family members. However, the addition of the COVID-19 pandemic to this process has increased the challenges. The COVID-19 pandemic affected children with cancer and their parents and caregivers (12,16,17). Mothers of children with cancer already feel fear, anxiety, and uncertainty. However, the COVID-19 pandemic exacerbated the situation as it caused additional financial, psychological, and social problems (18-20).

Healthcare professionals should determine the difficulties experienced by children with cancer and their parents to plan and provide healthcare services to them. They should also provide mothers with early psychosocial support. Therefore, this study investigated the challenges and advantages experienced by the mothers of pediatric oncology patients during the COVID-19 pandemic.

Material and Methods

Research Design and Population

This descriptive and cross-sectional study was conducted in the Pediatric Oncology and Hematology Clinic (Jacie accredited) of a university hospital in the Central Anatolia Region of Türkiye between March and September 2021. The study population consisted of all mothers of pediatric oncology patients in the university hospital. Mothers were the sample of choice because they undertake more duties than fathers in providing care during their children's treatment process and accompanying them in the hospital. The sample consisted of 300 participants. The inclusion criteria for children with cancer were (1) not being in the terminal stage and (2) not being in the stage of bone marrow transplantation preparation. The inclusion criterion for mothers was (1) volunteering (Figure 1). The information form was based on a literature review conducted by the researchers. It had 54 items and two parts. The first part consisted of 20 items on sociodemographic characteristics (age, education, employment status, family type, etc.). The second part consisted of 34 items on mothers' opinions, exposure, and difficulties regarding the COVID-19 pandemic (diagnosis and treatment, information about

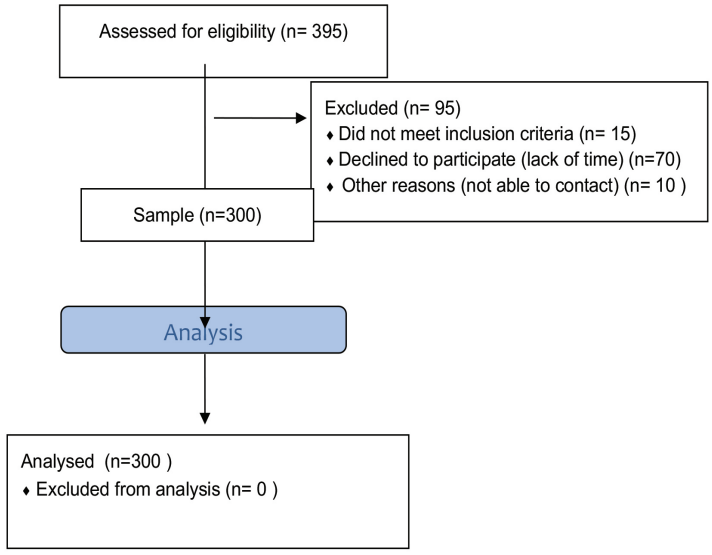

Figure 1. Flow Diagram

the disease, the impact of COVID-19 on the family and the child, the effect of COVID-19 on the cancer treatment process, etc.). The study was approved by the non-invasive ethics committee (date: 19.02.2021, approval number: 2021.03.71/2100005762). Written permission was obtained from the university hospital. All mothers were briefed about the research purpose and procedure. Informed consent was obtained from those who agreed to participate.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, IBM, version 18.0) at a significance level of 0.05. Numbers and percentages were used for descriptive statistics. The Chi-square test was used to compare categorical data.

Result

Less than half of the participants were 31 to 40 years of age (41.7%; n=125). Most participants were housewives (80%; n=240). More than half of the participants had a negative income (income < expense) (74%; n=222). Children with cancer had a mean age of 5.81±2.88 years. More than half of the children were girls (56%; n=168) diagnosed with leukemia (68.7%; n=206) (Table 1). More than a quarter of the participants viewed COVID-19 as a "fatal disease" (30%; n=90). Most participants considered COVID-19 risky for their children treated for cancer (96.7%; n=290) (Table 2).

More than a quarter of the participants stated that the COVID-19 pandemic adversely affected their financial well-being (28.7%; n=86). Less than a quarter of the participants noted that they or their spouses had to find new jobs during the COVID-19 pandemic (18.7%; n=56). Moreover, participants reported that they experienced additional stress during the COVID-19 pandemic (68.0%; n=204) as they were concerned about their healthy children catching the coronavirus (67.3%; n=202) or their children with cancer not surviving COVID-19 (38.7%; n=116). Less than a quarter of the participants also noted that the COVID-19 pandemic changed their sleep (16.3%; n=49) and dietary patterns (11.7%; n=35) (Table 3).

Most participants remarked that the COVID-19 pandemic helped them isolate their children with cancer (84.3%; n=249), wear masks (94%; n=282), and understand the reasons for using masks in the cancer treatment process (90.7%; n=272). They also noted that they believed that the COVID-19 pandemic relieved disease management (83.0%; n=249) (Table 3).

Less than a quarter of the participants stated they needed psychological support during the COVID-19 pandemic (12.3%; n=37) (Table 3). Diagnosis, gender (child), and income did not significantly affect the additional stress that all participants experienced during the COVID-19 pandemic (p>0.05) (Table 4).

Discussion

The COVID-19 pandemic changed the daily lives, habits, and routines of families, paving the way for a "new normal," which was a great challenge for parents (21). The lives of parents of children with chronic diseases, which are already distinct and challenging compared to those of other families even in ordinary circumstances, became even more arduous during the pandemic (22,23). Parents of children with chronic diseases have more challenging living conditions and lower quality of life than parents with healthy children (24-26). Mothers of children with chronic diseases in general, and mothers of children with cancer in particular, have a very low quality of life (27,28).

Pediatric cancer treatment and follow-up affect all family members (12). Like other types of cancer, pediatric cancers require special conditions for disease follow-up and treatment. Parents have to shift their roles and assume new responsibilities to provide those special conditions (12,29). As suggested Kadan, G. (30), the COVID-19 pandemic adversely affected the lives of children and their families. The COVID-19 pandemic had numerous direct and indirect effects on children with cancer and their family members (31,32). During the COVID-19 pandemic, they faced economic difficulties, changed their daily routines, and experienced sleep and dietary problems (33-35).

