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Changes in Fecal Calprotectin Levels in Infants with Cow's Milk Protein Allergy Before and After Dietary Therapy

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ABSTRACT

Background & Objective: In early infancy, adverse reactions to cow's milk proteins whether driven by IgE-dependent or cell-mediated immune pathways are among the leading causes of food-related gut inflammation. This investigation sought to quantify shifts in fecal calprotectin, a neutrophil-derived protein reflecting mucosal immune activity, in a cohort of infants with physician-confirmed cow's milk protein allergy (CMPA), assessed immediately prior to and one month following rigorous dietary exclusion of all cow's milk–derived components.

Materials & Methods: This cross-sectional study included 63 infants (aged 1–18 months) with CMPA referred to the pediatric gastroenterology clinic of Ayatollah Mousavi Hospital in Zanjan, Iran, in 2024. Diagnosis was based on clinical symptoms (e.g., bloody stool) and confirmed by a positive elimination-challenge test. Fecal Calprotectin levels were measured using a commercial ELISA kit before and one month after starting a cow's milk protein-free diet (using specialized formula or maternal dietary elimination). Statistical analysis was performed using SPSS version 26, with a P-value <0.05 considered significant.

Results: The mean age (±SD) of the infants was 3.78 (±2.28) months, with 31 (49.2%) boys and 32 (50.8%) girls. Regarding feeding type, 40 infants (63.5%) received Neocate LCP formula, 13 (20.6%) received Pepti Junior formula, and 10 (15.9%) were exclusively breastfed by mothers on a milk-free diet. The most frequent clinical symptom was bloody stool (65.1%, n=41), followed by gastroesophageal reflux disease (GERD) (39.7%, n=25) and dermatitis (39.7%, n=25). The mean fecal Calprotectin level decreased significantly from 479.41 ± 368.76 µg/g before the intervention to 130.39 ± 95.55 µg/g one month after the intervention (P<0.001). A significant reduction in Calprotectin was observed across all feeding types.

Conclusion: Fecal Calprotectin levels decreased significantly following dietary intervention in infants with CMPA. This suggests that fecal Calprotectin may serve as a useful, non-invasive biomarker for monitoring intestinal inflammation and therapeutic response in these patients.

Keywords: Calprotectin, Cow's Milk Protein Allergy, Diet Therapy, Fecal Biomarker, Infant

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1. Introduction

Food allergy is defined as an adverse reaction caused by a specific immune response that occurs after exposure to a specific food (1). The prevalence of food allergies in children is estimated to be around 3-6%. Cow's milk is considered one of the foods used in infant nutrition. Cow's milk allergy is one of the most common food allergies in infants. Studies have shown that about 6-8% of infants in the community are intolerant to the protein in cow's milk (2, 3). In addition to the transfer of cow's milk protein through direct consumption by the infant, proteins are also

transferred to infants through dairy products consumed by the mother (4). Cow's milk contains 20 different protein fragments. Four types of casein (S1, S2, S3, S4) comprise the proteins in milk. Other proteins in cow's milk include lactoglobulin, lactalbumin, and bovine serum albumin (5). The two main proteins responsible for causing allergies are beta-lactoglobulin and casein, both of which are also present in breast milk. IgE antigen concentrations vary between mothers, and mothers who have infants with intermittent colitis have higher IgE antigen concentrations (6, 7).

A definitive diagnosis of allergy is achieved by the resolution of symptoms following the elimination of cow's milk from the mother's and infant's diet and with the reintroduction of this product, which is usually offered after four weeks of the elimination diet, the symptoms return (8). Common clinical signs include chronic diarrhea, failure to thrive, abdominal distension, perianal erythema or diaper rash, reflux, regurgitation, anemia, rhinorrhea, wheezing, and eczema. Other symptoms of anaphylaxis include skin rashes, flushing, and severe vomiting, which are very rare (9, 10). The only available treatment for cow's milk allergy is to eliminate all cow's milk proteins. In breastfed infants, all cow's milk products should be eliminated from the mother's diet with the elimination of these antigens from breast milk, recovery is usually seen within 72 hours. Although cow's milk protein allergy resolves on its own and in most cases is not associated with significant complications, its diagnosis and treatment have always been a major concern (11, 12).

