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Original Article

Fecal Calprotectin and Cow's Milk-Related-Symptoms Score in Children with Cow's Milk Protein Allergy

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ABSTRACT

Purpose: The cow's milk-related-symptom-score (CoMiSS) tool was developed as an awareness tool for the assessment of cow's milk-related symptoms in infants or children. Fecal calprotectin (FC) is a noninvasive biomarker of gut inflammation that can be measured in serum and stool. This study aimed to investigate the relationship between FC levels and CoMiSS scores in infants with cow's milk protein allergy.
Methods: Infants (aged 6–12 months) who were allergic to cow's milk protein were enrolled prospectively. Following completion of the CoMiSS scoring, the infants were divided into group 1 (positive CoMiSS scores ≥12) and group 2 (negative CoMiSS scores <12). FC was measured using immunoassay.
Results: Of the 120 infants enrolled in this study, 60 (50.0%) had positive CoMiSS scores (group 1), while 60 (50.0%) had negative scores (group 2). The mean FC level was higher in the infants in group 1 than those in group 2 (2,934.57 μg/g vs. 955.13 μg/g; p<0.001). In addition, there was a positive correlation between FC and CoMiSS scores (R=0.168, p<0.0001). A FC level of 1,700 μg/g provided a sensitivity of 98.3%, specificity of 93.3%, and accuracy of 95.8% for the diagnosis of cow's milk protein allergy (CMPA).
Conclusion: FC measurement may have a role in the assessing infants with CMPA.

Keywords: The cow's milk-related symptom score; Calprotectin; Cow milk protein allergy

INTRODUCTION

Cow's milk protein allergy (CMPA) is an immune response to cow's milk proteins that commonly begins in infancy [1,2]. CMPA is a global issue that affects approximately 8% of children [3].

Atopic dermatitis, gastrointestinal symptoms, such as colic, vomiting, diarrhea, or constipation, and respiratory manifestations, such as wheezing or sneezing, are symptoms of CMPA. However, none of the symptoms of CMPA are specific, making CMPA difficult to diagnose. Lactose intolerance, gastroesophageal reflux disease, and infantile colic are other conditions with similar presentations [4]. The involvement of two or more organ systems increases the possibility of CMPA [5].

Conflict of Interest
The authors have no financial conflicts of interest.

Because of the difficulties in diagnosing CMPA, the cow's milk-related-symptom-score (CoMiSS) tool was developed and validated as an awareness tool to assist in diagnosing CMPA [6]. The CoMiSS tool contains various presenting symptoms and can be completed within 15 minutes. It has been used in various settings [1,2]. However, it does not replace a formal food challenge test [7].

Calprotectin is a cytosolic protein that binds calcium and zinc and has immunomodulatory and antimicrobial properties [8,9]. It is derived mainly from neutrophils and can be measured in various body fluids, including serum and feces. Calprotectin levels increased with inflammation, infection, and malignancy [10,11]. Fecal calprotectin (FC) is widely used in diagnostic procedures and for disease monitoring in individuals with inflammatory bowel disease [10,11].

The role of FC measurement in infants with suspected CMPA is unknown. This study aimed to measure FC in a group of infants with suspected CMPA and to determine if FC levels correlate with CoMiSS scores.

MATERIALS AND METHODS

Ethical consideration

The research ethics committee of the Faculty of Medicine of Suez Canal University (No. 4386) approved the study protocol. Each parent or caregiver provided written informed consent during recruitment.

Study design and study cohort

This cross-sectional comparative study was conducted at a single Pediatric Department in a tertiary teaching University Hospital. Infants receiving breast milk between aged 6 and 12 months were recruited prospectively and consecutively from pediatric out-patient clinics. The inclusion criteria included clinical features of suspected CMPA. Exclusion criteria included an infant or parent unable to comply with study procedures, known underlying gastrointestinal disease, or other conditions that may alter gut transit (such as bottle feeding, administration of an antibiotic or acid suppressant, surgery, or another medical intervention within the previous month). Infants who had previously received treatments for their symptoms (such as maternal dietary changes) were excluded.

Medical records (including birth details, medical history, symptoms, and feeding history) were collected from all infants using a predesigned questionnaire. CoMiSS scores were collected from all infants using the previously published scoring tool [6]. Investigations, such as skin prick tests, were not conducted routinely.

