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Comparison of Oral Desensitization with Heated Cow's Milk Products with Conventional Desensitization Method in Children with Cow's Milk Allergy

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ABSTRACT

Cow's milk allergy (CMA) is one of the most prevalent Immunoglobulin E (IgE)-dependent food allergies in children. Currently, the only accepted treatment for food allergy is avoiding the relevant allergen. The purpose of this study is to investigate the immunological changes following the consumption of heated cow's milk products compared to the usual method of oral desensitization in children aged over two years old with cow's milk allergy.

In a prospective double-blind clinical trial study, 25 children aged two years and older with a definite diagnosis of IgE-dependent cow's milk allergy referred to the allergy clinic of the Children's Medical Center from 2016 to 2017 were enrolled. The eligible patients were randomly divided into two groups: the first group was desensitized with raw milk (normal desensitization: n=13), and the second group was desensitized with heated cow's milk products (intervention group, n=12).

The mean ages in the raw milk group and heated milk group were 3.92±1.44 and 4.50±1.73 years, respectively. The rate of anaphylaxis in the heated milk group was higher than in the raw milk group (50% vs. 15.4%), although the incidence of urticaria and angioedema was not significantly different between the two groups. The mean concentration of serum IgE in the two groups decreased after desensitization compared to before, although there was no significant difference between the two groups. The increase in the number of CD4+Foxp3+ and CD4+ CD25+ cells was less in the heated milk group than the raw milk group, but this difference was not statistically significant. Additionally, the number of eosinophil cells was higher in the heated milk group than in the raw milk group, but this difference was not statistically significant difference.

We concluded that the changes in the level of eosinophil, IgE, and regulatory T cells in the conventional desensitization group were not significantly different compared to desensitization with heated milk. Further multicenter studies with a higher sample size are recommended to confirm these results.

Keywords: Allergy; Cow's milk allergy; Food allergy; Oral desensitization; Regulatory T cell

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INTRODUCTION

Food allergy is a complication caused by a specific immune response to a food that can be repeated upon re-exposure to that food and is the most common cause of anaphylaxis in all patients outside the hospital and can lead to death.[1,2]

Food allergies are more prevalent in the first few years of life, and one of the foods that accounts for the highest percentage of food allergies is cow's milk.[3-5] Prospective studies in several countries have shown that about 2.5% of infants experience allergic reactions to cow's milk in the first year of life.[6,7]

Most infants with a non-IgE-mediated reaction to cow's milk will recover by the third year of life, but 10-25% of infants with an IgE-mediated reaction to milk and eggs will continue to have food allergy into the second decade of life.[8,9]

Currently, there is no definitive treatment for allergies, and the current treatment is based on avoiding food and treating acute reactions.[10] Dietary restrictions can lead to growth disorders and lack of nutrients, including macronutrients and micronutrients, and affect the quality of life of patients.[11,12]

Immunotherapy and desensitization methods significantly benefit these patients, and one of the desensitization methods is the use of heated milk products.[13]

Food processing can both increase and decrease the allergenicity of proteins. The reduction of allergenicity is caused by the loss of spatial indicators as well as chemical reactions between proteins, fats and sugars that limit the availability of antigenic indicators of proteins against the immune system.[14] Therefore, it is possible to create or change spatial epitopes by heating milk, which causes the epitope to become unrecognizable by the immune system and prevents the occurrence of an immune reaction.[15,16]

The aim of this study is to evaluate the immunological changes following the consumption of heated cow's milk products compared to the usual method of oral desensitization in children over two years old with cow's milk allergy.

MATERIALS AND METHODS

Participant and Study Design

In a prospective double-blind clinical trial study, 25 children aged two years or older with a definite diagnosis of IgE-dependent cow's milk allergy were enrolled. These children were referred to the allergy clinic of the Children's Medical Center affiliated to Tehran University of Medical Sciences in 2016 to 2017.

The inclusion criteria were the presence of symptoms within≤2 hours after contact with cow's milk during the past 6 months: skin symptoms such as urticarial, erythema and angioedema, digestive symptoms such as acute vomiting, abdominal pain and diarrhea, respiratory symptoms such as rhinitis and bronchospasm. Additionally, the food challenge test had to be positive to cow's milk and the skin test or serum-specific IgE to cow's milk had to be positive.

Exclusion criteria were patients with malignancy, severe immunodeficiency, beta-blocker/ immunosuppressive drug use, severe uncontrolled asthma, eosinophilic gastroenteropathy produced by milk that had been previously diagnosed.

