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Fecal Calprotectin in Children Can Differentiate between Different Gastrointestinal Diseases

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1. Abstract

1.1. Background: Fecal calprotectin (FC) is increased in gastrointestinal inflammation due to increased permeability of the gastrointestinal mucosa. It could be used to differentiate between different gastrointestinal disorders.

1.2. Purpose: The aim of the present study was to examine the relationship between fecal calprotectin and different pediatric gastrointestinal diseases and to investigate if it could be helpful in differentiation between different GI diseases.

1.3. Methods: 180 patients in addition to 30 normal children as control, divided into groups according to the final diagnosis, 30 patients with Inflammatory bowel disease (IBD), 20 patients with eosinophilic colitis, 30 patients with helicobacter pylori infection, 40 patients with Functional constipation, 30 patients cow milk allergy, 30 patients with Celiac disease. All children were subjected to investigations that confirm their diagnosis in addition to measure fecal calprotectin according to the manufacturer's instructions.

1.4. Results: Fecal calprotectin in patients with inflammatory bowel disease had mean FC 4640 ± 850 µg/g, In Helicobacter pylori infection was 78.9 ± 25.1 µg/g. Celiac disease patients had mean FC 456 ± 123 µg/g. Eosinophilic esophagitis had mean FC4.2 ± 2.9 µg/g. Functional constipation patients had mean FC 23.6 ± 21.8 µg/g. while normal control children had mean fecal calprotectin 4.1± 6.9 µg/g. **Conclusion:** Fecal Calprotectin could be helpful in differentiating different pediatric gastrointestinal diseases.

2. Introduction

Calprotectin is a calcium and zinc binding protein, it constitute > 60% of cytosolic proteins in the neutrophils also found in smaller parts in the cytosol fluid of monocytes, and macrophages. It has antimicrobial effects against bacteria and fungi [1]. Calprotectin was first isolated in the 1970s [2]. Reports of calprotectin started in 1990s and early 2000s which focused mainly in its role to monitor inflammatory bowel disease (IBD). It can be measured in different body fluids as urine, plasma, saliva, faeces, synovial fluid and liquor [3] It contains one light chain that binds calcium and zinc and two heavy chains. Gastrointestinal inflammation is associated with release of calprotectin in large amount due to increased permeability of the gastrointestinal mucosa [4]. Some studies suggest that calprotectin can differentiate between functional and organic gastrointestinal disorders [5].

Meta-analyses of multiple studies concluded the accuracy of Calprotectin in patients with active endoscopic inflammatory bowel diseases with high sensitivity (70% to 100%) and specificity (44% to 100%) [6].

Fecal calprotectin testing can support diagnoses of relapsing inflammatory bowel disease in children. It is elevated in inflammation of gastrointestinal tract as cystic fibrosis and is the only biomarker elevated in cystic fibrosis compared to other inflammatory stool markers, as it predicts colorectal and intestinal inflammation in children [7]. Fecal calprotectin is also elevated in in adults with colorectal cancer or adenomatous polyp [8] in children with juvenile polyps, active

celiac disease, eosinophilic and lymphocytic colitis, multiple food allergy and cow milk allergy [9].

3. Aim of The Study

The aim of the present study was to examine the relationship between fecal calprotectin and different pediatric gastrointestinal diseases as IBD, eosinophilic gastroenteritis, cow milk allergy presented with bloody diarrhea, helicobacter pylori infection, functional constipation, Celiac disease, and to investigate if it could be helpful in differentiation between different GI diseases.

4. Methods

Children with significant gastrointestinal diseases, with total number of 180 patients in addition to 30 normal children as control, included in this study were divided into groups according to the final diagnosis, 30 patients with IBD, 20 patients with eosinophilic colitis, 30 patients with helicobacter pylori infection, 40 patients with functional constipation, 30 patients cow milk allergy and 30 patients with Celiac disease.