Table 1. Sociodemographic characteristics

Sociodemographic Characteristics	**n**	**%**
Child's age (year)		
2-4	107	35.7
5-7	125	41.7
≥8	68	22.6
Diagnosis		
Leukemia	206	68.7
Other malign	94	31.3
Gender		
Girl	168	56.0
Boy	132	44.0
Mother's age (year)		
20-30	28	9.3
31-40	125	41.7
41-50	94	31.3
51-60	53	17.7
Mother's education (degree)		
Literate	179	59.7
Primary school	121	40.3
Mother's working status		
Not working/Housewife	240	80.0
Working	60	20.0
Family's income satus		
Negative (income < expense)	222	74.0
Neutral (income = expense)	52	17.3
Positive (income > expense)	26	8.7
Family type		
Nuclear	262	87.3
Extended	38	12.7
Total	**300**	**100.0**

Table 2. Participants' information and opinions on COVID-19		
Information and opinions	**n**	**%**
What kind of disease is COVID-19?		
A mild disease that is easily treated	193	64.3
A deadly disease	90	30.0
A long-term disease	11	3.7
An incurable disease	6	2.0
Where do you get your information about COVID-19?		
TV	248	82.7
Health personnel	34	11.3
Friend/Relative	18	6.0
Has any of your family members tested positive for COVID-19?		
Yes	16	5.3
No	284	94.7
Do you consider COVID-19 risky for your children with cancer?		
Yes	290	96.7
No	10	3.3
Total	**300**	**100.0**

Table 3. The effect of COVID-19 on family life and treatment and disease management				
Impact of pandemic	**Yes**		**No**	
	n	**%**	**n**	**%**
The adverse impact of the pandemic on family life				
The adverse impact of the pandemic on family economy	86	28.7	214	71.3
Either parent had to change jobs	56	18.7	244	81.3
The adverse impact of the pandemic on mother's life				
Extra stress	204	68.0	96	32.0
Increased anxiety about healthy children catching COVID-19	202	67.3	98	32.7
Worrying that the child with cancer would not survive COVID-19	116	38.7	184	61.3
The ability to contribute to the online education of healthy children	78	26.0	222	74.0
The adverse impact of the pandemic on sleep patterns	49	16.3	251	83.7
Receiving psychological support	37	12.3	263	87.7
Being adversely affected by the diet	35	11.7	265	88.3
The child receiving cancer treatment and ıts effects on the management of the disease				
Thinking that it is useful to wear masks	282	94.0	18	6.0
Thinking that people understand why masks are necessary during cancer treatment	272	90.7	28	9.3
Thinking that it is easier to understand why it is necessary to be protected from infections during treatment.	271	90.3	29	9.7
Thinking that the pandemic helps with isolation	253	84.3	47	15.7
Thinking that the pandemic makes it easier to manage the disease	249	83.0	51	17.0
Disruption in treatment due to restrictions	29	9.7	271	90.3
Having difficulty accessing healthcare institutions for the treatment of the child with cancer due to restrictions	23	7.7	277	92.3

Table 4. Characteristics of mothers and children and mothers' extra stress during the pandemic					
Characteristics	**Mothers experiencing extra stress**				
	Yes	**No**	**Total**	**x^2**	**p-value**
Child's gender					
Girl	111 (66.1)	57 (33.9)	168 (100.0)	0.653	0.419
Boy	93 (70.5)	39 (29.5)	132 (100.0)		
Diagnosis					
Leukemia	142 (68.9)	64 (31.1)	206 (100.0)	0.262	0.608
Other malign	62 (66.0)	32 (34.0)	94 (100.0)		
Family income					
Positive (income > expense)	20 (76.9)	6 (23.1)	26 (100.0)	2.858	0.240
Neutral (income = expense)	39 (75.0)	13 (25.0)	52 (100.0)		
Negative (income < expense)	145 (65.3)	77 (34.7)	222 (100.0)		

Less than a quarter of our participants reported sleep problems (16.3%) and changes in their diets (11.7%) (Table 3). Most participants had a negative income (74%) (Table 1). Those participants stated that they faced additional challenges during the COVID-19 pandemic. More than a quarter of our participants reported that their financial situation worsened during the COVID-19 pandemic (28.7%). Less than a quarter of our participants stated that they or their spouses or both had to find new jobs during the COVID-19 pandemic (18.7%) (Table 3). These results suggest that the COVID-19 pandemic brought new challenges for children with cancer and their parents, who already went through a tough time during cancer follow-up and treatment, causing them to experience more stress and anxiety. Mothers experienced anxiety, fear, and stress during the COVID-19 pandemic. Mothers assume the care and responsibility of family members in every culture. Therefore, the fact that mothers experience anxiety, fear, and stress is not surprising. However, mothers of children with cancer experienced high levels of stress and anxiety even before the pandemic (17,28,36,37). During the pandemic, every mother in every corner of the world experienced a similar fear of losing her child. However, children with cancer fought hard and difficult battles for life even before the pandemic (18). Some children with cancer even came face to face with death. Considering all these, we can argue that the anxiety and fear experienced by mothers of healthy children and those of children with cancer during the pandemic cannot be the same. Research shows that parents of children with cancer experience high levels of stress and anxiety (17,28,36-37). Some of the most significant stressors for mothers are the risk of losing their sick child, enduring the difficulties caused by diagnosis and treatment, and a lack of maternal support for healthy children at home (17,28,36-37). More than half of our participants reported additional stress due to the COVID-19 pandemic for various reasons (Table 3). First, the more they knew about the pandemic, the more stress they might have experienced. Second, they were afraid of being exposed to infection. Third, preventive measures and fear of the unknown put a greater burden on their shoulders. Fourth, their children are disadvantaged. Fifth, people who come into contact with children have only recently begun to be vaccinated. However, diagnosis, gender (child), and income had no significant impact on their additional stress during the COVID-19 pandemic ($p>0.05$) (Table 4). In addition, less than a quarter of our participants stated that they needed psychological support during the COVID-19 pandemic (Table 3). These findings suggest that mothers of children with cancer need help and support during the COVID-19 pandemic but might be unable to receive social support due to social isolation.

Children with cancer have compromised immune systems and are at particular risk for COVID-19, which puts mothers in a precarious situation where they feel more stress during the COVID-19 pandemic. More than a quarter of our participants considered COVID-19 a fatal disease (30%). Almost

all participants believed that COVID-19 posed a significant risk to their children with cancer (96.7%) (Table 2). This may have exacerbated their fear of losing their children and resulted in increased anxiety and stress levels. More than half of our participants were concerned that their healthy children might contract the coronavirus (67.3%). More than a quarter of the participants worried that their children with cancer might not survive COVID-19 (Table 3). These findings suggest that mothers of children with cancer experienced more anxiety and struggled to cope with it during the COVID-19 pandemic. Dolunay (17) and Akoğlu et al. (38) found that children of mothers with high anxiety or stress during the COVID-19 pandemic also had high anxiety or stress. This situation could adversely affect the way families experienced the disease process (17,39,40). Similarly, how mothers perceive and cope with the COVID-19 disease affects how their children with cancer perceive the disease (41,42). Children make sense of the COVID-19 pandemic through their mothers' reactions because they experience such a challenging process for the first time (17,39,40). Multidisciplinary teams, including pediatric nurses, should provide mothers with training and counseling to help them learn about COVID-19, conquer uncertainties, experience less anxiety and stress, and prevent their children from being adversely affected by the situation.

Although the COVID-19 pandemic took a significant toll on children with cancer and their parents, our results showed that it helped mothers manage the treatment process. Chemotherapy affects children's immune systems (28,37). Therefore, protecting pediatric oncology patients with weakened immune responses from infections is one of the main goals of treatment (43-45). This means they need to be isolated. However, social isolation in response to the COVID-19 pandemic made it easier for mothers to cope with the current treatment process. Since everyone was wearing masks, their children wore masks without feeling stigmatized.

