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Cow's milk protein allergy damages not only the baby's health but also the mother's health

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Abstract
The aim of this study was to evaluate the nutritional status, especially calcium deficiency, of breastfeeding mothers of infants diagnosed with cow's milk food protein-induced allergic proctocolitis (FPIAP) on an elimination diet and to investigate the relationship between daily calcium intake and body composition of these mothers. Our prospective cohort study included 86 patients with cow's milk protein allergy (CMPA) and their mothers on an elimination diet. The mother's body fat, muscle, and water percentages were measured with the Bioelectrical Impedance Method (Tanita MC-580). All mothers were administered a diet containing an average of 2000 kcal (kilocalorie), 1000 mg of calcium, and 400 units per day of vitamin D. The final measurements were repeated 1 month later and the results of the first and second assessments were compared. The mean age of the patients included in the study was 3.8 ± 1.8 months, and the M/F (male to female) ratio was 18/17. In all, 54.3% of the patients had only CMPA, while 45.7% had multiple food allergies, including milk. A comparison of the mother's body measurements before and after the diet showed a significant decrease in fat but with an increase in calcium levels. In contrast, muscle and water ratios did not change ($p < 0.001$, $p = 0.332$, $p = 0.189$). Despite the recommendation of a 2000 kcal per day diet, the second evaluation found that calorie, fat, and protein ratios were significantly reduced ($p = < 0.001$, $p = 0.011$, $p = 0.009$, $p < 0.05$, respectively). The metabolic health of breastfeeding mothers who follow an elimination diet for CMPA is affected even with dietician support.

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Introduction

The foundation of treatment for cow's milk protein allergy (CMPA) is eliminating cow's milk in patients with a confirmed diagnosis.[1,2] The food allergy most commonly associated with cow's milk in infants is food protein-induced allergic proctocolitis (FPIAP); it is also observed in infants who receive only breast milk and not cow's milk. In the literature, more than 50% of the reported cases of FPIAP include exclusively breastfed infants.[3] The World Health Organization recommends breastfeeding until the age of two; hence, breastfeeding should be continued in these patients and cow's milk excluded from the mother's diet if it has been proven that there is a relationship between cow's milk consumption by the mother and allergic proctocolitis findings in the infant. This exclusion includes not only cow's milk but also all nutrients that may contain cow's milk protein and food groups that may be highly cross-reactive with cow's milk.[4,5] Mothers that are prescribed with an elimination diet should be given nutritional advice to ensure adequate amounts of protein, calories, trace elements, and vitamins for themselves and their growing infants. For example, if milk and dairy products are eliminated from the diet of a mother whose infant has CMPA, the mother should be supplemented with 1000 mg of calcium and 400 IU (international units) of vitamin D daily.[6]

It has been noted that the nutritional status of mothers who have a child with allergic proctocolitis deteriorates during the elimination diet, causing nutritional deficiencies both in them and in their infants.[7,8] Low-calcium diets suppress calcitriol levels, stimulating lipogenesis and suppressing fat breakdown; thus, lipid synthesis and adiposity increase.[9] In contrast, mothers who take calcium regularly are expected to have lower body fat levels.

The aim of this study was to assess nutritional status, especially calcium deficiency, and investigate the relationship between daily calcium intake and body components (muscle and adipose tissue) in breastfeeding mothers undergoing an elimination diet due to CMPA.

Materials and Methods

This study is a multicenter, prospective cohort study carried out from August 1, 2023, to February 29, 2024, at three tertiary care hospitals (Dr. Behçet Uz Children's Hospital, İzmir Tepecik Training and Research Hospital, and Bakırçay University Hospital) in central İzmir in Turkey.

Participants

This study included children aged 1–12 months with FPIAP related to cow's milk protein allergy who were admitted to the three centers of the pediatric allergy outpatient clinics and their breastfeeding mothers who were on a diet that eliminated milk and dairy products. Patients with chronic diseases or who refused to participate in the study and did not continue follow-up were excluded.

Sample size

Based on the population of children aged 0–2 years in the İzmir province in 2021 and a CMPA prevalence rate of 4%, the sample size was found to be 59 at a 95% confidence interval. A total of 65 patients were planned to be enrolled in the study, with an estimated 10% dropout during follow-up.