The eligible patients were divided into two groups: the first group underwent desensitization with raw milk (normal desensitization: n=13), and the second group underwent desensitization with heated cow's milk products (intervention group, n=12).

The immunotherapy protocol in this study was performed by Meglio et al' study (17). In all patients, serum IgE levels and the number of Treg cells ($CD4^+CD25^+FoxP3^+$) were measured by ELISA (Pars Gene Company, Iran) and flow cytometry before and after desensitization, in addition, the number of eosinophils was measured using a cell counter. Skin prick test was also performed using the conventional method.

Data Collection

In order to collect information, a special questionnaire was completed for each patients including age, sex, allergic symptoms, IgE serum level, number of eosinophils, number of Treg cells ($CD4^+CD25^+FoxP3^+$) before and after desensitization.

Statistical Analysis

SPSS version 22 software was used for statistical analysis. Quantitative variables are presented as mean±SD. Additionally, qualitative parameters were reported as number (percentage). Kolmogorov–Smirnov and, Shapiro–Wilk tests were applied to tests to evaluate the normal distribution of data. For statistical analysis, Mann-Whitney, Wilcoxon and Spearman correlation tests, chi-square and Fisher tests were used. P-value less than 0.05 was considered statistically significant.

RESULTS

The mean age of in the raw milk group and heated milk group were 3.92±1.44 and 4.50±1.73 years, respectively (p=0.373). Further, 61.5% and 83.3% of the raw milk group and heated milk group were male, respectively, there was no significant difference in gender between the two groups (p=0.378). During the immunotherapy, the rate of anaphylaxis was higher in the heated milk group than in the raw milk group (50% vs. 15.4%, p=0.097), although the incidence of urticaria and angioedema was not significantly different between the two groups, and digestive symptoms were not seen in either of the two groups. More details are provided in Table 1.

During the study, following multiple or severe anaphylactic reactions after consuming milk or muffins, 11 (44.0%) patients did not continue, due to parental concerns about frequent anaphylaxis (8 patients experienced recurrent anaphylaxis, but 3 other patients dropped out due to fear and concern about anaphylaxis and after observing adverse conditions in one of the patients). Only 14 patients (6 in the heated milk group and 8 in the raw milk group) had serum IgE concentrations and regulatory T cells were measured both a before and after desensitization, and these values were compared.

In the raw milk group, the mean IgE serum level before and after desensitization were 8.5±7.8 and 1.3±1.6 IU/mL, respectively (p=0.012). The mean serum IgE concentration in the heated milk group has decreased significantly after desensitization (p=0.028). There was no significant difference in the number of eosinophil cells in the raw milk group before and after desensitization(p=0.093). In the heated milk group, the mean eosinophil cell count before and after desensitization was 340.3±228.5 and 158.3±158.1, respectively, the level of eosinophils has decreased significantly after desensitization(p=0.028). The mean number of $CD4^+CD25^+$ cells increased in both groups after desensitization, but the increase was not significant in either group (P=0.058). In the heated milk group, the mean count of $CD4^+Foxp3^+$ cells before and after desensitization was 12.8±17.5 and 28.6±25.2, respectively (p=0.012). The mean number of $CD4^+Foxp3^+$ cells increased after desensitization in the heated milk group, but the increase was not statistically significant (p=0.600) (Table 2).

Table 1. Frequency of allergic symptoms in the intervention and control groups

Variable	Raw milk group (n=13)	Heated milk group (n=12)	*p*
Age, year (mean±SD)	3.92±1.44	4.50±1.73	0.373
Sex (Male), n (%)	8 (61.5%)	10 (83.3%)	0.378
Anaphylaxis, n (%)	2 (15.4%)	6 (50.0%)	0.097*
Urticaria, n (%)	7 (53.8%)	6 (50.0%)	0.848
Angioedema, n (%)	1 (7.7%)	0 (0.0%)	1.0
Respiratory symptoms, n (%)	2 (15.4%)	1 (8.3%)	1.0
Gastrointestinal symptoms, n (%)	0	0	-

Table 2. Immunoglobulin E serum level, number of eosinophil cells, number of regulatory T cells before and after desensitization