Wearing masks is an essential measure for the protection of pediatric oncology patients from infections. Before the COVID-19 pandemic, only pediatric oncology patients and their parents wore masks, However, other people around those children have worn masks since the COVID-19 pandemic (4,46). Most of our participants believed that the COVID-19 pandemic facilitated the use of masks (94%). They noted that the COVID-19 pandemic helped other people better understand why masks were integral to the cancer treatment process (90.7%). They also added that the COVID-19 pandemic relieved the disease management process (83.0%) (Table 3). We think that these are original findings that contribute to the literature. Our results showed that the COVID-19 pandemic had both positive and negative impacts on children with cancer and their parents.

Study Limitations

This study had several limitations. First, the sample size was small. Second, the results are sample-specific and cannot be generalized to all pediatric cancer patients. Third, the treatment stage of the disease (induction, maintenance, etc.) may have affected the results, but this study did not evaluate the findings by disease stage. However, this is one of the first studies to investigate the difficulties experienced by pediatric cancer children and their mothers during the COVID-19 pandemic. Moreover, our results also show that the COVID-19 pandemic made it easier for mothers to cope with the current treatment process and helped them manage it. We think this study will contribute to the literature and pave the way for further research on the impact of the COVID-19 pandemic on pediatric cancer patients and their parents.

Conclusion

Although the COVID-19 pandemic caused mothers of pediatric oncology patients to experience new stressors, it contributed to the isolation process and disease management. Multidisciplinary teams should adopt family- and child-centered care approaches and support mothers of pediatric oncology patients emotionally and psychologically to help them experience less stress and anxiety. Researchers should recruit larger samples and conduct multicenter qualitative and quantitative studies to investigate the impact of the COVID-19 pandemic on pediatric cancer patients and their parents.

Ethics

Ethics Committee Approval: The study was approved by the non-invasive ethics committee (date: 19.02.2021, approval number: 2021.03.71/2100005762). Written permission was

obtained from the university hospital. All mothers were briefed about the research purpose and procedure.

Footnotes

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

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ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

The Relationship Between System Involvement and Vitamin D Level in Cases Diagnosed with Multisystem Inflammatory Syndrome in Children

Multisistemik Enflamatuvar Sendrom Tanılı Olgularda Sistem Tutulumlarının D Vitamini Düzeyi ile İlişkisi

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Abstract

Introduction: Vitamin D is a steroid prehormone that is produced in the skin as a result of exposure to UV-B, is fat-soluble and has immunomodulatory properties by affecting immune cells. In this study, it was aimed to evaluate the relationship between systemic involvement and 25-OH vitamin D level in patients with a diagnosis of MIS-C.

Materials and Methods: It is a descriptive research. It was conducted at AFSÜ, Health Application and Research Center between 01.10.2020-01.04.2022. Patients aged between 1 month and 18 years who were diagnosed with MIS-C and who applied to AFSU, Child Health and Diseases Clinic, and whose serum 25-OH vitamin D was in the electronic archive system, were included in this study. The collected data were analyzed with the Statistical Package for Social Sciences version 26.0. It was done with the S package program.Since vitamin D was not distributed normally, the Mann-Whitney U test was used when comparing between paired groups. The Kruskal Wallis test was used when comparing vitamin D levels between more than two groups.

Results: This retrospective study was conducted with 34 patients diagnosed MIS-C. The mean age of the cases was 7.03±3.9 years; 52.9% (n=18) were males and 47.1% (n=16) were females. Gastrointestinal system (58.8%; n=20), cardiac (64.7%; n=22) and neurological involvement (23.5%; n=8) were found. Mean 25-OH vitamin D were found to be 15.7 (IQR= 18) ng/mL in gastrointestinal system, 16.8 (IQR=17) ng/mL in cardiac, and 12 (IQR= 8) ng/mL in neurological system involvement. No statistically significant differences were found between systemic involvement of the cases and their 25-OH vitamin D (p values: 0.779, 0.957, 0.144, respectively). A negative correlation was found between 25-OH vitamin D of the cases and their age (r=-0.414; p=0.015). When the relationship between 25-OH vitamin D and fibrinogen in patients diagnosed with MIS-C was evaluated, a negative correlation was also found (r=-0.414; p=0.015).

Conclusion: As a result, mean 25-OH vitamin D levels in cases diagnosed with MIS-C were found to be at an insufficient level. It was suggested that prophylactic vitamin D should be administered in children. In addition, no significant difference was found between system involvement and vitamin D level.

Keywords
MIS-C, 25-OH vitamin D, COVID-19, SARS-CoV-2.

Anahtar kelimeler
MIS-C, 25-OH vitamin D, COVID-19, SARS-CoV-2

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Öz

Giriş: D vitamini, ultraviole B ışınlarına maruz kalma sonucu ciltte üretilen, yağda çözünen ve bağışıklık hücrelerini etkileyerek immünomodülatör özelliği olan steroid ön hormondur. Bu çalışmada Multisistemik inflamatuar sendrom (MIS-C) tanılı hastaların sistemik tutulumları ile 25-OH vitamin D düzeyi arasındaki ilişkinin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Tanımlayıcı bir araştırmadır. Afyonkarahisar Sağlık Bilimleri Üniversitesi, Sağlık Uygulama ve Araştırma Merkezi'nde 01.10.2020-01.04.2022 tarihleri arasında yürütüldü. Bu araştırmaya AFSÜ, Çocuk Sağlığı ve Hastalıkları Kliniği'ne başvuran MIS-C tanısı almış olan 1 ay-18 yaş arasındaki hastalardan serum 25-OH vitamin D düzeyi eletronik arşiv sisteminde olan hastalar dahil edildi. Toplanan veriler Statistical Package for Social Sciences versiyon 26.0 (SPSS IBM, Armonk, NY, Amerika Birleşik Devletleri) ile analiz edildi. S paket programı ile yapıldı. Kategorik değişkenler yüzde ve frekanslar ile sunuldu. Sürekli değişkenlerin normal dağılıma uygunluğu Shapiro Wilk testi ve görsel histogramlar ile kontrol edildi. Normal dağılan sürekli değişkenler ortalama±standart sapma olarak, normal dağılmayan sürekli değişkenler ise ortanca ve çeyrekler-arası aralık (IQR=interquartile range) olarak ifade edildi. D vitamini normal dağılmadığı için ikili gruplar arasında karşılaştırılırken Mann-Whitney U testi kullanıldı. İkiden fazla grup arasında D vitamini düzeyi karşılaştırılırken Kruskal Wallis testi kullanıldı. Anlamlılık düzeyi $p<0.05$ olarak kabul edildi. 25-OH vitamin D ile laboratuvar değerleri arasındaki korelasyon için Spearman korelasyon testi kullanıldı.

