ORIGINAL ARTICLE

TJG
The Turkish Journal of Gastroenterology

Fecal calprotectin concentration is increased in children with celiac disease: relation with histopathological findings

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Background/aims: *The aim of this study was to compare the fecal calprotectin concentration in children with newly diagnosed celiac disease, children with celiac disease strictly adhering to a gluten-free diet and healthy controls. We also tried to correlate the fecal calprotectin concentration with the clinical presentation, degree of neutrophilic infiltration and the severity of histopathological injury (Marsh grade) in the small bowel mucosa.* ***Material and Methods:*** *The study included three groups: children with untreated celiac disease, children with treated celiac disease, and healthy controls. Moreover, we obtained a second fecal sample from nine newly diagnosed children when their endomysial antibody became negative after gluten-free diet.* ***Results:*** *Fecal calprotectin concentrations were significantly higher in newly diagnosed celiac patients (n=31) compared to patients on gluten-free diet (n=33) and healthy controls (n=34) (117.2 µg/g (3.2-306) vs. 3.7 µg/g (0.5-58.2) and 9.6 µg/g (1-70), respectively, p<0.001). Patients presenting with gastrointestinal symptoms had higher fecal calprotectin concentration compared to the patients presenting with non-gastrointestinal symptoms [142.8 (12.2-306) vs. 79.7 (3.2-243.2) respectively, p=0.04]. Nine newly diagnosed patients gave a second fecal sample after starting gluten-free diet when endomysial antibody became negative. Their fecal calprotectin concentration had decreased from 113.7 µg/g (8.7-295.2) to 4.2 µg/g (0.5-20.7) (p<0.01).* ***Conclusions:*** *Increased fecal calprotectin concentration can be used as a non-invasive marker that might aid in the diagnosis of celiac disease, especially in patients with gastrointestinal presentation. Fecal calprotectin concentration returns to normal on a strict gluten-free diet. Fecal calprotectin may be used as a marker of diet adherence and improvement in gastrointestinal inflammation in children with celiac disease. Additionally, it may be used for the differentiation of celiac disease from functional disorders of the gastrointestinal system.*

Key words: Celiac disease, children, fecal calprotectin

Çölyaklı çocuklarda dışkı kalprotektin konsantrasyonu artmıştır: Histopatolojik bulgularla ilişkisi

Amaç: *Bu çalışmanın amacı yeni tanı ve diet altındaki çölyak hastaları ile sağlıklı çocuklardaki fekal kalprotektin miktarını karşılaştırmaktır. Ayrıca histopatolojik evre (Marsh evresi) ve nötrofilik infiltrasyon derecesi ile de fekal kalprotektin oranı karşılaştırıldı.* ***Gereç ve Yöntem:*** *Çalışma, yeni tanı, glutensiz diet altındaki hastalar ve sağlıklı kontrol grubu olmak üzeri 3 grubu içermektedir. Ayrıca çalışma boyunca glutensiz diet ile serolojisi negatifleşen 9 hastada fekal kalprotektin miktarı tekrar çalışıldı.* ***Bulgular:*** *Fekal kalprotektin oranı yeni tanı çölyak hastalarında (n=31), diet altındaki hastalara (n=33) ve sağlıklı kontrol grubuna (n=34) göre yüksek saptandı (sırasıyla 117,2 µg/g (3.2-306), 3.7 µg/g (0.5-58.2) ve 9.6 µg/g (1-70), p<0.001). Gastrointestinal semptomu olan hastalarda diğer gastrointestinal semptomu olmayan hastalara göre yüksek saptandı [142.8 (12.2-306) ve 79.7 (3.2-243.2), p=0.04]. Diet ile serolojisi negatifleşen 9 hastada fekal kalprotektin konsantrasyonun azalmış olduğu saptandı; 113.7 µg/g (8.7-295.2)'den 4.2 µg/g (0.5-20.7)'ye (p<0.01).* ***Sonuç:*** *Artmış dışkı fekal kalprotektin miktarı çölyak hastalığında non-invazif yardımcı tanı yöntemi olarak kullanılabilir. Glutensiz diyet tedavisi ile fekal kalprotektin düzeyinin normale döndüğü görülmektedir. Fekal kalprotektin bir belirteç olarak çölyak hastalarının diyete uyumunun ve bunun göstergesi olan gastrointestinal inflamasyonun azalmasını göstermede yararlıdır. Ek olarak; çölyak hastalığını gastrointestinal sistemin fonksiyonel hastalıklarından ayırmada da kullanılabilir.*

Anahtar kelimeler: Çölyak hastalığı, çocuklar, fekal kalprotektin

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Manuscript received: 21.02.2011 **Accepted:** 13.07.2011

Turk J Gastroenterol 2012; 23 (5): 503-508
doi: 10.4318/tjg.2012.0366

INTRODUCTION

Fecal calprotectin, a granulocyte neutrophil-predominant cytosolic protein, is a new marker for the assessment of gastrointestinal tract inflammation. Its high concentration in feces, resistance to bacterial degradation in the gut, and stability in stool for up to a week at room temperature make it a valuable laboratory marker (1). Measurement of fecal calprotectin concentration (FCC) is simple, non-invasive and inexpensive. It might be used for the diagnosis and monitoring of the response to medical treatment in patients with inflammatory gastrointestinal tract diseases.