Definitions

Allergic proctocolitis

FPIAP is considered in babies with stools containing bloody mucus. The diagnosis is made when the infant's symptoms improve upon elimination of cow's milk from the infant's and mother's diet for 2–4 weeks and the recurrence of the infant's symptoms upon resumption of cow's milk.[10]

Nutrition information system (BeBİS)

This program was developed to determine calories, protein, carbohydrates, fats, vitamins, minerals, amino acids, fatty acids, antioxidants, and other nutrients depending on the portion and amount of food intake (Nutrition Information System [BeBİS] Version 9; Ebispro for Windows, Stuttgart, Germany; and Turkish version BeBİS Version 9 [http://www.bebis.com.tr Access Date: 06.09.2023]).

Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) is one of the most common approaches for body composition measurements. This method primarily determines the ratio of fat and muscle mass and the analysis is based on the principle that different tissues in the human body conduct electric currents differently. A low-level electric current is applied to measure changes in the electrical conductivity of body tissues such as water, muscle, fat, and bone. This allows for determining body weight, body fat percentage, total body water, fat-free mass, muscle mass, and bone mass.[11]

A Tanita MC-580 device was used for BIA measurement in this study.

Study design

Questionnaire

A questionnaire was administered to the mothers of infants with FPIAP. Delivery method (vaginal delivery, cesarean section), gestational week, duration of breastfeeding, and nutritional status (breast milk, supplementary food, formula) were recorded. In the questionnaire, demographic data of the mothers (age, education, employment status, etc.), presence or absence of an elimination diet, how they started the diet (doctor, others—friends and family elders—social media), whether they received dietitian support during the diet, and whether they received calcium and/or vitamin D replacement.

Dietitian assessment

After a dietitian evaluated the mother's 3-day nutritional history, the amount of calories, fat, carbohydrate, and protein intake was measured with the Nutrition Information

System (BeBIS 9.0) program. The mother's body fat, muscle, and water percentage were measured by the Bioelectrical Impedance Method (Tanita MC-580). An average 2000 kcal (kilocalorie) diet consisting of 50–55% carbohydrate, 10–15% protein, and 30–35% fat was recommended to all mothers by a dietitian, and 1000 mg of calcium and 400 units per day of vitamin D were added to their diet. At the end of the first month, the Nutritional Information System and Bioelectrical Impedance measurements of the mothers who were followed up by a dietitian were repeated.

Statistical analysis

Statistical data were evaluated using the SPSS 20 (Statistical Package for Social Sciences V 20; SPSS Inc, Chicago, USA) package program. Parametric methods were used to analyze data fitting the normal distribution and nonparametric methods were used to analyze data not fitting the normal distribution and categorical data. Normalization was evaluated based on the Kolmogorov-Simirnov analysis. Independent group Student's t-test was used for comparisons between two independent groups, Mann Whitney U test was used when nonparametric conditions were met, Chi-square test was used for comparison of ratios of nominal data, dependent group Student's t-test was used for comparison of baseline and control data, Wilcoxon's test was used when nonparametric conditions were met, and McNemar's test was used for comparison of ratios.

Ethical considerations

This study was approved by the Izmir Dr. Behçet Uz Training and Research Hospital Clinical Research Ethics Committee in Türkiye and was conducted in accordance with all rules of the Declaration of Helsinki (decision number: 2023/1003). All participants filled out and assigned individual consent forms at the beginning of the research.

Results

Between August 2022 and August 2023, 86 patients were diagnosed with FPIAP in three different centers, and their mothers were included in the study; however, only 41% (n = 35) of the participants completed the study. The mean age was 3.8 ± 1.8 months and the M/F (male to female) ratio was 18/17. Nineteen (54.3%) patients had only milk allergy and 16 (45.7%) had multiple food allergies along with milk. All patients were breastfed, but 12 (28%) were receiving amino acid-based formula in addition to breast milk. The mean age of the mothers included in the study was 29 ± 3.8 years where 15 (43%) were high school graduates. Before the study, 27 (80%) mothers started a diet with the advice of a doctor, 5 (14%) received dietitian support, and 18 (51%) received calcium and vitamin D (Table 1).

The mothers had low daily caloric, macro-, and micronutrient intake in their initial assessments. A comparison of the body measurements of the mothers before and after a dietitian adjusted the diet revealed a significant decrease in fat ratios after the diet but no change in muscle and water ratios ($p < 0.001$, $p = 0.332$, $p = 0.189$, respectively). The effect of the recommended regular calcium and vitamin D was observed in the initial assessment and an increase in body calcium levels was found ($p < 0.05$) in the second assessment. Similarly, the body fat ratio was thought to have decreased in parallel (Table 2).