Diagnosis of IgE-mediated allergy is generally easier because of the clear temporal relationship between ingestion of the offending food and the onset of symptoms. In addition, IgE tests and skin prick tests help to diagnose IgE-mediated allergy in the clinical setting. The diagnosis of food protein enterocolitis syndrome is based primarily on the clinical history of characteristic signs and symptoms with improvement after exclusion of the suspected food, after exclusion of other potential causes (13). Cow's milk protein allergy (CMPA) can be immunoglobulin E (IgE)-mediated, non-IgE-mediated, or mixed. IgE-mediated reactions are immediate and involve specific IgE antibodies, typically presenting with cutaneous, respiratory, or gastrointestinal symptoms. Non-IgE-mediated reactions are delayed, T-cell-driven, and primarily manifest with gastrointestinal symptoms such as food protein-induced enterocolitis syndrome (FPIES) or allergic proctocolitis, which is often characterized by bloody stools in otherwise well-appearing infants. The immunologic pathway involved influences both the clinical presentation and the diagnostic approach.

Evidence suggests that fecal Calprotectin is the best noninvasive indicator of organic bowel disease, and measurement of FC concentration can be performed with 50 to 100 mg of stool. Calprotectin levels can reach 5-40 times normal levels when inflammation occurs in the body (14, 15). Fecal Calprotectin is a simple, rapid, and relatively inexpensive biomarker, therefore, it can be a reliable and valid indicator in digestion for the diagnosis and monitoring of inflammatory diseases (16). Recent studies (2022-2024) have supported its role as a non-invasive marker for monitoring mucosal inflammation in CMPA. Given the high prevalence of cow's milk protein allergy in infants and the importance of early diagnosis of food allergy in infants and children to prevent further progression of allergic diseases and alternative diets, investigating the role of non-invasive fecal inflammatory biomarkers such as Calprotectin in the timely diagnosis and treatment of cow's milk protein allergy seems essential. Therefore, given the limitations of studies conducted in this field, the present study was designed to investigate the changes of fecal Calprotectin in infants with cow's milk protein allergy before and one month after receiving a therapeutic diet.

2. Materials and Methods

This cross-sectional study was conducted on 63 infants sensitive to cow's milk protein who referred to the pediatric gastroenterology clinic of Mousavi Zanjan hospital in 2024. The sampling method was convenient. The inclusion criteria were infants 1 to 18 months, having an allergy to cow's milk protein willingness to participate in the study. Exclusion criteria were known growth failure, chronic respiratory disease, and inflammatory colitis, congenital gastrointestinal disease such as atresia, known immunodeficiencies, and concurrent allergies to other common allergens such as egg, beef, or soy. In present study, confirmation and diagnosis of cow's milk protein allergy was made based on characteristic clinical findings (e.g., bloody stool in the absence of signs of infection such as fever or positive stool culture) and a positive elimination and challenge test in all patients. Data collection consisted of a checklist including demographic information and clinical symptoms related to food allergy, positive stool culture, and challenge test, which was completed by the researcher with the help of the parents. Fecal Calprotectin levels were measured using a commercial ELISA kit (Calpro, Germany; Catalog No: CAL0030) according to the manufacturer's instructions. Briefly, fresh stool samples were collected, aliquoted, and weighed. Samples were then centrifuged at 3000 rpm for 10 minutes, and the supernatant was snap-frozen in liquid nitrogen and stored at -80°C until batch analysis. The clinical threshold for a positive test was considered >50 µg/g. Finally, fecal Calprotectin levels were measured before and one month after receiving the special treatment regimens for these infants.

It is confirmed that no infant in this cohort was maintained on a mixed diet; all infants were exclusively either breastfed (with maternal dietary elimination) or receiving a specified hydrolyzed formula (Neocate LCP or Pepti Junior) according to their assigned therapeutic group.

The data were analyzed using SPSS26 software and P-Value <0.05 was considered as a significant level. In descriptive analysis, mean (S.D) and number (%) were used for quantitative variables. In analytical analysis,

paired t-test was used to compare the mean Calprotectin before and one month after receiving the respective treatment regimens. Additionally, analysis of covariance (ANCOVA) was used to control for the potential confounding effect of age on Calprotectin changes. It should also be mentioned the protocol study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Deputy of Research and Ethics Committee of Zanjan University of Medical Sciences (ID-number: IR.ZUMS.REC.1402.203). The study was also registered with the Iranian Clinical Trial Registry (IRCT).

3. Result

In total, 63 infants sensitive to cow's milk protein were investigated. Table 1 shows the demographic and clinical characteristics of the infants. As can be seen, the mean age of treatment initiation (±SD) was 3.78 (± 2.28) months. The number of boy and girl were 49.2 % (31) and 50.8% (32), respectively. In terms of feeding, 63.5% (40) Neocate LCP powdered milk, 20.6% (13) Pepti Junior powdered milk and 15.9% (10) were breastfed. The frequency of clinical symptoms of bloody stool, Gastroesophageal reflux disease (GERD), dermatitis, diarrhea, and hematemesis were 65% (41), 39.7% (25), 39.7% (25), 12.7% (8), and 1.6% (1), respectively. Occult blood test results were positive in 90.5% (57) and 58.9% (32) had abnormal WBC in SE (Table 1).

