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The survey of factors associated with the development of immune tolerance in children with cow's milk allergy; a cross-sectional study in Gorgan, Iran

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Abstract

Introduction: Cow's milk allergy (CMA) is a common complication and one of the most common food allergies in infants, and it is estimated that this disease affects 2-3% of children under five years old.

Objectives: This study investigated the factors associated with developing immune tolerance in children with CMA in Gorgan.

Patients and Methods: In this cross-sectional study, 50 children with CMA referred to the asthma and allergy clinic of Taleghani Hospital in Gorgan during 2019-2020 were investigated. Multiple logistic regression was applied to measure odds ratios (ORs) and 95% confidence intervals (CIs) of the associations between the selected factors and tolerance to cow's milk.

Results: The results suggested that factors including standard delivery ($P=0.024$, OR=4.03, 95% CI: 1.20-13.53) and breastfeeding in the first month of birth ($P=0.003$, OR=6.73, 95% CI: 1.94-23.36), and gastrointestinal symptoms ($P=0.026$, OR=3.78, 95% CI: 1.17-12.19) were associated with immune tolerance to CMA. Younger age of starting complementary feeding ($P<0.001$, OR=0.070, 95% CI: 0.02-0.30), younger age of starting cow's milk feeding ($P=0.003$, OR=0.520, 95% CI: 0.32-0.83), skin symptoms ($P=0.012$, OR=0.211, 95% CI: 0.06-0.71) and respiratory symptoms ($P=0.013$, OR=0.219, 95% CI: 0.07-0.72) were associated with allergy persistence.

Conclusion: The findings of our study demonstrated natural childbirth, breastfeeding, and gastrointestinal symptoms, including bloody stools, are factors related to immune tolerance in children with CMA. The findings showed that decreasing the onset of complementary feeding, reducing the age of cow's milk feeding, and clinical manifestations of respiratory and skin are associated with persistent CMA in children.

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Introduction

Cow's milk allergy (CMA) is defined as a reproducible adverse event to one or more cow's milk proteins, immunoglobulin-mediated E or non-IgE-mediated are two broad categories depending on the immunological mechanism. Mediated reactions IgE-mediated reactions are later delayed by IgE that appear a few hours-days after ingestion of food (1).

Accurate diagnosis of CMA is essential to avoid only the risk of rickets, bone loss, anemia, growth retardation, and hypoalbuminemia but that of immediate clinical reactions or chronic gastroenteropathy resulting in malabsorption. Milk is formed from of average of 87% water, lactose, 3% protein, 3-4% mineral matter, and 0.1% vitamins (2, 3). Milk is commonly

Key point

Natural childbirth, breastfeeding, gastrointestinal symptoms, and bloody stools are factors related to immune tolerance in children with CMA. Likewise, decreasing the age of onset of complementary feeding, decreasing the age of cow's milk feeding, and clinical manifestations of respiratory and skin are associated with persistent CMA in children.

advised to be a leading source of protein in the human diet, providing about 32 g/L. Its protein fraction can be divided into insoluble soluble proteins. Soluble proteins, called whey proteins, represent 20 of the protein fraction of milk, soluble protein, called whey protein, makes up 20 of the milk protein fraction, while soluble protein, namely caseins, makes up 80% (3, 4). Significant biological actions include antibacterial,

antiviral, antioxidant, antihypertensive, antimicrobial, antithrombotic, opioid, and immunomodulatory roles, in addition to the absorption of other nutrients, antioxidants, antihypertensives, antimicrobials, antithrombotics, opioids and immunomodulators as well as the absorption of other nutrients. It can develop in the neonatal period or the first years of life, and it usually tends to subside during childhood and is quite rare in adults; milk allergy has been shown to affect between 1.8% and 7.5% of infants during the first year of life (5).

About 10-15% of children with cow's milk protein allergy (CMPA) are allergic to soy, and the risk of cross-allergy is higher if symptoms start before the age of a month. Differential diagnoses Common conditions such as infectious colitis, celiac disease, gastroesophageal reflux, eosinophilic esophagitis, and immunodeficiency persistent diarrhea should be kept in check mind (6).

Diagnosis of CMPA is based on a detailed symptom history, skin test and specific serum cow's milk protein IgE, deletion diet, and oral food challenge. Removal of allergens followed by oral food provocation has been advocated as the cornerstone of diagnosis. IgE levels are specific for milk and are helpful in predicting clinical reactivity. Also, we observed in the follow-up that casein is the protein that best discriminates between persistence, allergy, and tolerance, as commented on (7). As treatment: food avoidance, choice of a milk substitute, and replacement of calcium, reintroduction, and oral tolerance are different treatments. It is common for pediatricians to modify the formula when symptoms of intolerance appear, such as acids formula, partially hydrolyzed formula, extensively hydrolyzed formula, or whey, rice partially and/or eHF, soy, hydrolyzed soy formula, and others from mammals. Intolerance appears, such as acid formula, partially hydrolyzed formula, primarily hydrolyzed formula, or whey, partially rice and/or eHF, soybean, hydrolyzed formula from soybeans and other mammals.