In recent years, high levels of FCC have been found in several gastrointestinal diseases, including inflammatory bowel disease (IBD), colorectal cancer, non-steroid anti-inflammatory drug enteropathy, alcoholic enteropathy, chronic pancreatitis, and cirrhosis (2-4).

Celiac disease (CD) is an autoimmune enteropathy caused by intolerance to gluten-derived peptides of wheat, rye and barley. This autoimmune condition results in inflammatory injury to the mucosa of the small intestine. There are few reports published about FCC in patients with CD and their results are inconclusive (5-7).

The aim of this study was to compare the FCC in children with newly diagnosed (untreated) CD and children receiving strict gluten-free diet (GFD, treated) CD and to determine the relationship between FCC and clinical presentation, degree of neutrophilic infiltration and the severity of histopathological injury in the small bowel mucosa. We also investigated whether FCC differs after GFD is implemented.

MATERIALS AND METHODS

The study was carried out in Hacettepe University İhsan Doğramacı Children's Hospital between February 2008 and June 2009. The study included three groups: children with untreated CD, children with treated CD and healthy controls. Moreover, we obtained a second fecal sample from nine newly diagnosed children when their endomysial antibody (EMA) became negative after GFD.

All subjects were evaluated for cardiopulmonary, hepatic, renal, neurological, psychiatric, endocrine (including type 1 diabetes mellitus, autoimmune thyroiditis and other autoimmune diseases), rheumatologic diseases, malignancy, a positive family history of IBD, consumption of non-steroidal anti-inflammatory drugs, gastric acid inhibitors, antibiotics, or drugs influencing gut motility, and menstrual or nasal bleeding in the last three weeks. Patients were excluded from the study in the presence of these diseases and conditions. Children within the treated group were EMA-negative and were asymptomatic. Children without gastrointestinal symptoms and negative celiac serology were included in the control group.

We evaluated and noted the presenting signs and symptoms. Thereafter, they were divided into two categories based upon the clinical presentation. Patients who presented with gastrointestinal symptoms including diarrhea, abdominal pain, bloating, and constipation were accepted as the gastrointestinal presentation subgroup and patients who presented with symptoms such as short stature and refractory iron deficiency anemia were accepted as the non-gastrointestinal presentation subgroup.

Stools of the subjects were examined to exclude infectious diseases such as giardiasis and amebiasis. Giardiasis was also examined in the duodenal biopsy samples of the patients. Serum immunoglobulin A (IgA) levels and EMA were measured in all three groups. Serum levels of EMA were analyzed by indirect immunofluorescence with the use of a section of monkey liver (Euroimmune GmbH, Lübeck, Germany). CD was diagnosed based on the revised criteria of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (8).

A minimum of five biopsy specimens were obtained from the bulbus and second part of the duodenum. The samples were embedded and stained with hematoxylin and eosin, and examined by an expert pathologist blind to the study data. Biopsy specimens were assessed for histopathological injury based upon modified Marsh criteria (9). Marsh 1, 2 and 3a were grouped as mild and Marsh 3b and 3c as severe histopathological injury. The neutrophilic infiltration in the lamina propria and epithelium was graded as absent, mild, moderate, or severe according to the updated Sydney system, which was updated by Dixon et al. (10).

Fecal samples were collected from each subject using a disposable plastic bucket-type device to avoid contact with toilet water and to simplify laboratory sampling. In children with diapers, samples were collected directly from the rectum

into a test tube. Fecal samples were obtained before upper gastrointestinal endoscopy in newly diagnosed patients. Samples were stored at -20°C until the laboratory analysis. FCCs were determined by ELISA (PhiCal®Calprotectin ELISA Kit, Bensheim, Germany). Normal FCC was accepted as <50 µg/g feces. Parental consent was obtained before the endoscopic procedures.

