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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