

ORIGINAL ARTICLE

Role of Fecal Calprotectin in Differentiating between Hirschsprung's Disease and Functional Constipation

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Background/Aims: Calprotectin is a 36.5 kD calcium and zinc binding protein in the S100 protein family. Fecal calprotectin levels are elevated in patients with inflammatory bowel disease and some other gastrointestinal disorders such as colorectal carcinoma. We decided to evaluate the fecal calprotectin level to see if it was able to distinguish between functional and organic causes of constipation.

Methods: Seventy-six children aged 1 to 120 months that all underwent deep rectal mucosa biopsies at Children Medical Center from November 2010 till September 2011 were recruited. Nineteen cases were diagnosed as Hirschsprung's disease and 57 of the patients had nerve ganglion cells in their biopsies. Calprotectin concentration was analyzed by the ELISA method.

Results: Although there was a significant difference between the median of the two groups ($p=0.036$), the median was not above the predetermined cutoff value of 50 $\mu\text{g/g}$.

Conclusions: We propose that fecal calprotectin, using the above cutoff value, has limited value in differentiating functional constipation from Hirschsprung's disease. (*Korean J Gastroenterol* 2013;62:288-291)

Key Words: Constipation; Hirschsprung disease; Calprotectin; Feces; Pediatrics

INTRODUCTION

Calprotectin is a 36.5 kD calcium and a zinc binding protein in the S100 protein family. Its molecular structure consists of a heterotrimer made of two heavy chains and one non-glycosylated light chain.¹ This protein is found primarily within cells derived from the myelomonocytic cell lineage, such as neutrophil granulocytes, monocytes and activated macrophages.² The protein constitutes about 60% of the soluble proteins in the cytosol fraction of neutrophils. Its exact biological function is not known. It may be involved in modulating inflammation and tissue destruction, as it has both bactericidal and fungicidal properties.² A faecal calprotectin ELISA has been available since 1994.³ Fecal calprotectin levels are elevated in patients with inflammatory bowel disease (IBD) and may be used to evaluate the degree of inflam-

mation in these patients.⁴ Some investigators have assessed fecal calprotectin in the differentiation of colorectal cancer from benign lesions and have concluded that it is a sensitive non-invasive marker of colorectal cancer and adenomatous polyps; however, it has somewhat lower specificity in comparison to occult blood testing.⁵

Constipation is one of the frequent complaints in children that has different etiologies.

The commonest type of constipation is functional constipation. The other types are called organic constipation including Hirschsprung's disease which is a frequent congenital disorder (1 in 5,000 newborns) that results from lack of coordinated propulsive movement of the distal portion of the large bowel resulting from the absence of parasympathetic ganglion cells in the intramural and submucosal plexuses.⁶ The gold standard for diagnosis is a rectal biopsy

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under general anesthesia,⁶ but it is largely replaced by suction or mucosal biopsies nowadays.⁷

Previous studies have demonstrated that the severity of mucosal inflammation, especially the presence of crypt abscesses, could predict the risk of Hirschsprung's associated enterocolitis.⁸ In another study, 47 pediatric patients with gastrointestinal symptoms were studied and the authors concluded that fecal calprotectin was higher in children with IBD than those with organic non-IBD and healthy children, and also higher in functional pathologies rather than in normal controls.¹ It was also elevated in one child with Hirschsprung's disease.¹ Bremner et al.⁹ studied 100 children referred to the pediatric gastroenterology service for fecal calprotectin. The results showed that calprotectin was higher in IBD than in normal children or in those with functional constipation. Also 3 of 31 (9.7%) of the children with chronic functional constipation had fecal calprotectin levels higher than 50 µg/g.

Fernell et al.¹⁰ studied autistic patients for fecal calprotectin and rectal nitric oxide and found no relation with autism and these markers; however, in one patient with severe constipation, fecal calprotectin was elevated. While we had several pediatric patients who were referred due to severe constipation and underwent rectal biopsies which proved the cause to be ganglionic later, we decided to perform the evaluation using a non invasive method in these cases as this can aid in diagnosis. Based on these studies, we were determined to evaluate the fecal calprotectin level in order to distinguish between the functional and organic causes of constipation. To the best of our knowledge, no systematic study for differentiating these two conditions by fecal calprotectin has been reported yet.

SUBJECTS AND METHODS

1. Subjects

Seventy-six children aged 1 to 120 months who all underwent deep rectal mucosal biopsies at Children Medical Center in Tehran University of Medical Sciences from November 2010 till September 2011 were recruited. The study was approved by Tehran University of Medical Sciences Research Center (8801308388).