Collection of fecal samples for measurement of FC levels

Stool samples were requested at the time of study enrolment. All samples were collected and processed following a standard protocol. Parents were instructed to collect a feces sample from the infant's diaper immediately after defecation using a sterile collection tube at home. The sample was briefly stored at −20°C (home freezer) before being transported frozen to the laboratory, where it was stored at −80°C until analysis. After thawing, a portion of each fecal sample (50–100 mg) was collected and transferred into a disposable crew cap tube using a disposable, breakable inoculation loop. The feces were weighed, and the loop handle was cut off,

leaving the loop and 4–6 cm of the handle inside the tube. The extraction solution containing urea and citrate was added in a weight: volume ratio of 1:50. Following 30 s agitation on a mixer and 20 minutes of homogenization at 1,400 rpm on a shaker, 1 mL of the homogenate was transferred to an Eppendorf tube and then centrifuged for 20 minutes. The supernatant (0.5 mL) was then collected and analyzed immediately using an enzyme-linked immunosorbent assay with a test device (Nycomed Pharma AS) following the manufacturer's instructions.

Sample size calculation

The sample size was calculated according to the following equation:

$$n = 2\left[\frac{(Z_{\alpha/2} + Z_{\beta}) * \sigma}{\mu_1 - \mu_2}\right]^2$$

In which n denotes the sample size required in each group, σ refers to the estimation of the standard deviation in the study group, $Z_{\alpha/2}$ of 1.96 denotes the critical value that divides the central 95% of the Z distribution from the 5% in the tail, Z_{β} is 0.84 (i.e., the critical value separating the lower 20% of the Z distribution from the upper 80%), μ_1 signifies the mean in group 1 (516 μg/g in CMPA) and μ_2 refers to the mean in group 2 (296 μg/g). As a result, the required sample size was calculated to be 100 participants plus a 20% dropout rate.

Statistical analysis

Statistical package for social science IBM SPSS Statistics for Windows, Version 24.0 (IBM Co., Armonk, NY, USA) was used to code, enter, and process the data. The results were expressed in a tabular and diagrammatic form before being interpreted.

Descriptive statistics (mean, standard deviation, range, frequency, and percentage) were calculated. Chi-square test was used to calculate the association of variables in categorical data. Fisher's exact test was used for two or more nominal variables. Student's *t*-test and Pearson's correlation coefficient values were calculated. The accepted level of significance was $p<0.05$.

RESULTS

Demographic characteristics

We enrolled 120 infants that are receiving breast milk with suspected CMPA. Sixty infants were included in group 1 based on their positive CoMiSS scores (≥12), and 60 infants with negative CoMiss scores (<12) were included in group 2.

Table 1 shows there was no significant difference between the two groups regarding age at diagnosis, current age, birth weight, current weight, or parental history of consanguinity. Group 1 had more girls (62% vs. 35%; $p=0.003$), a higher mean gestational age (38.2 weeks vs. 37.7 weeks; $p=0.045$), and was more likely to have a family history of CMPA (18.3% vs. 0.0%; $p=0.001$) than group 2.

FC and CoMiSS scoring

The mean FC level in the infants with a positive CoMiSS score was higher than in those with a negative CoMiSS score (2,934.6 μg/g vs. 955.1 μg/g; $p<0.001$). In addition, FC levels were associated with CoMiSS scores (R=0.931; $p<0.0001$) (**Fig. 1**). FC was correlated with gestational age and the duration of symptoms (R=0.168; $p<0.05$).

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Table 1. Background and demographic data of 120 infants with potential cow's milk protein allergy according to their CoMiSS

Demographic characteristics	Group 1 positive CoMiSS ≥12	Group 2 negative CoMiSS <12	*p*-value
Age of diagnosis (mo)	2.90±1.94	3.43±1.74	0.116
Age at the recruitment (mo)	10.02±5.29	9.90±4.26	0.895
Gestational age (wk)	38.18±1.24	37.73±1.19	0.045
Birth weight (kg)	2.74±0.662	2.67±0.542	0.550
Weight (kg)	8.31±1.69	8.70±1.80	0.223
Length (cm)	69.70±5.97	70.81±6.82	0.346
Sex			0.003
Female	37 (61.7)	21 (35.0)	
Male	23 (38.3)	39 (65.0)	

Values are presented as mean±standard deviation or number (%).
CoMiSS: cow's milk-related-symptom-score.