Variable	Raw milk group			Heated milk group		
	Before desensitization	After desensitization	*p*	Before desensitization	After desensitization	*p*
IgE serum level (IU/mL)	8.5±7.8	1.3±1.6	0.012	3.2±2.8	1.1±1.2	0.028
Eosinophil cells numbers	242.9±181.8	177.0±111.9	0.093	340.3±228.5	158.3±158.1	0.028
$CD4^+$ $CD25^+$ cell count	4.4±3.9	5.8±4.6	0.236	6.3±5.5	9.2±5.8	0.058
$CD4^+Foxp3^+$ cell count	12.8±17.5	28.6±25.2	0.012	20.8±25.4	22.8±11.7	0.600

The reduction in serum IgE levels was less in the heated milk group compared to the raw the raw milk group, but this difference was not statistically significant (p=0.345). The increase in eosinophil cell count was higher in the heated milk group was higher than in the raw milk group, but the difference was no statistically significant (p=0.108). Regarding the difference in the increase in the number of $CD4^{+}CD25^{+}$ cells between the two groups, no significant difference was observed (p=0.662). Moreover, the increase difference in the number of $CD4^{+}Foxp3^{+}$ cells was less in the heated milk group than in the raw milk group, but this difference was not statistically significant (p=0.228) (Figure 1).

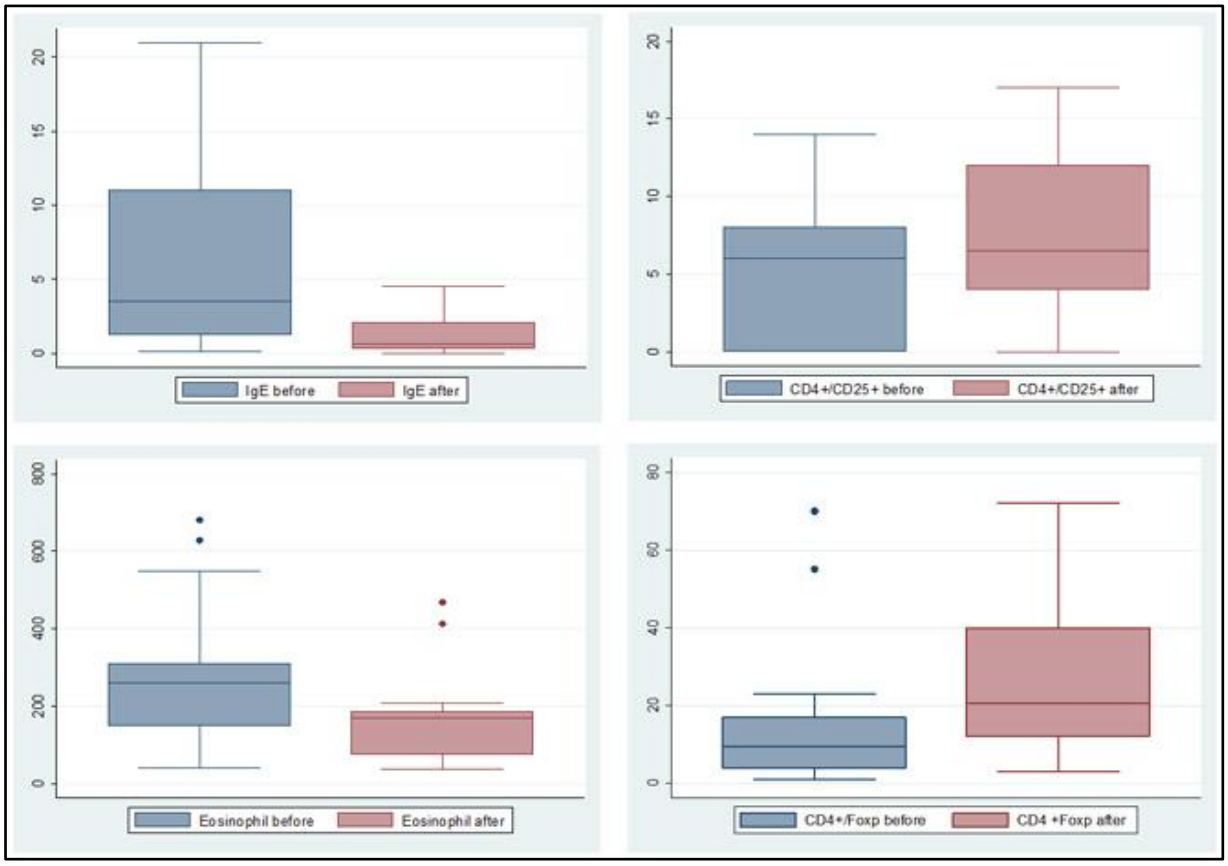

Figure 1. Serum Immunoglobulin E level, number of eosinophil cells and regulatory T cells before and after desensitization

DISCUSSION

Allergy to cow's milk is one of the most prevalent immediate-type food hypersensitivities.[18] It seems that the consumption of baked milk products in children with cow's milk allergy accelerates the induction of tolerance in these children.[19-21] In addition, a comprehensive meta-analysis indicated that most of these studies were observational and lacked suitable control groups.[18] Therefore, the current study was conducted to investigate the immunological changes following the consumption of heated cow's milk products compared to the traditional method of oral desensitization in children with cow's milk allergy.