Bulgular: Bu retrospektif çalışma MIS-C tanılı 34 hasta ile yapıldı. Olguların yaş ortalaması 7.03±3.9, %52.9 (n=18)'u erkek, %47.1 (n=16)'i ise kız idi. Olgularda gastrointestinal sistem tutulum (%58.8;n=20), kardiyak tutulum (%64.7;n=22), nörolojik tutulum (%23.5;n=8) olduğu tespit edildi. Ortalama 25-OH vitamin D düzeyleri; gastrointestinal sistem tutulumu olanlarda 15.7 (IQR= 18) ng/mL, kardiyak tutulumu olanlarda 16.8 (IQR= 17) ng/mL, nörolojik sistem tutulumu olanlarda 12 (IQR= 8) ng/mL saptandı. Olguların sistem tutulumları ile 25-OH vitamin D düzeyleri arasında istatiksel olarak anlamlı fark saptanmadı.(p değerleri sırasıyla; (p= 0.779), (p= 0.957), (p=0.144).) Olguların 25-OH vitamin D düzeyleri ile yaş arasındaki ilişki değerlendirildiğinde negatif bir korelasyon saptandı. (r=-0.414; p=0.015) MIS-C tanılı olguların 25-OH vitamin D düzeyleri ile fibrinojen arasındaki ilişki değerlendirildiğinde negatif bir korelasyon saptandı. (r=-0.414; p=0.015)

Sonuç: Sonuç olarak MIS-C tanılı olgularda 25-OH vitamin D düzeyi ortalaması yetersizlik seviyesinde olduğu saptandı. Çocuklarda profilaktik D vitamini yapılması gerektiği düşünüldü. Sistem tutulumlarının D vitamini ile ilişkisi incelendiğinde anlamlı bir fark bulunmadı.

Introduction

The coronavirus disease first appeared in late 2019 and was declared as a pandemic in March 2020. During this period, many studies have been initiated to understand the disease and to find an effective and an appropriate treatment (1). Although children and adolescents are as susceptible to infection by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) as adults, COVID-19 disease rarely causes serious illness among them. However, a proportion of children suffer from a life-threatening condition following 4-6 weeks of primary COVID-19 infection, so-called multisystemic inflammatory syndrome (MIS-C) (2). Multisystemic inflammatory syndrome was first described in the United Kingdom in April 2020. The Pediatric Intensive Care Association issued an alert describing a recognized increase in inflammation and evidence of SARS-CoV-2 infection among the critically ill patients admitted with hyperinflammatory shock. The United States Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have issued case definitions for multisystemic inflammatory syndrome. The incidence of MIS-C in populations with COVID-19 infection is 5.1 in 1.000.000 (3). All of the definitive case criteria must be met for multisystemic inflammatory syndrome. Exact case criteria were identified by CDC, WHO.

1,25 dihydroxy vitamin D, also known as calcitriol, is the active form of vitamin D. When interacting with the vitamin D receptor gene (VDR) on the immune system (B cells, T cells, and antigen presenting cells) and in pulmonary epithelial cells, the calcitriol-VDR complex induces the transcriptional expression of antimicrobial peptides such as cathelicidins and defensins (4). Cathelicidins serve to disrupt bacterial cell membranes as well as enveloped viruses such as SARS-CoV-2, while defensins promote chemotaxis of inflammatory cells through increased capillary permeability. Although vitamin D promotes the expression of various inflammatory cytokines through T cell inactivation and interferon-γ activation, it simultaneously downregulates the proinflammatory markers as interleukin-6 and tumor necrosis factor-α. According to this study, it can be said that cases with 25-OH vitamin D deficiency have a high risk of developing more severe symptoms and/or worse prognosis of

COVID-19 (5). In this study, it was aimed to evaluate the relationship between systemic involvement of patients with multisystemic inflammatory syndrome (MIS-C) and their 25-OH vitamin D levels.

Among the patients who admitted to Afyonkarahisar Health Sciences University, who were aged between 1 month-18 years old and diagnosed with MIS-C and those who had a recorded serum 25-OH vitamin D level in the electronic archive system were included in the study. The MIS-C definition of the patients was made according to the clinical guidelines of the American Academy of Pediatrics, WHO and CDC (6). Patients under 1 month and over 18 years of age, patients whose serum 25-OH vitamin D level has not been studied, patients who did not fully meet the diagnostic criteria of MIS-C, patients with a chronic disease (asthma, diabetes, tuberculosis, juvenile idiopathic arthritis, chronic renal failure, hematological and oncological diseases), patients receiving immunosuppressive treatment or taking medications continuously and patients who have received vitamin D therapy in the last 3 months were excluded from the study.

Echocardiographic findings included cardiac function, coronary artery abnormalities, cardiac valve insufficiency, and pericardial effusion. Standard echocardiographic parameters such as left ventricular ejection fraction (LVEF) assessed by two methods (Simpson biplane and M-mode methods), spectral doppler mitral inflow peak velocities, early diastolic septal and lateral mitral ring peak velocities assessed by tissue doppler imaging (TDI), tricuspid circular plane systolic excursion (TAPSE) and lateral tricuspid circular peak velocity assessed by TDI (TAPSV) were included. Coronary arteries were evaluated according to the American Heart Association (AHA) Guidelines for Kawasaki disease (7). Abnormalities were classified using the Boston z-score system. When the coronary artery Z-score was between +2.0 and +2.5, it was defined as mild coronary dilatation. The treatments of patients diagnosed with MIS-C were arranged according to the criteria determined by the American Society of Rheumatology and Erciyes MIS-C guidelines (8-10).

Material and Methods

Data Collection Technique and Tools

Patients aged between 1 month and 18 years who were diagnosed with MIS-C and who were retrospectively admitted to Afyonkarahisar Health Sciences University, Child Health and Diseases Clinic, and whose serum 25-OH vitamin D level was in the electronic archive system were included in this study retrospectively. The data of the patients diagnosed with MIS-C were accessed from the hospital electronic archive system (Nucleus-HBYS). A separate data form was created for each patient; and their demographic characteristics, diagnoses, fever history and duration, age, gender, 25-OH vitamin D, inflammation markers (C-reactive protein level, ferritin, lactate dehydrogenase), platelet, albumin, amylase, lipase, GGT, total bilirubin, direct bilirubin, creatinine kinase, INR, troponin-T and CK-MB, presence and characteristics of gastrointestinal findings, presence of cardiac findings and characteristics, treatments of given, serum 25-OH vitamin D levels, echocardiographic findings, abdominal ultrasonography and computed tomography imaging findings were recorded. Ethics committee approval was taken from Afyon Health Sciences University Clinical Research Ethics Committee (date: 04.01.2022, approval number: 2022/4).

RT-PCR test

Combined nasopharyngeal and oropharyngeal swab samples were collected from children with suspected COVID-19 and they were sent to the medical microbiology laboratory. A rapid antibody test was used to detect SARS-CoV-2 immunoglobulin (Ig) M and G (Nadal COVID-19 IgM/IgG Rapid Test). , BioServUK Ltd., UK).

Evaluation of Serum Vitamin D Level

25-OH vitamin D level was measured by electrochemiluminescence immunoassay (ECLIA) method. Vitamin D levels of the patients were considered as adequate at >20 ng/ml, inadequate between 12-20 ng/ml and deficient at <12 ng/ml (11).

Statistical Analysis

Collected data were analyzed with the Statistical Package for Social Sciences version 26.0 (SPSS IBM, Armonk, NY, United States). S package program was used. Categorical variables were presented with percentages and frequencies. Normality assumption of continuous variables was carried out with the Shapiro Wilk test and visual histograms. Normally distributed continuous variables were expressed as mean±standard

deviation, and non-normally distributed continuous variables were expressed as median and interquartile range (IQR=interquartile range). Since vitamin D was not normally distributed, Mann-Whitney U test was used when comparing the two groups. The Kruskal Wallis test was used when comparing vitamin D levels between more than two groups. Significance level was accepted as $p<0.05$. Spearman correlation test was used to analyze the correlation between 25-OH vitamin D and laboratory values.