All patients were subjected to thorough clinical history with thorough clinical examination which was done with special interest on weight, height, and nutritional status and proper abdominal examination. Full blood count, Liver function tests, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), Stool routine, culture and sensitivity, occult blood in stool, stool PH and reducing substances if needed, total Ig E, tissue transglutaminase IgA (tTg-IgA), endomysial antibodies (EMA), helicobacter pylori stool Ag, upper GI and lower GI endoscopies done when needed. Fecal calprotectin expressed as µg/g of feces. The stool samples were prepared and analyzed according to the manufacturer's instructions.

5. Fecal Calprotectin Measurement

The stool samples were prepared and analyzed according to the manufacturer's instructions (Calprest; Eurospital SpA, Trieste, Italy). A portion of each sample (40-120 mg) was measured and an extraction buffer containing citrate and urea was added in a weight per volume ratio of 1:50. The samples were mixed for 30 s by a vortex method and homogenized for 25 min. One milliliter of the homogenate was transferred to a tube and centrifuged for 20 min. Finally, the supernatant was collected and frozen at -20℃. In most cases, time from sampling to preparation and freezing was estimated to be 1-3 d, except for a few samples that took 4-6 d before handling. The supernatants were thawed and analyzed later with Calprest, a quantitative calprotectin ELISA, for determination of calprotectin in stools. The within assay coefficient of variation was 1.5%. Calprotectin was expressed as µg/g of feces.

6. Statistical Analysis

Statistical analyses were performed using SPSS (SPSS, Inc., Chicago, Illinois), Epi lnfo (CDC, Atlanta, Georgia), and Log Xact (Cytel Software Corporation, Cambridge, Massachusetts).

7. Ethical Points

The study followed the ethical standards of national liver institute-Menofiya university- Egypt, committee and international Review Board (IRB) of National Liver Institute. The study followed the ethical standards of National Liver Institute – Menofiya University – Egypt, committee (IRB00003413).

8. Results

In cow milk protein allergic patients with marked GI presentation in the form bloody diarrhea and / or abdominal distension, the mean fecal calprotectin (FC) was 1260 ± 625 µg/g. (Table 2) FC was decreased after 2-4 weeks elimination of cow milk products to 420 ± 190 µg/g. Patient with inflammatory bowel disease had mean FC 4640 ± 850 µg/g (Table2), decreased after medical treatment and resolution of symptoms to 1360 ± 520 µg/g. In Helicobacter pylori infection detected by upper GI endoscopy and histopathology with positive stool antigen the mean FC was 78.9 ± 25.1 µg/g. Celiac disease patients had mean fecal calprotectin 456 ± 123 µg/g. Eosinophilic esophagitis had mean fecal calprotectin 4.2 ± 2.9 µg/g. Functional constipation patients had mean fecal calprotectin 23.6 ± 21.8 µg/g. Normal control children had mean fecal calprotectin 4.1± 6.9 µg/g. (Table2).

Table 1: Number and percent of the studied patients.

Diagnosis (total cases 180)	No.	%
IBD	30	16.66
Cow milk allergy (GI symptoms)	30	16.66
Helicobacter pylori infection	30	16.66
Eosinophilic esophagitis	20	11.11
Functional constipation	40	22.22
Celiac disease	30	16.66

Table 2: Fecal calprotectin of the studied patients.

Diagnosis	Fecal Calprotectin µg/g (*n* = 180) Mean ± SD
IBD	4640 ± 850
Cow milk allergy (GI presentation)	1260 ± 625
Helicobacter pylori infection	78.9 ± 25.1
Eosinophilic esophagitis	4.2 ± 2.9
Functional constipation	23.6 ± 21.8
Celiac disease	456 ± 123

9. Discussion

There are different laboratory markers used to assess systemic inflammation, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). ESR and CRP cannot localize itself in to the gut. Calprotectin is the protein content of the cytosol in neutrophils. Intestinal inflammation lead to polymorphonuclear neutrophils migration to the intestinal mucosa. Inflammation cause disturbance of the mucosal architecture causing leakage of neutrophils, hence, calprotectin into the lumen and its subsequent excretion in feces [10].