Categorical variables between groups were compared by chi-square test and continuous variables by Mann-Whitney U, one-way ANOVA and Kruskal-Wallis tests where appropriate. FCC values were expressed as median (range). The area under the receiver operating characteristic (AUROC) curves was used to determine the discriminative power of the FCC in the diagnosis of CD. A p value <0.05 was accepted as statistically significant. The Statistical Package for the Social Sciences (SPSS) version 15.0 was used for all the statistical analyses.

RESULTS

Overall, 98 children were enrolled in the study; 31 with untreated CD, 33 with treated CD, and 34 healthy children as the control group. Male/female ratios for the untreated patient group were 14/17, for the treated group 16/17 and for the control group 16/18. Mean ages (±SD) of the untreated CD, treated CD patients and the control group were 7.7±2.1, 6.7±4.1, and 10.1±3.8 years, respectively (p=0.001). Within the newly diagnosed patients, 18 (58.1%) had gastrointestinal and 13 (41.9%) had non-gastrointestinal presentation.

Among the newly diagnosed patients, there was one patient with Marsh grade 1, two patients with Marsh grade 2, and 28 patients with Marsh grade 3 (10 with 3a, 14 with 3b, and 4 with 3c). When biopsies were evaluated for neutrophilic infiltration, three patients (9.7%) had no infiltration (1 patient per each Marsh group), whereas the remaining 28 (90.3%) patients had neutrophilic infiltration (14 mild, 12 moderate, 2 severe). There was a positive correlation between the degree of neutrophilic infiltration and Marsh grade (r^2=0.4, p=0.02).

FCCs were significantly higher in untreated celiac patients compared to patients already on GFD and controls (117.2 µg/g (3.2-306) for untreated vs. 3.7 µg/g (0.5-58.2) for treated patients and 9.6 µg/g (1-70) for control group, p<0.001) (Table 1). Within the untreated CD group, patients presenting with gastrointestinal symptoms had higher FCC when compared to patients presenting with non-gastrointestinal symptoms (142.8 (12.2-306) vs. 79.7 (3.2-243.2), respectively, p=0.04). In untreated patients, there was no correlation between FCC and Marsh histologic grading or degree of neutrophilic infiltration (Table 2).

Table 1. Demographic data and fecal calprotectin concentration in study groups

	Number (n)	Gender (Male/Female)	Mean Age (Year)	Median FCC (µg/g)
Untreated Patients	31	14/17	6.1±3.8	117.2 (3.2-306)
Treated Patients	33	16/17	10.1±3.8	3.7 (0.5-58.2)
Controls	34	15/18	7.5±2.0	9.6 (1-70)

Table 2. Fecal calprotectin concentration with regard to presentation type, neutrophilic infiltration in the duodenum mucosa and histopathological severity in newly diagnosed celiac patients

	Number	Median (Min-Max)	P
Presentation types			
Gastrointestinal symptoms	18	142.8 (12.25-306.00)	p<0.05
Non-gastrointestinal symptoms	13	79.7 (3.25-243.20)	
Neutrophilic infiltration			
None	3	20.50 (16.50-130.00)	p>0.05
Mild	14	121.75 (3.25-306.00)	
Moderate	12	72.35 (8.70-295.25)	
Severe	2	79.75 (16.50-143.00)	
Histopathologic severity			
Mild (Marsh 1, 2, and 3a)	13	99.72 (16.50-306.00)	p>0.05
Severe (Marsh 3b and 3c)	18	119.00 (3.25-295.25)	

The FCC was significantly decreased from 113.7 µg/g (8.7-295.2) to 4.2 µg/g (0.5-20.7) in nine patients from whom second fecal samples were obtained ($p<0.01$) (Figure 1).

The AUROC for predicting CD was 0.91 ($p=0.0001$, 95% confidence interval [CI]: 0.85-0.97). With a cut-off value of 50.4 µg/g, the sensitivity, specificity, and positive and negative predictive values of FCC were 67.7, 94, 87.5, and 75.6%, respectively.

DISCUSSION

In this study, we found that FCC was higher in children with newly diagnosed CD compared to CD patients under GFD and healthy controls. Moreover, patients presenting with typical gastrointestinal symptoms had higher FCC with respect to patients presenting with non-gastrointestinal symptoms.

In the recent years, FCC has been demonstrated to be useful for the determination of inflammation throughout the gastrointestinal tract. Fecal calprotectin is related to the neutrophils and neutrophil migration in the gut wall and is not a disease-specific marker (11,12). FCC can be used alone, in which it has been shown to be superior to the other tests, or with other tests such as C-reactive protein (CRP) and erythrocyte sedimentation rate in IBDs (13,14).