Results

This study was conducted with 34 patients diagnosed with MISC. 52.9% (n= 18) of the cases were males and 47.1% (n= 16) were females. Mean age of the cases was 7.03±3.9 years old. The clinical, radiological and echocardiographic characteristics of the patients were shown in Table 1, and their laboratory characteristics were shown in Table 2.

There was a history of contact with COVID-19 in 82.4% (n= 28) of the cases, and 11.8% (n= 4) had SARS-Co-V PCR positivity in the nasal swab, and 35.3% (n= 12) had positive SARS CoV IgG serology. When the clinical classification of the cases was made, 82% (n= 28) were evaluated as having mild, 8.8% (n= 3) as moderate and 8.8% (n=3) as severe MIS-C. When the systems, signs and symptoms of the cases were evaluated; mucocutaneous (73.5%; n=25), cardiac (64.7%; n=22), mesenteric lymphadenopathy (61.8%; n=21), gastrointestinal (58.8%; n=20), limb edema (32.4%; n=11) and neurological (23.3%; n=8) involvement were observed. Fever symptoms were also observed in all patients (100%; n=34). It was determined that the duration of fever was longer than 72 hours in 91.1% of patients with symptoms, and longer than 24 hours in 8.8%.

It was determined that all MIS-C cases were treated with human immunoglobulin, acetylsalicylic acid and proton pump inhibitor. It was observed that acetylsalicylic acid was given to 67.6% (n=23) at 5 mg/kg dose and to 32.4% (n= 11) at 50 mg/kg dose. Antibiotic treatments of the cases were given as cefuroxime axetil (73.5%; n= 25), vancomycin (73.5%; n= 25), metronidazole (64.7%; n= 22), meropenem (29.4%; n= 10), amikacin (17.6%; n= 6) and ampicillin-sulbactam (5.9%; n= 2). It was observed that methylprednisolone was administered at a dose of 2 mg/kg in 85.3% (n=29) and at a dose of 10mg/kg in 14.7% (n=5). 11.7% (n=4) of the cases also needed inotropes; and they received dopamine infusion (8.8%; n=3) and adrenaline infusion (2.9%; n=1) as inotropic treatment. It was observed that low molecular weight heparin (enoxaparin sodium) treatment was given to the cases (38%; n=13). It was also observed that fresh frozen plasma (70.6%; n= 24), albumin (38.2%; n= 13), vitamin K (17.6%; n= 6) were given to the cases. The treatment characteristics of patients diagnosed with MIS-C are shown in Table 3.

When the distribution of the cases with mucocutaneous involvement was evaluated, conjunctivitis (55.9%; n=19), strawberry tongue (52.9%; n=18) and rash (58.8%; n= 20) were found. Mean 25-OH vitamin D levels of the cases were found to be 15.3 (IQR= 11) ng/mL in those with mucocutaneous involvement and 20 (IQR= 20) ng/mL in those without mucocutaneous involvement.

Table 1. Clinical, radiological and echocardiographic characteristics of the patients diagnosed with MIS-C

Symptom	**(n; %)**
Fever	34; 100
Duration of fever > 24 hours	3; 8.8
Duration of fever > 72 hours	31; 91.1
Rash	20; 58.8
Conjunctivitis	19; 55.9
Strawberry tongue	18; 52.9
Abdominal pain	27; 79.4
Diarrhea	9; 26.5
Vomiting	10; 29.4
Headache	8; 23.5
Change of consciousness	3; 8.8
Abdominal ultrasonographic findings	**(n; %)**
İleitis	16; 47.1
Free fluid in the abdomen	20; 58.8
Mesenteric lymphadenopathy	23; 67.6
Transient invagination	2; 5.9
Echocardiographic findings	**(n; %)**
Dilatation of coronary artery	19; 55.9
Pericardial effusion	11; 32.4
Valve anomaly	9; 26.5
Enlargement in left spaces	6; 17.6
Myocarditis	5; 14.7
Aneurysm	5; 14.7

MIS-C: Multisystem inflammatory syndrome in children

Table 2. Laboratory characteristics of MIS-C patients	
Laboratory parameter	**Mean ± SD; IQR, (lower-upper limits)**
Leukocyte (mcL)	13.081±6945; (12.053); (4000-10.000)
Lymphocyte (mcL)	1195±1500; (1680); (1.200-4000)
Thrombocyte (mEq/L)	241±132; (178.8); (160.000-370.000)
Sodium (mEq/L)	133.5±3.9; (132.5); (135-145)
Albumin (g/dL)	3.65±0.5; (3.6); (3.5-5.2)
LDH (U/L)	324±117.5; (115); (135-225)
Amilase (U/L)	43±168.4; (37); (28-100)
Lipase (U/L)	28.5±179.2; (39); (13-60)
AST (U/L)	72.1±139.1; (27); (5-41)
ALT (U/L)	55.3±95; (36); (5-40)
GGT (U/L)	18±54.1; (13); (5-60)
ALP (IU/mL)	148.5±67.3; (77); (35-130)
Total bilirubin (mg/dL)	0.57±0.8; (0.21); (0.3-1.2)
Direct bilirubin (mg/dL)	0.32±0.7; (0.13); (0-0.3)
Creatine kinase (U/L)	93.67±91.1; (61); (0-190)
Sedimentation (mm/saat)	56.7±24.6; (34); (1-15)
CRP (mg/L)	32.39±62.1; (13.5); (0-5)
Fibrinogen (mg/dL)	493.8±121.8; (166); (200-400)
INR	1.08±0.09; (0.14); (0.8-1.25)
D-dimer (ng/L)	4.41±2.28; (4.72); (0-0.5)
Troponin-T (ng/mL)	0.015±0.017; (0.007); (0-0.014)
CK-MB (pg/mL)	1.81±2.64; (1.14); (1.72-6.22)
Pro-BNP (pg/mL)	4313.3±7657; (2949); (0-125)
Vitamin D (ng/mL)	18.92±13.3; (14); (15-20)

MIS-C: Multisystem inflammatory syndrome in children, IQR: Inter quantile range, LDH: Lactate dehydrogenase, U/L: unit/ liter, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, CRP: C-reactive protein, INR: International normalized ratio, CK-MB: Creatine kinase-MB, Pro-BNP: pro-B-type natriuretic peptide

When the mean 25-OH vitamin D levels were compared in cases with and without mucocutaneous involvement, no statistically significant difference was found (p= 0.545) (Figure 1).

At least one cardiac pathology was detected in 67.6% (n= 23) of the cases with cardiac involvement in the echocardiographic assessment. Coronary artery dilatation (55.9%; n=19), pericardial effusion (32.4%; n= 11), valve anomaly (26.5%; n= 9), left cavities enlargement (17.6%; n=6), myocarditis (%) 14.7; n= 5) and aneurysm (14.7%; n= 5) were observed among the cases. Mean 25-OH vitamin D levels of the cases were found to be16.8 ng/mL (IQR=17) in cases with cardiac involvement, and 14.27 ng/mL (IQR= 11) in cases without cardiac involvement. When the mean 25-OH vitamin D levels were compared in cases with and without cardiac involvement, no statistically significant difference was found (p= 0.957) (Figure 2).