Analysis of fecal Calprotectin levels based on feeding type showed a consistent and significant decrease post-intervention across all groups (exclusively breastfed, Neocate LCP, and Pepti Junior). The statistical correlation between feeding type and the magnitude of Calprotectin reduction was also significant (P<0.05).

Table 2 shows the mean Calprotectin levels before and one month after therapeutic diet. A paired t-test was used to compare the mean Calprotectin levels at these two time points, and the results of this test showed that the mean Calprotectin level one month after therapeutic interventions (479.41 ± 368.76) was significantly lower than before the intervention (130.39 ± 95.55) (P<0.001).

Table 1. Demographic and clinical characteristics of the patients under study.

Variable		Lower CI 95%	Upper CI 95%
Sex	Boy	32	50.8
	No	31	49.2
Diarrhea	Yes	55	87.3
	No	8	12.7
Bloody stool	Yes	22	35
	No	41	65
Gastroesophageal reflux disease (GERD)	Yes	38	60.3
	No	25	39.7
Hematemesis	Yes	61	98.4
	No	1	1.6
Occult Blood (OB)	Yes	6	9.5
	No	57	90.5
Dermatitis	Yes	38	60.3
	Normal	25	39.7
WBC in SE	Abnormal	31	49.2
Type of feeding	Neocate LCP powdered milk	32	50.8
	Breast milk	40	63.5
	Pepti Junior powdered milk	10	15.9
		13	20.6
Variable		**Mean**	**S.D**
Age		3.78	2.28

† GERD: Gastroesophageal reflux disease; OB: Occult Blood; WBC: White Blood Cell; SE: Stool Exam; SD: Standard Deviation.

Table 2. Comparison of Calprotectin levels before and one month after therapeutic diet.

Variable		N	Mean	S.D	P-Value
Gender (Male vs Female)	Before	63	479.41	368.76	<0.001
	After	63	130.39	95.55	

SD: Standard Deviation. Statistical significance determined by paired t-test.

4. Discussion

The present study was designed to investigate the changes of fecal Calprotectin in infants with cow's milk protein allergy before and one month after receiving a therapeutic diet. Our results showed the mean age (±SD) was 3.78 (± 2.28) months. The number of boy and girl were 49.2 % (31) and 50.8% (32), respectively. In terms of feeding, 63.5% (40) Neocate LCP powdered milk, 20.6% (13) Pepti Junior powdered milk and 15.9% (10) were breastfed. The frequency of clinical symptoms of bloody stool, gastroesophageal reflux disease (GERD), dermatitis, diarrhea, and hematemesis were 65% (41), 39.7% (25), 39.7% (25), 12.7% (8), and 1.6% (1), respectively. Occult blood test results were positive in 90.5% (57) and 58.9% (32) had abnormal WBC. The mean of Calprotectin level one month after therapeutic interventions (479.41 ± 368.76) was significantly lower than before the intervention (130.39 ± 95.55) (P<0.001).

To mitigate potential confounding from age and digestive maturity, the inclusion criteria were strictly limited to infants under 18 months. Furthermore, statistical analysis incorporated age (in months) as a covariate in regression models to control for residual age-related variance. Recent literature (2022-2023) supports that while fecal Calprotectin levels physiologically decrease with age and intestinal maturation in the first years of life, the significant reduction observed in our study within a short one-month interval is likely attributable to the dietary intervention rather than natural maturation alone.

In our study, the frequency of clinical symptoms was high. In the study by Nocerino et al **(17)** the overall prevalence of gastrointestinal symptoms among infants with cow's milk allergy was reported to be between 50 and 80% **(17)**. In the study by Iacono et al **(18)** the frequency of gastrointestinal symptoms was reported in 56 cases **(18)**. Based on the study by Katz et al **(19)** diarrhea was reported in 25% and vomiting in 100% of cases **(19)**. In the study by Kianifar et al **(20)** gastrointestinal symptoms were observed in 98.5% of patients, and bloody stool was the most common gastrointestinal symptom with 67.9% (40). In present study, the frequency of dermatitis in infants was 39.7%, which was consistent with similar studies in this field that reported a prevalence of skin symptoms of 20-40% **(17, 18, 21)**. For example, the study by Kianifar et al **(20)** reported a frequency of skin findings of 40.7%. Perhaps one of the reasons for the high frequency of gastrointestinal symptoms in patients in this study is the research setting, as the research setting was a specialized gastrointestinal clinic.