Objectives

The present study was conducted to determine immune resistance in children with CMA who had visited asthma and allergy center of Taleghani educational and therapeutic center in Gorgan.

Patients and Methods

Study design

The present cross-sectional study with a descriptive approach was conducted in Gorgan city, northeast of Iran. The studied population included all children aged 24 to 36 months with CMA who had visited asthma and allergy clinic of Taleghani educational-therapeutic center in Gorgan during 2019-2020.

We have defined CMA as the European Society of Pediatrics, Hepatology, and Nutrition. The first step is a thorough medical history and physical examination. If symptoms and signs related to CMPA occur in an infant

or child and cannot be explained by another cause, CMPA may be considered a potential diagnosis. Thus, this diagnosis must be confirmed or ruled out by an allergen removal and provocation procedure (6). Our studied population was between Ages 24 to 36 months. Our inclusion criteria were:

- Age 24 to 36 months
- Positive specific IgE antibody test (ELISA) against cow's milk protein (IgE specific to casein, β -lactoglobulin and α -lactalbumin with IgE cut-off >0.35 kU/L) or confirmation of diagnosis by skin scratch test
- History of receiving oral treatment

Data collection

The tool of data collection was a researcher-made checklist based on the patient's medical records, which included information such as age, sex, type of delivery, type of nutrition received in the first month of life, receiving nutritional supplements and allergic symptoms and other clinical manifestations at the time of diagnosis.

Tolerance to cow's milk was determined based on the negative challenge of oral cow's milk, following the regular consumption of age-appropriate amounts of cow's milk at home, without any clinical symptoms caused by allergy, and if approved by pediatric asthma and allergy specialist.

Statistical analysis

After we collected the data, it was entered into SPSS version 18 software. The quantitative variables are described by mean \pm standard deviation, and the frequency table describes the qualitative variables. The chi-square test was used to analyze the qualitative variables. To compare the average scores in the two groups, if the data were standard, the independent t-test was used. If the data distribution was not normal, the non-parametric Mann-Whitney test (Kruskal Wallis) was used. A logistic regression test was used to estimate the risk ratio of different variables in immune resistance to cow's milk. A significance level of less than 0.05 was considered.

Results

A total of 50 patients participated in the study; subjects were divided into two groups: positive cow's milk immune tolerance cow (25 people) and negative cow's milk immune tolerance cow (25 people). The mean and SD age of children was 29.74 ± 5.8 months with a range of 1-36 months. Demographic, quantitative, and qualitative nutritional characteristics of patients in two groups with and without cow's milk immune tolerance cow presented in [Table 1](#). Gender and place of residence were not statistically significant in the two groups. As shown in [Table 2](#), bloody stools were the most frequent symptom, followed by wheezing and hives. Avoidance (48%) was the most frequently prescribed treatment for referring patients in the studied community, followed

Table 1. Comparison of demographic, quantitative, and qualitative nutritional characteristics of patients in two groups with and without immune tolerance to cow's milk

Variable	Frequency (%)	Tolerance to cow's milk		P value	Test results	
		No. Positive (%)	No. Negative (%)		Odds ratio	95% CI
Gender	Female	22 (44)	10 (40)	0.569	1.38	0.45-4.25
	Male	28 (56)	15 (60)			
Place of residence	City	33 (66)	15 (60)	0.372	1.71	0.52-5.6
	Village	17 (24)	10 (40)			
Delivery type	Natural	30 (60)	19 (76)	0.024	4.03	1.2-13.53
	Cesarean section	20 (40)	6 (24)			
Type of nutrition received in the first month of life	Breast milk	23 (46)	17 (68)	0.003	6.73	1.94-23.36
	Milk powder	27 (54)	8 (32)			
Contact with cigarette smoke	5 (10)	2 (8)	3 (12)	0.639	0.638	0.1-4.19
Contact with animals and bird	12 (24)	5 (27)	7 (28)	0.509	0.643	0.17-2.39

Table 2. Description of quantitative demographic and nutritional variables in two groups with and without immune tolerance to cow's milk

Variable	Tolerance to cow's milk		P value	Test result	
	No. Positive (%)	No. Negative (%)		Odds ratio	95% CI
Age (month)- mean (SD)	29.20 (2.34)	30.28 (3.21)	0.514	0.967	0.87-1.07
Age at the start of complementary feeding (month)-mean (SD)	5.26 (1.74)	5.76 (1.92)	<0.001	0.07	0.02-0.3
Age of starting feeding with cow's milk (month)-mean (SD)	9.88 (2.24)	11.08 (3.25)	0.007	0.520	0.32-0.83

by casein hydrolysate (28%) and casein hydrolysate with *Lactobacillus rhamnosus*.