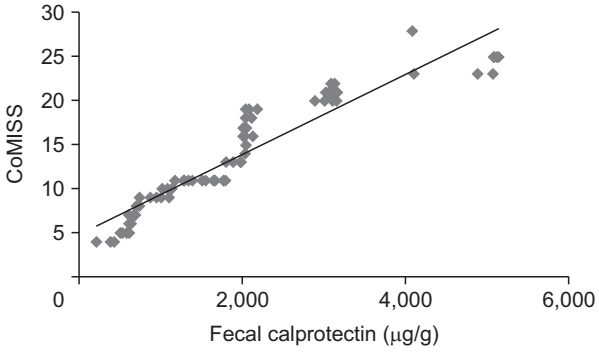

Fig. 1. Strong positive correlation between fecal calprotectin and CoMiSS. Fecal calprotectin levels correlated with CoMiSS (R=0.931; $p<0.0001$).
CoMiSS: cow's milk-related-symptom-score.

Accuracy of FC in the diagnosis of CMPA

Receiver operator curve analysis was used to define the test characteristics of FC in this population. FC provided a sensitivity of 98.3%, specificity of 93.3%, positive predictive value of 93.7%, negative predictive value of 98.2%, and accuracy of 95.8% for the diagnosis of CMPA with a cut-off value of 1,700 μg/g.

DISCUSSION

The study aimed to establish the possible role of FC measurement in infants with suspected CMPA and to determine the relationship between FC and cow's milk-related symptoms, measured with CoMiSS scores. FC levels correlated with symptom severity, whether these were considered dichotomously (<12 vs. ≥12) or as a continuous variable. The findings suggest a role for FC measurement in investigating of infants with suspected CMPA.

We enrolled 120 prospective infants for this study. There were more girls than boys in the groups (p=0.003). Previous studies did not report a sex difference in the development of CMPA [2]. This study demonstrated a strong relationship between the infant's diagnosis and family history of CMPA, which is supported by the existing literature [2]. Furthermore, this study found no relationship between anthropometric status and symptom severity. Although the interruption of normal feeding patterns and the resulting influence on weight gain is a significant clinical concern, other researchers have found no relationship between symptom severity and weight or length [1].

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Given that the initial presentation of CMPA can be diverse or ambiguous, a group of experts developed the CoMiSS score in 2014 to provide a simple clinical awareness tool based on symptoms commonly exhibited by infants who react adversely to cow's milk proteins [6]. It was seen as a tool to aid in the evaluation of these infants and to track their response to nutritional treatments, rather than as a replacement, rather than as a replacement for a formal food challenge. It has now been used in various studies from different locations [1,2,12,13]. CoMiSS have been studied in healthy infants [14], and as a guide to identifying those with CMPA [12]. Other reports have evaluated CoMiSS following the dietary intervention [8,15]. One study reported that the standard cut-off of ≥12 was too high, raising concerns about possible underdiagnosis of CMPA [2]. However, the relationship between FC and CoMiSS scores has not been evaluated.

Several researchers have already investigated FC in the context of CMPA. Merras-Salmio et al. [16] measured FC in 57 infants with gastrointestinal symptoms and suspected CMPA. FC levels were higher in infants with a positive food challenge than those with a negative challenge. A serial assessment of FC in 82 Spanish infants revealed that infants with CMPA had a higher level of FC than control infants [17]. This study demonstrated a cut-off value that may be used to rule out CMPA. Ataee et al. [18] reported a reduction in FC levels in 29 infants with CMPA treated with a change in the maternal diet. An earlier Italian study reported that excluding cow's milk for 4 weeks reduced FC levels in infants with rectal bleeding caused CMPA [19].

These findings suggest that measuring FC may be a useful, cheap, simple, and noninvasive test to demonstrate and assess disease activity in infants with CMPA. However, FC can be variable in infancy, and elevated levels are reported in other gastrointestinal disorders [20-23]. The strengths of this study included a consistent recruiting design from a single pediatric unit and a relatively large sample size. The limitations of this study were: first, the patients included had a variable duration of symptoms. Second, the assessments were only conducted once, with no reassessment of symptoms or FC after any dietary modification. Third, the infants were not subjected to any objective examination (such as skin prick testing) to identify potential allergens and were recruited entirely based on their symptoms. Furthermore, the cohort might not represent infants with CMPA in other areas.

In conclusion, this cross-sectional study of infants with suspected CMPA found a strong relationship between symptom severity using CoMiSS and levels of FC. These findings should be investigated in further longitudinal studies in other areas.

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