According to our findings, the serum level of IgE decreased significantly after desensitization in both groups, but no statistically significant difference was observed between these two methods in terms of reducing IgE antibody levels.

In the study by Kim et al despite a significant increase in casein-specific IgG4 levels in the group tolerant to highly heated milk, milk-specific IgE levels did not change significantly.[22] It seems that, despite the absence of a statistically significant difference in IgE concentration, increased IgG4 levels through antigen neutralization and antibody feedback effects may inhibit IgE production and prevent its increase in these patients.

Antibody feedback effect refers to the downregulation of antibody production by secreted IgG antibodies. IgG antibodies inhibit B cell activation by forming complexes with antigen, and these complexes bind to the B cell receptor for the FC portions of IgG, called FCγRII or CD32. Through the FCγRII engagement mechanism, the B cell response to the antigen is terminated.[23,24]

A study by Wegrzyn et al demonstrated that children who digested heated milk products had higher levels of casein-specific IgG4 antibodies.[14] In our study, although the serum level of IgG antibody was not measured, the significant decrease in the serum IgE levels suggests a feedback mechanism antibodies contributing to observed decrease in both methods. The reduction of IgE in the desensitization group with raw milk was greater than that with heated cow's milk. This could be clinically significant; the lack of statistical significance is likely due to the small sample size.

In both groups, a decrease in the number of eosinophils was observed after desensitization, with the decrease being significant in the group desensitized with heated cow's milk products. Eosinophils activated by mast cells and basophils produce and release lipid mediators such as platelet-activating factor (PAF), prostaglandins and C4 leukotrienes and its derivatives, leukotriene D4 (LTD4) and leukotriene E 4 (LTE 4).[25,26]

The number of regulatory T cells ($CD4^+$ $CD25^+$ $FoxP3^+$), before and after desensitization did not differ significantly in either method. In the study of G. Shreffler et al the number of specific regulatory T cells was higher in children who were able to tolerate highly heated milk. There was a higher percentage of regulatory T cells with $CD25^+FoxP3^+$ markers in the peripheral blood of these children compared to other groups.[27]

Contrary to our results, Tosca et al showed that oral immunotherapy probably acts on tolerogenic mechanisms, independent of the type 2 response, but involves B regulatory and Treg cells as actors of natural immune tolerance.[28] It appears that the role of regulatory T cells in exerting the effects of oral desensitization has not yet been conclusively confirmed. To clarify this, it would be necessary to examine the phenotype of T helper-1 (TH1), T helper-2 (TH2) cells, cytokines and signaling molecules involved in the development process of these cells, which were not measured in the present study.

The mean quantitative indices of swelling and redness in the skin prick test did not differ significantly between the two methods. Additionally, the incidence of complications resulting from oral immunotherapy and the use of highly heated cow's milk products, such as anaphylaxis, angioedema, respiratory symptoms, gastrointestinal symptoms, and urticaria, did not differ statistically. Based on the findings of this study, there is a consistency and agreement among the results.

In our study, there was an initial bias, as patients with more symptoms and expected to have more severe reactions were placed in the heated milk group, likely because they could not tolerate raw milk. Therefore, the higher incidence of reactions in the heated group may reflect this bias.

The study limitations included a very small sample size, potential biases in participant selection, individual variability in responses to desensitization, and a short follow-up duration, all of which could influence the results and limit the generalizability of the findings.

We conclude that the immunological changes induced by a diet containing heated milk were not significantly different from those observed with conventional oral desensitization. Further, it is recommended that multicenter studies with larger sample sizes be conducted to investigate the number and function of mast cells and basophils, as well as the phenotype of TH1 and TH2 cells.

STATEMENT OF ETHICS

This study was approved by the Ethical Committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.MEDICINE.REC.1395.1076) and this trial was registered in the Iranian clinical trial system with the registration number of IRCT20170426033658N2). Written informed consent was obtained from parents of all participating patients.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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