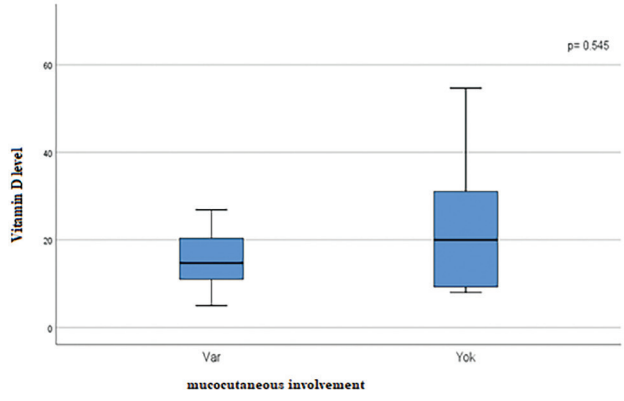

Figure 1. The comparison of mean 25-OH vitamin D levels based on the presence of mucocutaneous involvement

While mean 25-OH vitamin D level was 15.3 ng/mL (IQR= 23) in cases with mesenteric lymphadenopathy, it was 16.1 ng/mL (IQR=12) in cases without mesenteric lymphadenopathy. When mean 25-OH vitamin D levels were compared in cases with and without mesenteric lymphadenopathy, no statistically significant difference was found (p= 0.901) (Figure 3).

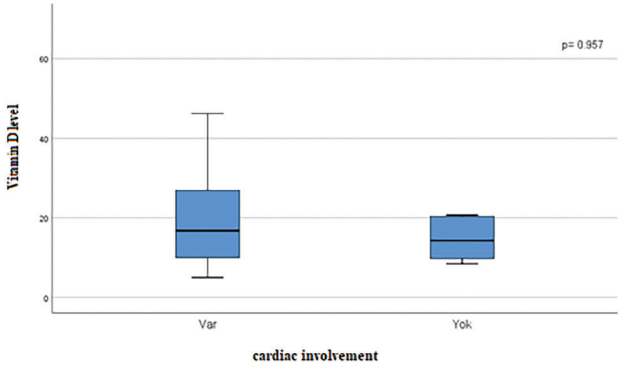

Figure 2. The comparison of mean 25-OH vitamin D levels based on the presence of cardiac involvement

Table 3. Treatment characteristics of patients diagnosed with MIS-C	
Treatment	**(n; %)**
Human immunoglobulin	34; 100
Methylprednisolone (2mg/kg)	29; 85.3
Methylprednisolone (20mg/kg)	5; 14.7
Acetylsalicylic acid (5mg/kg)	23; 67.6
Acetylsalicylic acid (50mg/kg)	11; 32.3
Albumin	13; 38.2
Fresh frozen plasma	24; 70.6
Vitamin K	6; 17.6
Dopamine infusion	3; 8.8
Adrenalin infusion	1; 2.9
Low molecular weight heparin (Enoxaparin sodium)	13; 38
Antıbıotherapy	(n; %)
Cefuroxime axetil	25; 73.5
Vancomycin	25; 73.5
Metronidazole	22; 64.7
Meropenem	10; 29.4
Amikacin	6; 17.6
Sulbactam- ampicillin	2; 5.9
MIS-C: Multisystem ınflammatory syndrome in children	

When the gastrointestinal system involvement of the cases was evaluated, abdominal pain (79.4%; n= 27), vomiting (29.4%; n= 10) and diarrhea (26.5%; n= 9) were detected. Mean 25-OH vitamin D level of the cases was found to be 15.7 ng/mL (IQR= 18) in the cases with gastrointestinal system involvement and 16.3 ng/mL (IQR= 11) in the cases without gastrointestinal involvement. When mean 25-OH vitamin D levels of cases with and without gastrointestinal system involvement were compared, no statistically significant difference was found (p= 0.779) (Figure 4).

While mean 25-OH vitamin D level was found as 14.1 ng/mL (IQR=13) in cases with extremity edema, it was 16.1 ng/mL (IQR=18) in cases without limb edema. When mean 25-OH vitamin D levels were compared in cases with and without extremity edema, no statistically significant difference was found (p= 0.912) (Figure 5).

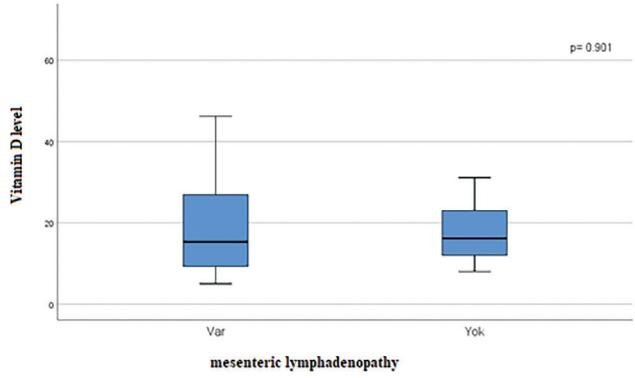

Figure 3. The comparison of mean 25-OH vitamin D levels based on the presence of mesenteric lymphadenopathy

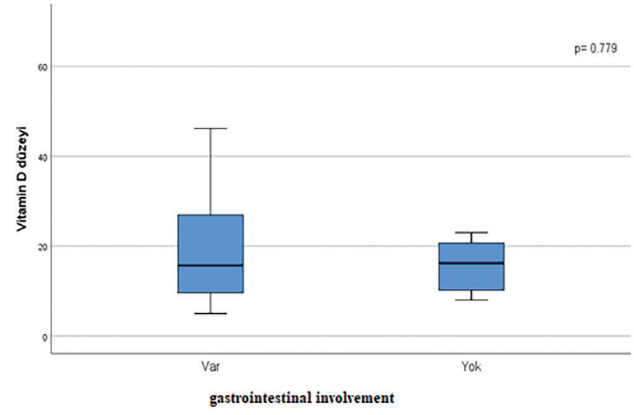

Figure 4. The comparison of mean 25-OH vitamin D levels based on the presence of gastrointestinal involvement

Headache (23.5%; n=8) and altered consciousness (8.8%; n= 3) were found in cases with neurological involvement. While mean 25-OH vitamin D levels were 12 ng/mL (IQR= 8) in cases with neurological involvement, it was 18.3 ng/mL (IQR= 18) in cases without neurological involvement. When mean 25-OH vitamin D levels were compared in cases with and without neurological involvement, no statistically significant difference was found (p=0.144) (Figure 6).

Considering 25-OH vitamin D levels of the cases, they were found to be <12 ng/mL in 38.2% (n= 13), 12-20 ng/mL in 23.6% (n=8) and >20 ng/mL in 38.2% (n= 13). Figure 7 shows the distribution of cases based on their 25-OH vitamin D levels.

Correlation analysis was performed between 25-OH vitamin D levels and age, CRP, lymphocyte count, fibrinogen, D-dimer, albumin, sodium and leukocyte counts. Vitamin D levels were found to have a statistically significant, moderately strong negative correlation with age and fibrinogen (Figures 8 and 9).