Additional studies such as barium enema were performed in some patients and deep rectal mucosal biopsy was the last

resort to differentiate between Hirschsprung's disease, other organic disorders and functional constipation. The rectal biopsy was taken two centimeters above the dentate line in a surgical room under general anesthesia, and an adequate specimen, usually 0.8×0.8 cm in size, was sent to our ward (no suction biopsies were taken in our center). Generally, several nerve plexuses were present in the biopsy and ganglion cell detection was rather easy. If ganglion cells were not found in the specimen, we prepared several cut sections (up to 60) and examined the cuts thoroughly. In nineteen cases, no ganglion cells were present in the deep rectal mucosa. These cases were diagnosed as Hirschsprung's disease and for some of these cases, a colostomy was performed. Fifty-seven of the patients had nerve ganglion cells in the rectal biopsies and were diagnosed as having functional constipation with no further surgical procedures performed, but after our report, they received a Botox shot for the relief of constipation. A fecal sample was collected from all of the patients, and this was stored in freezer set at -70°C.

2. Measurements of calprotectin

Before analysis, the samples were thawed at room temperature. The calprotectin concentration was analyzed by the ELISA method (HK325; Hycult Biotech kit, Uden, Netherlands). Calprotectin concentrations were calculated from a 4th order polynomial regression curve obtained with the standards of a kit (6.25, 12.50, 25.00, 50.00, 100.00 ng/mL). Samples were diluted 1 : 50 with an extraction buffer and 1 : 800 with a dilution buffer, as indicated by the manufacturer. The lower limit of detection was 1.6 ng/mL. Results were expressed in µg/g. Based on previous studies and also on the information in the brochure of the kit, the cut-off value of 50 µg/g was applied as the upper limit of normal.^{1,9,11}

3. Statistical methods

We used the SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) for the statistical analysis of the data. A Kolmogorov-Smirnov test showed that the calprotectin concentration values did not follow a normal distribution. Therefore, the results were expressed in median values and interquartile ranges. Comparisons between the groups were performed using the Mann-Whitney U statistical test. We assumed a statistically significant difference when $p < 0.05$.

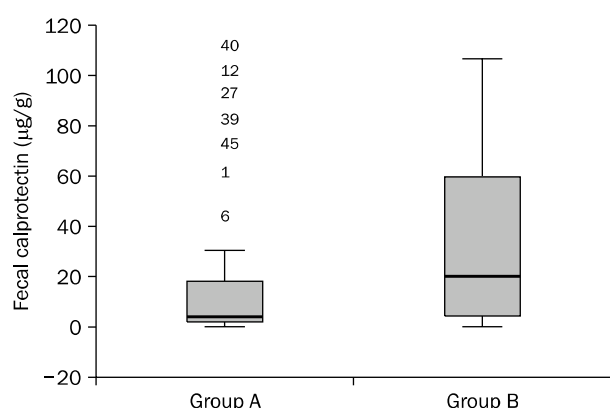


Fig. 1. Box plot of fecal calprotectin concentration in children with functional constipation (group A, n=57) and Hirschsprung's disease (group B, n=19).

RESULTS

Of 76 patients, 46 were male (60.5%) and 30 were female (39.5%). Nineteen patients were diagnosed as having Hirschsprung's disease, with an age range of 1 to 84 months (median, 18 months) and 57 were diagnosed as having functional constipation, with an age range of 3 to 120 months (median, 42 months). No statistically significant difference was found between the two groups with regard to sex and age. None of our patients had clinical symptoms of enterocolitis at the time of the biopsy or fecal specimen collection.

Microscopic examination of the rectal biopsies also showed some degree of mucosal inflammation which did not differ significantly between the two groups. In the Hirschsprung's disease group, the median concentration of fecal calprotectin was 20 µg/g (under 0.5 to 106.0 µg/g) and in the functional group, the median concentration was 4 µg/g (under 0.5 to 110.8 µg/g).

Although the range of fecal calprotectin in the two groups was rather wide and the difference between the two groups was not statistically significant, there was a significant difference between the medians of the two groups ($p=0.036$) (Fig. 1); however, the medians were not above the predetermined cutoff value of 50 µg/g. Also, the difference between the two groups was statistically significant in males (under 0.5 to 100 µg/g, mean 3.6 µg/g in the functional group; and under 5 to 106 µg/g, mean 27.2 µg/g in the Hirschsprung's disease group).

DISCUSSION

Constipation is a common problem in the pediatric age group and while there are no specific tools available to discriminate organic from functional constipation clearly, collaborative studies are necessary to determine the pathophysiology of constipation.¹²

Functional constipation is one of the major complaints in the pediatric age group. These patients undergo rectal deep mucosal biopsy which is an invasive method. Based on previous studies in which fecal calprotectin was elevated in patients with constipation, whether it was functional constipation⁹ or Hirschsprung's disease,¹ we were determined to assess the ability of fecal calprotectin to differentiate patients with Hirschsprung's disease from those with functional constipation, which if successful will lead to lower expenses and less invasive methods in the functional group. First, this was based on the supposition that prolonged constipation may lead to a mucosal inflammatory process and this process maybe more marked in Hirschsprung's disease than in functional constipation, although we could not prove it with microscopic examination of rectal biopsies. In those with functional constipation, no colonic resection was performed, and so we could not determine the difference in the degree of mucosal inflammation between the two groups in the larger specimens.