In addition to detection of calprotectin in stool, Calprotectin has been detected in different body fluids. Reference ranges in serum/ plasma 0.12 to 0.66 mg/L, CSF calprotectin concentrations were 0.3 to 0.35 mg/L, Saliva: 22.0 mg/L. Calprotectin concentrations in urine 0.024 mg/L. In Meconium concentration of Calprotectin 78.5 to 145µg/g. Fecal calprotectin (FC) ≤50.0 mcg/g is considered normal [11,12]. Increased FC can be related to inflammation, but not disease specific, so we in this study we investigated level of FC in different pediatric GI diseases. It was well known that FC is a useful biomarker to accurately assess the degree of inflammation. In this study mean FC in children was (17 patients with Crohn's disease and 13 patients with ulcerative colitis with 4640 ± 850 µg/g (Table 3). Studies showed that FC values >600µg/g are strongly associated with IBD, although no consistent CP cut-off is established that would allow to diagnose IBD with high accuracy [13]. Our study showed mean FC level in patients with GI presentations of cow milk allergy including abdominal distension and bloody diarrhea was 1260 ± 625 µg/g (Table 3), it was statistically significant when compared to normal control group P value 0.001(Table 3), other studies [14] found the mean FC value before the CMP elimination diet was 886±278 µg/g in the non-IgE-mediated group, the elevated level of FC in our results compared to their results may be explained by the fact that our cases were selected with more severe GI presentations. Patients positive for H. Pylori by histopathology, rapid test during endoscopy (CLO test) and positive stool Ag for H. pylori, had mean FC 78.9 ± 25.1 µg/g (Table 3), nearly same findings reported by [15] who reported mean FC 74.8 ± 67µg/g. Explained mild elevation of FC in H. pylori infected patients because of Fecal calprotectin represents gastric neutrophilic inflammation. Current study showed children diagnosed as Celiac disease had mean FC 456 ± 123 µg/g, there was statistically significant difference between celiac and normal control children ($P < 0.1$) (table 7), this completely was in difference with other studies who found no significant difference, this difference in results may be due to age difference in both studies [16]. While other studies came in harmony with our study by [17] who in which it was reported Increased fecal calprotectin concentration may be considered non-invasive marker which can help to diagnose celiac disease. Eosinophilic esophagitis (EoE) (Table 3) patients enrolled in current study showed mean fecal calprotectin 4.2 ± 2.9µg/g, which did not show statistically significance with FC of normal control children, the other studies who found increased FC, this because they studied eosinophilic gastrointestinal diseases as enteritis and colitis, whereas our study enrolled only eosinophilic esophagitis (EoE) [18].

Functional constipation is common problem in children, this group of children enrolled in our study had mean FC 23.6 ± 21.8 µg/g, (Table 3) it was statistically insignificant with FC level of normal children, other studies reported nearly the same with mean fecal calprotectin in children with functional constipation 0.5 to 100 µg/g.

Fecal Calprotectin in this study could be helpful in differentiating different pediatric GI diseases, although there is no cut off level, but it could be start for future researches.

Table 3: Calprotectin level in diseased groups and normal control group.

Diseased groups (Number of patients)	Calprotectin level in diseased groups mean ± SD	Calprotectin level in Control group *n* = 30 mean ± SD	*P*-value
IBD *n* = 30	4640 ± 850 µg/g	4.1± 6.9 µg/g	0.0001
Cow milk allergy (GI presentation) *n* = 30	1260 ± 625 µg/g		0.001
Helicobacter pylori infection *n* = 30	78.9 ± 25.1 µg/g		0.01
Eosinophilic esophagitis *n* = 20	4.2 ± 2.9µg/g		> 0.5
Functional constipation *n* = 40	23.6 ± 21.8 µg/g		> 0.5
Celiac disease *n* = 30	456 ± 123 µg/g		< 0.1

10. Conclusion

Fecal CP could be able to differentiate between different pediatric GI diseases, as it is noninvasively, inexpensive and remains stable at room temperature in stool for at least 3 days. Calprotectin testing can reduce the need for endoscopy.

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