Although mothers received nutrition counseling from a dietitian during the elimination diet and a daily 2000 kcal diet was recommended, their calorie, fat, and protein ratios decreased significantly in the second evaluation ($p = < 0.001$, $p = 0.011$, $p = 0.009$, respectively) (Table 2).

Discussion

In this study, it was found that body fat decreased with increased calcium intake in mothers on an elimination diet whose infants had CMPA. Unexpectedly, although the

Table 1 Demographic and clinical characteristics of infants with FPIAP and their mothers.

Age of the infant	3.8 ± 1.8 months
Gender of the infant (Male), n (percentage)	18 (51%)
Delivery week (mean ± SD)	37.9 ± 2.2
Delivery method, n (percentage)	Vaginal delivery: 8 (23) Cesarean section: 27 (77)
Responsible allergen, n (percentage)	Cow's milk: 19 (54) Multiple foods: 16 (46)
Mother's age (year)	29 ± 3.8 years
Mother's education level, n (percentage)	High School: 15 (43) University: 20 (57)
Mother's employment status, n (percentage)	Housewife: 23 (66) Working: 6 (17) Working but on leave: 6 (17)
Initiation of the elimination diet, n (percentage)	Doctor's recommendation: 28 (80) Other: 7 (20)
Prior dietitian support, n (percentage)	5 (14)
Prior recommendation of calcium + vitamin D to the mother, n (percentage)	18 (51)

Table 2 Comparison of metabolic analysis of mothers before and after diet.

	First Assessment (mean ± SD)	Second Assessment (mean ± SD)	*p*
Body Fat Percentage (%)	19.1 ± 6.2	17.7 ± 6.7	**<0.001**
Body Muscle Percentage (%)	43.4 ± 4.3	43.6 ± 4.4	0.332
Body Water Percentage (%)	30.7 ± 3.9	30.5 ± 3.8	0.189
BeBiS Calories (kcal)	1472.20 ± 492.158	1018.70 ± 328.503	**<0.001**
BeBiS Calcium (mg)	939.92 ± 573.197	1322.18 ± 179.099	**0.040**
BeBiS Fat (gr)	49.27 ± 24.225	37.69 ± 13.667	**0.011**
BeBiS Protein (gr)	49.55 ± 21.378	38.16 ± 14.518	**0.009**
BeBiS Carbohydrates (gr)	198.7 ± 100	149.4 ± 150.7	0.058

nutrition of these mothers was supported under the guidance of a dietitian, the mothers continued to be adversely affected metabolically in the second assessment.

FPIAP is a non-IgE(immunoglobulin E)-mediated food allergy characterized by bloody stool, often in a healthy infant. Symptoms begin in the first months of life, usually at 2–8 weeks of age. Breastfed infants accounted for 60% of the cases, all of whom were healthy and showed adequate weight gain—most patients develop tolerance before the age of one.[12] In FPIAP, an elimination diet is recommended for 3–6 months after diagnosis or until the baby is 9–12 months old, and the nutritional status of patients should be monitored periodically.[13] Growth retardation, especially stunting, and micronutrient deficiency beyond calcium and vitamin D have been observed in infants on elimination diets.[14] These deficiencies and their degree vary, depending on many factors such as the patient's age, gender, other accompanying allergic diseases, and the duration of elimination.[15,16]

Cow's milk has significant protein, mineral, and vitamin content, and when its nutritional equivalent is excluded from the diet, macronutrient deficiencies such as protein, carbohydrates, and fat or micronutrient deficiencies such as vitamins and minerals may occur. The macronutrients found in cow's milk are carbohydrates, protein, and fat; the micronutrients are calcium, iodine, vitamin A, vitamin B12, and vitamin D.[17,18] In addition, cow's milk and dairy products are good sources of some immunomodulatory nutrients, especially vitamin A, zinc, and magnesium.[19] In fact, certain nutrients such as calcium, iron, zinc, copper, and folate are not highly dependent on the mother's diet. Other nutrients such as thiamine, riboflavin, vitamin B6, vitamin B12, choline, vitamin A, vitamin D, selenium, and iodine and fatty acids such as docosahexaenoic acid are dependent on the maternal diet, and therefore, deficiency is important.[20]