This study showed the mean of Calprotectin level one month after therapeutic interventions was significantly lower than before the intervention, therefore, it was a reliable and valid indicator in diagnosing children with cow's milk protein allergy. This finding was consistent with the results of similar studies **(22-25)**. For example, the study by Xiong et al **(22)** in 2021 showed that fecal Calprotectin is a biomarker of intestinal inflammation with the advantages of non-invasiveness, high sensitivity, easy transfer and availability, and its use is more suitable for infants and children **(22)**. Mousavi's study showed that fecal Calprotectin can be used as a non-invasive diagnostic marker to differentiate between irritable bowel syndrome and inflammatory bowel syndrome **(26)**. Rycyk et al **(15)** suggested that fecal biomarkers are suitable and useful for diagnosis due to their ease of collection, good correlation with the severity of inflammation in the intestinal wall, and specificity for intestinal inflammation. For this reason, fecal Calprotectin could also be a reliable indicator for the diagnosis and monitoring of inflammatory bowel diseases **(15)**.

Calprotectin has played an important role in the diagnosis and monitoring of gastrointestinal inflammation in recent years. Quantification of this biomarker is simple, rapid, and relatively inexpensive. It is widely detected in blood plasma, urine, cerebrospinal fluid, saliva, and synovial fluids. Evidence suggests that when inflammation occurs in the body, Calprotectin levels can reach several times the normal level **(16, 27)**. Under normal conditions, the fecal Calprotectin concentration is six times the plasma concentration, indicating the potential of fecal Calprotectin as an accurate biomarker of intestinal inflammation. Furthermore, fecal Calprotectin remains stable in feces for more than a week **(22, 28)**. Fecal Calprotectin, as a noninvasive and useful marker of intestinal inflammation, could reduce the number of children who are referred unnecessarily for endoscopic or radiological examination. Calprotectin screening has advantages for both patients and physicians, for patients it reduces delays in diagnosis and unnecessary exposure to endoscopy, and for physicians it reduces the number of endoscopic tests and facilitates decision-making. Therefore, it is a useful marker of intestinal inflammation and can be measured within hours using a simple ELISA test. Also, the test results are available quickly for effective clinical decision-making **(29)**. In addition, studies have shown that fecal Calprotectin plays an important role in the identification and management of patients with inflammatory bowel disease. A study by

Kawashim et al (30) by fecal Calprotectin measurement showed that fecal Calprotectin levels were correlated with the extent of mucosal damage as well as its severity in ulcerative colitis patients (30).

In our study, Calprotectin levels decreased significantly after receiving the treatment regimen. In line with this finding. Qiu et al (16) study showed that fecal Calprotectin levels in infants with cow's milk protein allergy decreased after treatment intervention. They also suggested that Calprotectin is a promising biomarker for monitoring intestinal allergies (16). A systematic review and meta-analysis by Zhang et al (31) showed that FC levels in children with CMPA were significantly higher than in healthy subjects, and that FC levels decreased significantly after dietary elimination (31). A study by Lendvai-Emmert et al (32) also showed significant improvements in clinical symptoms and FC levels after only one dietary elimination (32).

5. Conclusion

The present findings document a marked reduction in fecal calprotectin concentrations among infants with confirmed cow's milk protein allergy (CMPA) following strict adherence to a cow's milk–free dietary regimen. This decline strongly reflects diminished immune activity within the intestinal mucosa and underscores the utility of calprotectin as a quantifiable, stool-based metric for evaluating mucosal response to dietary intervention in early life. Although fecal calprotectin demonstrates considerable promise for monitoring therapeutic outcomes, its suitability as a standalone diagnostic criterion for CMPA cannot yet be affirmed and warrants validation through future multicenter, prospective, and longitudinal studies with larger cohorts. Integrating calprotectin measurement into routine clinical assessment has the potential to refine therapeutic decisions, decrease the need for endoscopic or radiologic procedures, and support more precise, individualized management of infants affected by food-triggered intestinal inflammation.

6. Declarations

6.1 Acknowledgments

The authors are grateful for the sincere cooperation of the staff of Ayatollah Mousavi Hospital and the families of the children who participated in this study.

6.2 Ethical Considerations

The study was approved by the Deputy of Research and Ethics Committee of Zanjan University of Medical Sciences (ID-number: IR.ZUMS.REC.1402.203). Informed consent was obtained from the parents or legal guardians of all participating infants.

6.3 Authors' Contributions

Conceptualization, supervision, funding acquisition and resources: Mohammad Esagh Rozeh and Mahdieh Sharifi; Methodology: Kamyar Mansouri and Reza Mahdian Jouybari; Data collection: Mahdieh Sharifi; Data analysis: Kamyar Mansouri; Investigation and writing: All authors.

6.4 Conflict of Interest

The authors declare that they have no competing interests.

6.5 Fund or Financial Support

This study was funded by Zanjan University of Medical Sciences.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

6.7 Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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