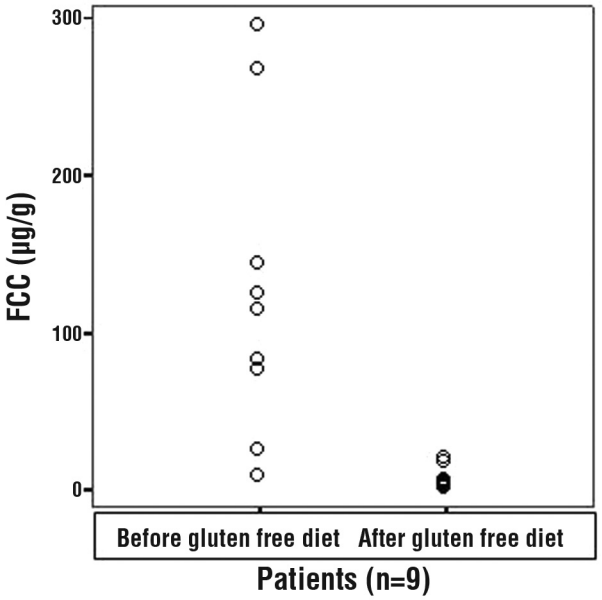

Figure 1. Comparison of the fecal calprotectin concentration at the time of diagnosis and after the gluten free diet in nine patients.

The studies that investigated the role of the FCC in pediatric gastroenterology practice have focused mainly on IBD and reported that FCC was a reliable tool in the diagnosis and follow-up of IBD. FCC was correlated with the severity of the histopathological injury and sufficient to demonstrate subclinical inflammation (15,16). Therefore, it was suggested that the determination of FCC in the follow-up of IBD might decrease the need for invasive methods like colonoscopy and increase the quality of life in patients with IBD.

Another research area for FCC is the differentiation of inflammatory diseases of the gastrointestinal tract from the functional disorders, such as irritable bowel syndrome (17-20). Li et al. (21) reported that calprotectin appears to be the most accurate marker to discriminate between two common causes of chronic diarrhea: IBD and irritable bowel syndrome. When FCC is found to be normal, unnecessary diagnostic colonoscopy could be avoided (22).

Data on FCC in CD are still scarce in both adults and children. Limited numbers of reports are available in the literature. Montalto et al. (5) investigated FCC in adult patients with untreated CD. They did not find any difference between healthy controls and CD patients. They reported that FCC did not correlate with the severity of clinical symptoms, degree of neutrophilic infiltration, or histopathological injury. However, only four of 28 patients showed granulocyte infiltration in their study group, which might explain the results (5). Bremner et al. (23) measured FCC in 100 children with chronic gastrointestinal symptoms and compared them with healthy controls. Their study included only four children with untreated CD. FCC was found to range between 50-200 µg/g in three of them. Canani et al. (6) studied FCC in 281 children with various gastrointestinal diseases, including 38 CD patients, and compared them with the FCC levels of 76 healthy children. They reported increased FCC in all of the pediatric gastrointestinal diseases characterized by mucosal inflammation. In their study, which did not include the features of the patients, FCC values showed a decreasing pattern toward normal values after at least four weeks of exclusion diet in patients with CD and allergic colitis. Nevertheless, they did not specify neutrophilic infiltration. Ertekin et al. (7) reported that FCC was increased in children with CD, related to the severity of histopathological findings, and responsive to GFD. In our study, we did

not find any correlation between FCC and severity of the histopathological injury or degree of neutrophilic infiltration. As CD-related inflammation is patchy, we assume that the degree of duodenal infiltration might not necessarily reflect the extent of whole gut inflammation, which might explain our results. The small number of patients might have influenced our results as well. However, FCC was significantly higher in children presenting with gastrointestinal symptoms. This condition decreases the utility of the test in patients with non-gastrointestinal symptoms. We also demonstrated that FCC decreased after GFD as EMA became negative, which might show the usefulness of FCC in the follow-up of GFD in patients.

In both adult and pediatric CD patients, neutrophilic infiltration was observed in various parts of the intestinal mucosa (24). Dhesi et al. (25) reported that there was a 20-fold increase in neutrophils in untreated CD; neutrophils were restored to normal after GFD. However, it was shown to be due to increased granulocyte turnover in the gut, and those neutrophils were not considered to have any role in the pathogenesis (26). Kristjánsson et al. (27) showed that gluten challenge increased neutrophilic infiltration in the rectal mucosa of CD patients. We found no relation between neutrophilic infiltration in the duodenum and FCC. However, we do not know the neutrophilic activation status, which might be more important than the quantity of these cells.

In conclusion, FCC might be useful in the diagnosis and follow-up of children with newly diagnosed CD. Its utility and cost-effectiveness and the impact on the patient's quality of life must be investigated in further studies.

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