The type of natural delivery ($p=0.024$, Odds ratio [OR]: 4.03, 95% CI: 1.20-13.53) and breastfeeding in the first month of birth ($p=0.003$, OR: 6.73, 95% CI: 23.36-1.94) was statistically significant with positive cow's milk immune tolerance cow. Younger age at the start of complementary feeding ($P<0.001$, OR: 0.070, 95% CI: 0.02-0.30) and younger age at the start of cow's milk feeding ($p=0.003$, OR: 0.520, 95% CI: 0.83-0.32) was statistically significant and had a countervailing effect for positive immune tolerance to the cow (Table 2). In positive cases of immune tolerance to cow's milk, all three types of treatment were used in a balanced manner, while in the group of negative immune tolerance, the most treatment was avoidance.

Bloody stools, urticaria, and wheezing had a significant relationship with cow's milk immune tolerance cow. The bloody stool was directly related to positive cow's milk immune tolerance cow ($p=0.026$, OR: 3.78, 95% CI: 1.17-12.19), while the clinical symptoms of urticaria ($p=0.012$, OR: 0.71 - 0.06: CI 95%) and wheezing ($p= 0.013$, OR: 0.219 95% CI: 0.72-0.07) with Positive to cow's milk immune tolerance cow had an inverse relationship, and in adverse cases of cow's milk immune tolerance cow, immune symptoms were dominant.

Discussion

The present study was conducted to determine immune resistance in children allergic to cow's milk who had

visited asthma and allergy center of the Taleghani educational and therapeutic center in Gorgan. The study's results showed that the type of natural delivery and breastfeeding in the first month of birth were associated with positive cow's milk immune tolerance. On the other hand, starting supplementary feeding at a younger age and starting feeding with cow's milk at a younger age had countervailing and adverse effects in developing immune tolerance in children with CMA.

Allergic diseases are caused by the activation of basophil mast cells mediated by IgE bound to the surface, resulting in the release of histamine and other mediators and inflammation. Chronic allergic inflammation typically involves cellular infiltration of eosinophils and lymphocytes associated with chronic lesions. In allergic people, TH cells secrete cells that motivate the production of IgE antibodies against allergens. The IgE secretion condition in response to typical environments is called atopy (8).

Cow's milk allergy has variable presentations, from immediate responses such as vomiting, abdominal pain, and cramps to chronic indications such as dermatitis atopy, gastrointestinal symptoms, and difficulties (9). The study by Hossein Eslamian and colleagues in 2017 showed that the most common clinical manifestations of cow's milk allergic patients included skin symptoms, followed by the digestive and respiratory systems. Immune tolerance occurred in nearly half of the children. Family history and atopic dermatitis were associated with the possibility

of intolerance to cow's milk. Still, there was no difference between sex, age, birth weight, age at onset of allergy, type of delivery, breastfeeding, or initiation of solid food. There was no relationship between smoking in people around and contracting this disease (10).

According to a study by Metsälä and colleagues in 2010, cesarean delivery and advanced maternal age increase the risk of CMA in children (11). In 2018, Topal et al (12) found that concomitant food allergies, IgE-related reactions, and respiratory symptoms can lead to immune intolerance and persistent sensitivity to cow's milk protein in children under three years old. On the other hand, gastrointestinal discomfort was a risk factor for CMA but was also independently associated with immune tolerance in these children. The study showed that skin and respiratory symptoms were independent factors for the persistence of CMA, while bloody stool was an independent risk factor for immune tolerance in children with CMA. As our study included children under three years old, it aligns with the findings of other studies. (12-15).

In a 2019 study by Sánchez-Valverde et al, it was shown that non-IgE-dependent CMA and receipt of casein hydrolyzate with *Lactobacillus rhamnosus* GG were factors associated with rapid immune tolerance and delayed immune tolerance with breastfeeding for a period of at least three It was fasting (16). The study's results cleared that breastfeeding in the first month of birth leads to 6.73 times more immune tolerance than formula feeding in children with CMA, which was inconsistent with the findings of Sanchez and colleagues' study. However, it is widely accepted that the timing of the initiation of breastfeeding, in addition to its duration, has an effect on protection against atopy in general and against CMA in particular (17). The results of the study confirmed this issue.

Conclusion

The study showed that natural childbirth, breastfeeding, gastrointestinal symptoms, and bloody stools are factors related to immune tolerance in CMA children. The findings showed that the decrease in the age of initiation of supplementary feeding, the reduction in cow's milk feeding, and respiratory and skin clinical manifestations are associated with the durability of CMA in children.

Limitations of the study

One of the limitations of this study is the limited number of children with CMA.

Authors' contribution

Conceptualization: Seyed Ali Aghapour.

Data curation: Khadijeh Kazemi, Edris Ghezalje.

Formal analysis: Mousa Ghelichi-Ghojogh.

Funding acquisition: Mohsen Ebrahimi.

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Conflicts of interest

The authors decline any kind of conflict of interest.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Golestan University of Medical Sciences approved this study (Ethical code# IR.GOUMS.REC.1400.370). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from MD., thesis Khadijeh Kazemi at this university (Thesis #111729). Additionally, ethical issues (including plagiarism, data fabrication, and double publication) were completely observed by the authors.

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