Although calprotectin is a marker for neutrophils and is mostly used for differentiating IBD from other gastrointestinal disorders, in a study by Meucci et al.,¹³ in 870 patients undergoing colonoscopy, 37% of those with a normal colonoscopy or with trivial findings also had elevated fecal calprotectin levels. Interestingly, in a newly published article about bacterial enteric infections, calprotectin level detected by the ELISA method (the same kit we used) did not differ between those with bacterial infections and the control subjects.¹⁴ So we suggested that other mechanisms may have a role in the elevation of fecal calprotectin. We decided to use this new marker for another purpose; differentiating functional constipation and Hirschsprung's disease.

To the our best of our knowledge, no systematic studies have been done in regard to this subject as yet. In our study, patients with functional constipation were three times the number of patients with Hirschsprung's disease. The median concentration of fecal calprotectin was significantly higher in

patients with Hirschsprung's disease ($p=0.036$); however, the median range was not above $50 \mu\text{g/g}$, which was stated in other studies as the cutoff of normality.

In Bremner et al.'s study,⁹ in cases with functional constipation, the fecal calprotectin level was above $50 \mu\text{g/g}$ in 9.7% in comparison to our study, in which 12.3% had a fecal calprotectin level that was above this level.

One child with Hirschsprung's disease in the Bonnín study had fecal calprotectin between 50 and $200 \mu\text{g/g}$,¹ but in our cases, the highest value was $106 \mu\text{g/g}$. This difference may be explained by ethnic differences, analytical procedures or differences in the assays.

None of our patients with Hirschsprung's disease developed enterocolitis and so we could not assess the role of calprotectin as a prognostic marker, as stated by Elhalaby et al.⁸

We propose that fecal calprotectin may be useful in differentiating the functional form from organic causes of constipation if the cutoff value is considered lower than the previously stated one. In further studies with a larger number of cases, we can establish a new cutoff value with high sensitivity and specificity in differentiating these two conditions.

REFERENCES

1. Bonnín Tomàs A, Vila Vidal M, Rosell Camps A. Fecal calprotectin as a biomarker to distinguish between organic and functional gastrointestinal disease. *Rev Esp Enferm Dig* 2007;99:689-693.
2. Olafsdottir E, Aksnes L, Fluge G, Berstad A. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. *Acta Paediatr* 2002;91:45-50.
3. Johne B, Fagerhol MK, Lyberg T, et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. *Mol Pathol* 1997;50:113-123.
4. Røseth AG, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997;58:176-180.
5. Tibble J, Sigthorsson G, Foster R, Sherwood R, Fagerhol M, Bjarnason I. Faecal calprotectin and faecal occult blood tests in the diagnosis of colorectal carcinoma and adenoma. *Gut* 2001;49:402-408.
6. Swenson O. Hirschsprung's disease: a review. *Pediatrics* 2002;109:914-918.
7. Hirose R, Hirata Y, Yamada T, Kawana T, Taguchi T, Suita S. The simple technique of rectal mucosal biopsy for the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 1993;28:942-944.
8. Elhalaby EA, Teitelbaum DH, Coran AG, Heidelberger KP. Enterocolitis associated with Hirschsprung's disease: a clinical histopathological correlative study. *J Pediatr Surg* 1995;30:1023-1026.
9. Bremner A, Roked S, Robinson R, Phillips I, Beattie M. Faecal calprotectin in children with chronic gastrointestinal symptoms. *Acta Paediatr* 2005;94:1855-1858.
10. Fernell E, Fagerberg UL, Hellström PM. No evidence for a clear link between active intestinal inflammation and autism based on analyses of faecal calprotectin and rectal nitric oxide. *Acta Paediatr* 2007;96:1076-1079.
11. Fagerberg UL, Lööf L, Merzoug RD, Hansson LO, Finkel Y. Fecal calprotectin levels in healthy children studied with an improved assay. *J Pediatr Gastroenterol Nutr* 2003;37:468-472.
12. Myung SJ, Lee TH, Huh KC, Choi SC, Sohn CI; Korean Society of Neurogastroenterology and Motility. Diagnosis of constipation: a systematic review. *Korean J Gastroenterol* 2010;55:316-324.
13. Meucci G, D'Incà R, Maieron R, et al. Diagnostic value of faecal calprotectin in unselected outpatients referred for colonoscopy: a multicenter prospective study. *Dig Liver Dis* 2010;42:191-195.
14. Bennett WE Jr, González-Rivera R, Puente BN, et al. Proinflammatory fecal mRNA and childhood bacterial enteric infections. *Gut Microbes* 2010;1:209-212.