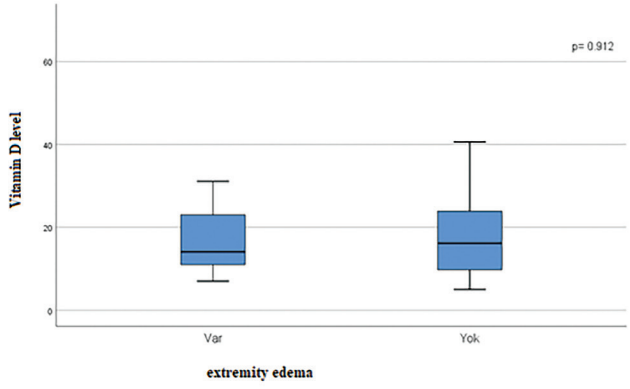

Figure 5. The comparison of mean 25-OH vitamin D levels based on the presence of extremity edema

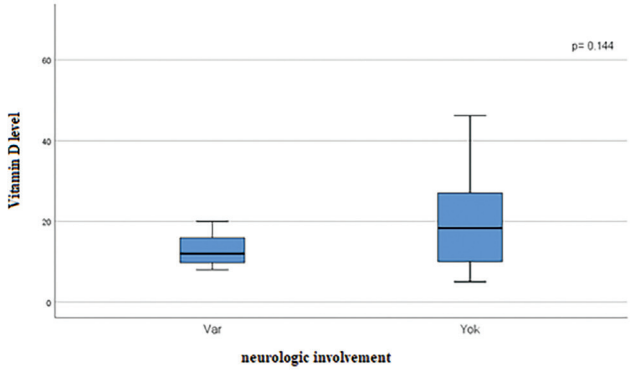

Figure 6. The comparison of mean 25-OH vitamin D levels based on the presence of neurological involvement

Table 4 shows the correlation between vitamin D and other parameters.

In our study, 3 patients received treatment in the pediatric intensive care unit and those all patients were covered well.

Discussion

In this study, we aimed to evaluate the relationship between system involvement rates and 25-OH

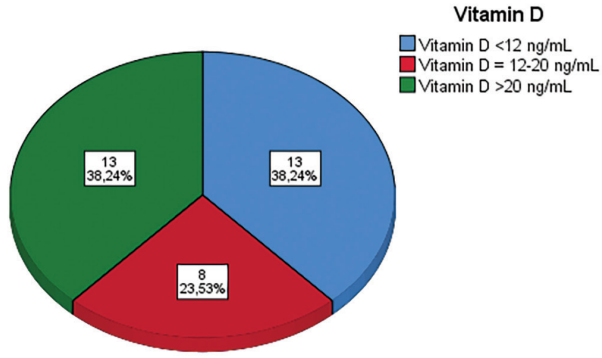

Figure 7. The distribution of 25-OH vitamin D levels of the cases

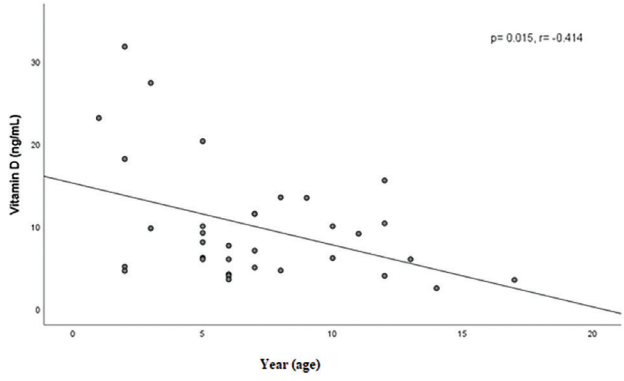

Figure 8. The correlation between 25-OH vitamin D level and age

Table 4. The correlations of 25-OH vitamin D levels with age and several parameters

Parameters	p and r values
25-OH vitamin D- Age	**0.015; -0.414**
25-OH vitamin D- CRP	0.680; 0.073
25-OH vitamin D - Lymphocyte	0.513; 0.116
25-OH vitamin D -Fibrinogen	**0.004; -0.482**
25-OH vitamin D -D-Dimer	0.983; -0.004
25-OH vitamin D -Albumin	0.662; 0.078
25-OH vitamin D -Sodium	0.817; 0.041
25-OH vitamin D -Leukocyte	0.621; -0.088

OH: Hydroxyl, CRP: C-reactive protein

vitamin D levels in patients with multisystemic inflammatory syndrome. Mean age of 34 cases evaluated in our study was 7.03±3.9 years old. Radia et al. (12) identified 783 separate MIS-C cases in 35 documented articles about MIS-C cases, which they evaluated among 1726 articles, and reported the mean age of the cases as 8.6 years old (IQR, 7-10 years) (12). The mean age of patients with MIS-C was reported as 8.9 years old (IQR: 0.3-14.6) among 18 cases by Darren et al. (13), as 6.4±4 years old among 122 cases by Munshi et al. (14), as 8.8 years old (IQR: 5.6-12.3) among 51 cases by Ekemen Keles et al. (10), and as 6.9 years among 23 cases by Hadžić-Kečalović et al. (15). Our study is similar to the relevant studies in the literature.

Patients included in study, 52.9% (n= 18) were males and 47.1% (n= 16) were females. Radia et al. (12) evaluated 1726 articles and described 783 separate MIS-C cases in 35 documented articles on MIS-C cases, and found that 55% (n = 435) of the cases were males and 45% (n=348) were females (12). Male and female ratios were reported as 55% (n=10) and 45% (n=8) among 18 patients in the study by Darren et al. (13), as 64.7% (n=74) and 39.3% (n=48) among 122 patients in the study by Munshi et al. (14), as 64.7% (n=33) and 35.3% (n=18) among 51 patients in the study by Ekemen Keles et al. (10), as 56.6% (n=13) and 43.4% (n=10) among 23 cases in the study by Hadžić-Kečalović et al. (15), and Torpoco Rivera et al. (16) reported 45% as males and 58% as African American origin among 31 cases diagnosed with MIS-C. Morover reported, 67% (n=14) and 33% (n=7) among 21 patients in the study by Petrovic et al. (17) Our study is similar to the studies in the literature.

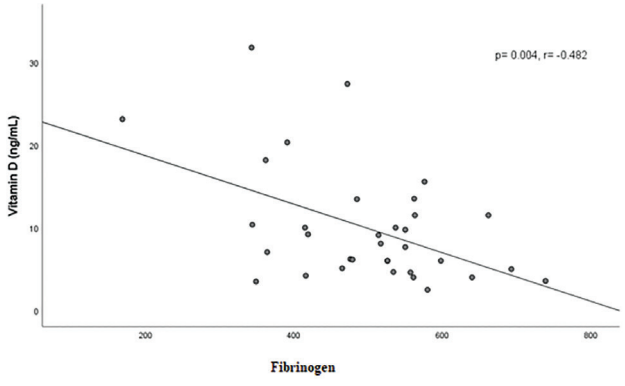

Figure 9. The correlation between 25-OH vitamin D level and fibrinogen

When mean values of laboratory findings were evaluated for the diagnosis of MIS-C in our study, platelet, albumin, amylase, lipase, GGT, total bilirubin, direct bilirubin, creatinine kinase, INR, troponin-T and CK-MB levels were evaluated as within the normal range. The studies in the literature have reported common laboratory abnormalities such as lymphocytopenia, neutrophilia, anemia, thrombocytopenia, elevated CRP, sedimentation, D-dimer, fibrinogen, ferritin, PCT, IL-6, troponin T, NT-pro-BNP and lactate in patients diagnosed with MIS-C (18-20). The results of our study is not sımılar to other studıes.