Cow's milk protein allergy may also be observed in breastfed infants.[21] Hence, mothers are put on elimination diets, which can affect not only the health of the infant but also the health of the breastfeeding mother. The maternal intake of nutrients such as energy, protein, calcium, phosphorus, riboflavin (B2), pantothenic acid (B6), cyanocobalamin (B12), and vitamins A and D may be significantly reduced. During lactation, a woman requires an additional energy intake of 2000 kJ (kilojoule) per day (approximately 500 cal per day) and 1.1 g/kg protein above her usual energy and protein daily nutritional requirement. There is also an increased need for many other vitamins and minerals (especially vitamins A, group B, C, D, E, and zinc). Although calcium requirements do not increase during lactation, the regular intake of many women is less than the recommended 1000 mg per day.[22] In a study conducted by Adams et al.,[23] increased bone turnover was found in breastfeeding mothers on an elimination diet despite adequate calcium supplementation of 1000 mg daily. These mothers had lower dietary intakes of energy, protein, and phosphorus than mothers on an unrestricted diet. Previous studies have shown that bone loss during breastfeeding is reversible after discontinuation of breastfeeding; however, the situation in long-term breastfeeding mothers has not been sufficiently studied.[20]

Calcium has an essential role in regulating fat mass, mainly through effects such as increasing thermogenesis, decreasing lipogenesis, or inhibiting fat absorption. It has been reported that intracellular calcium signals regulate the adipocyte mechanism through calcium-dependent hormones. Diets with low calcium levels result in elevated calcitriol levels (1,25-dihydroxy vitamin D), which act on adipocytes by upregulating a specific vitamin D membrane receptor and increasing Ca2+ influx into the cell. Increased intracellular Ca2+ levels stimulate lipogenesis and at the same time suppress fat breakdown; thus, lipid synthesis and adiposity increase.[24] In addition, calcitriol acts through a nuclear vitamin D receptor to suppress the increased expression of adipocyte uncoupling protein 2. This decreases mitochondrial fatty acid transport and oxidation and suppresses fat cell apoptosis.[25,26] In contrast, high calcium diets suppress calcitriol levels, decreasing lipogenesis and increasing lipolysis and adipocyte apoptosis, thereby reducing lipid synthesis. Furthermore, a higher dietary calcium intake promotes fat excretion in the feces by forming insoluble compounds with fats and bile acids in the intestines. In our study, consistent with the above information, a decrease in the fat ratio of mothers was observed with calcium replacement.

Providing adequate substitution or supplementation to the maternal diet can improve the nutritional quality of both maternal diet and human milk for the allergic infant. Therefore, the mother's diet was regulated under the dietitian's supervision and the necessary supplements were administered; however, the negative metabolic effects in the mothers still persisted after one month. This may be explained

by the mother's appetite problems or the stress of caring for a chronically ill baby. As the allergen-free foods that need to be replaced are usually high-cost foods, lack of access to these foods may also be related to low socioeconomic status.[27]

One limitation of our study is that the number of participants was initially consistent with the sample size, but was reduced due to mothers withdrawing from the second assessment. The second limitation was that we included not only mothers with cow's milk allergy but also mothers with cow's milk allergy and egg and multiple food allergies. This may have increased the negative consequences of the elimination diet. Another critical limitation of our study is the lack of a control group of breastfeeding mothers of healthy infants without food allergies. Inclusion of such a group in our study would have allowed for more robust comparisons and strengthened the attribution of the observed metabolic changes specifically to the elimination diet. However, due to our financial constraints and the lack of external funding for our study, we were unable to establish a control group and compare the metabolic changes in breastfeeding mothers who followed the elimination diet with those who did not follow the elimination diet. Previous studies have shown that calcium supplementation alone does not significantly affect fat loss or body composition in postpartum lactating women without dietary restrictions.[28] This suggests that the changes observed in our study may be more pronounced and specific to the context of elimination diets. However, future studies should include a control group to confirm and extend our findings.

Our study showed that despite the nutritional support provided to mothers of infants with CMPA on elimination diets under the guidance of a dietitian, the mothers were adversely affected in terms of metabolism. As shown by numerous studies, children with CMPA are at risk for inadequate nutrient intake and growth retardation. While ensuring a balanced diet for infants with CMPA, the mother's health on the elimination diet should not be neglected. Providing adequate substitution or supplementation to the maternal diet may improve the nutritional quality of the maternal diet and breast milk. The development of industrial products containing calcium and balanced nutrition for mothers on elimination diets should be considered.

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Mandatory Disclosure on Use of Artificial Intelligence

The authors declare that no AI-assisted tools were used in the preparation of this manuscript. All references have been manually verified for accuracy and relevance.

Authors Contribution

All authors contributed equally to this article.

Conflicts of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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