25-OH vitamin D below 12 ng/ml is vitamin D deficiency and causes serious health problems. As shown in the study by Topal et al. (21), 65% of children between the ages of 1-18 years old in Turkey have vitamin D deficiency. In our study, mean vitamin D level of 34 MIS-C patients was found as 13.2 ng/mL, and it was found to be below 12 ng/mL among 38.2% (n= 13). The results of our study were similar to the study by Topal et al (21).

Oscanoa et al. (22) reported in a meta-analysis including 23 studies (n= 2692) evaluating the effect of 25-OH vitamin D concentrations in COVID-19 patients that vitamin D deficiency appeared to be associated with increased severity and mortality. However, these studies did not show causality.

Mercola et al. (23) have provided evidence that 25-OH vitamin D levels are inversely related to the incidence or severity of COVID-19 in 14 studies.

In the study by Mohan et al. (24), it has been reported that vitamin D, an immunomodulatory hormone with proven efficacy against various upper respiratory tract infections, can stop hyperinflammatory responses and accelerate the healing process of affected areas, especially lung tissue. Since there is currently no curative drug for COVID-19, it was thought that the potential of vitamin D to change the course of disease severity should be investigated.

Rhodes et al. (25) compared the mortality rate of COVID-19 in relation to the latitude of various nations to establish a precise relationship between vitamin D levels and COVID-19. After comparison by age, they found a 4.4% increase in mortality for each degree of latitude north of 28°. This finding suggests that indirect vitamin D from UV light may play a role in protection against COVID-19.

Pereira et al. (26) selected 27 articles on COVID-19 and vitamin D out of 1542 articles, and vitamin D deficiency was not found to be associated with the risk of getting infected with COVID-19 (OR = 1.35; 95% CI = 0.80-1.88). However, vitamin D deficiency was observed in severe cases of COVID-19 more than mild cases at a rate of 64% (OR = 1.64; 95% CI = 1.30-2.09). Vitamin D deficiency was found to increase hospitalization (OR = 1.81, 95% CI = 1.41-2.21) and death rate due to COVID-19 (OR = 1.82, 95% CI = 1.06-2.58). It has been observed that there is a positive relationship between vitamin D deficiency and the severity of the disease.

Vitamin D modulates both innate and adaptive immunity and can also potentially prevent or reduce the complications associated with SARS-CoV-2 infection by increasing the concentrations of anti-inflammatory cytokines (IL-10) and Th2 cytokines as IL-4 and IL-5 (27).

25-OH vitamin D levels of our cases diagnosed with MIS-C were found to be below 12 ng/mL in 38.2% (n=13), between 12-20 ng/mL in 38.2% (n=13), and above 20 ng/mL in 23.6% (n=8), and its mean level was found as 13.6 ng/mL. In the study by Torpoco Rivera et al. (16) including 31 patients with MIS-C, 25-OH vitamin D levels were found to be 7.2±0.42 ng/ml in 10 (32.3%) patients with severe MIS-C, and severe vitamin D deficiency was found. They also reported severe disease in 90% of the patients with vitamin D deficiency (n=9) (p<0.001) (16). Although this study resembles the study by Torpoco Rivera et al. (16), future prospective studies at the basic science and clinical levels should be continued to better describe this relationship.

In the study conducted by Petrovic et al. (17) where they evaluated the relationship between 25-OH vitamin D levels and the clinical severity of MIS-C in 21 patients, it was found that vitamin D level was low in 95% (n=20), and severe vitamin D deficiency was found in 70% (n=14). Mean 25-OH vitamin D level was found to be 14.1 ng/mL. Hadžić-Kečalović et al. (15) conducted a study with 23 patients diagnosed with MIS-C, and they found mean 25-OH vitamin D level to be 17.8 ng/mL (IQR: 38.40-72.2). Ekemen Keles et al. (10) also reported a 25-OH vitamin D level of 14.6 ng/mL among 51 patients with MIS-C. Vitamin D deficiency was found at an insufficient level in 74.5% (n=38) of 51 patients and at a sufficient level in 25.5% (n=13) patients. In the study by Darren et al. (13) including 18 patients diagnosed with MIS-C, mean 25-OH vitamin D level was found to be 12 ng/mL in 78% (n=14) of the patients (13). Our study was found to be compatible with the literature.

When the relationship between 25-OH vitamin D levels and age of patients diagnosed with MIS-C was evaluated, a negative correlation was found (r=-0.414; p=0.015). In the study by Bayramoğlu et al. (28) with 103 COVID-19 positive pediatric patients, age and 25-OH vitamin D level were found to be negatively correlated (r = -0.496; p = <0.001) (28). Hadžić-Kečalović et al. (15) did not find any statistically significant correlation between the ages of the patients and their 25-OH vitamin D levels in their study including 23 patients with a diagnosis of MIS-C (r=0.11, P=0.26; 95%). Although our study resembled the study by Bayramoğlu et al. (28), more studies are needed to understand the correlation between 25-OH vitamin D level and age.

When the relationship between 25-OH vitamin D level and fibrinogen in cases diagnosed with MIS-C was evaluated, a negative correlation was found (r=-0.414; p=0.015). In the study by Heidari et al. (29) conducted on 144 COVID-19 positive adult patients, a negative correlation was reported between fibrinogen and 25-OH vitamin D levels (r=-0.52; p=<0.001) (29). Our study is very similar to the study carried out by Heidari et al. (29).

In their literature review Feketea et al. (30) concluded that vitamin D might not only be a biomarker but also a nutritional support product, and it might potentially have a positive effect on the clinical picture of MIS-C through correction of serum 25-OH vitamin D insufficiency with supplementation in very severe MIS-C cases (30).

This study is valuable because there are few publications regarding the relationship between MIS-C and vitamin D. In addition, it is also valuable in terms of the classification of laboratory, ultrasonographic findings, cardiac involvement, neurological involvement and gastrointestinal involvement rates in MIS-C patients.

It was determined that mean of 25-OH vitamin D level in cases diagnosed with MIS-C was at an insufficient level. It is thought that prophylactic vitamin D supplementation should be given according

to the age in children and should be recommended by healthcare professionals.

Study Limitations

Our study was limited to only 34 patients who admitted to the Department of Pediatric Health and Diseases in Health Practice and Research Center of Afyonkarahisar Health Sciences University. More cases are needed in terms of the relationship between MIS-C and vitamin D. In our study, were not found to be statistically significant because the number of cases was limited to 34, the mean vitamin D levels were far below the normal values and fewer patients receiving treatment in intensive care.

Conclusion

In cases diagnosed with MIS-C, the average 25-OH vitamin D level was found to be at the insufficiency level. It is thought that prophylactic vitamin D supplementation should be given to children according to age and recommended by healthcare professionals.

Ethics

Ethics Committee Approval: Ethics committee approval was taken from Afyon Health Sciences University Clinical Research Ethics Committee (date: 04.01.2022, approval number: 2022/4).

Footnotes

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

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