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Relationship Between Fecal Calprotectin and Upper Endoscopy Findings in Children With Upper Gastrointestinal Symptoms

Pedram Ataee,[1] Vahidreza Afrasiabi,[2] Bahram Nikkhoo,[3] Mehri Najafi Sani,[4,5] Ramesh Rahehagh,[3] Ebrahim Ghaderi,[6] Maryam Monajemzadeh,[5,7] Asadollah Fathollahpour,[2] Banafsheh Sedaghat,[8] Froozan Kariminejhad,[9] Jaleh Parizad,[5] and Kambiz Eftekhari[10,*]

[1]Liver and Digestive Research Center, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[2]Department of Pediatrics, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[3]Department of Pathology, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[4]Department of Pediatrics, Tehran University of Medical Sciences, Tehran, IR Iran
[5]Children's Medical Center, Pediatrics Center of Excellence, Tehran, IR Iran
[6]Social Determinants of Health Center, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[7]Department of Pathology, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, IR Iran
[8]Department of Pediatrics, Shariati Hospital, Social Security Organization, Isfahan, IR Iran
[9]Department of Endoscopy, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
[10]Department of Pediatrics, Bahrami Children's Hospital, Tehran University of Medical Sciences, Tehran, IR Iran

[*] *Corresponding author*: Dr. Kambiz Eftekhari, Pediatrics Department Number 2, Bahrami Children's Hospital, Tehran, IR Iran. Tel: +98-2173013210, E-mail: k-eftekhari@sina.tums.ac.ir

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Abstract

Background: Chronic abdominal pain in children is a common disorder. For an accurate diagnosis of its cause, sometimes invasive diagnostic procedures such as endoscopy should be performed.
Objectives: The purpose of the study was to evaluate the fecal calprotectin in children with upper gastrointestinal signs and symptoms and to compare it with endoscopic findings.
Methods: A total of 131 children aged 1-14 years with upper gastrointestinal symptoms were enrolled during 2012 - 2013 at two centers. One hundred and twenty patients underwent endoscopy and biopsy. Before endoscopy, the level of calprotectin was measured by the enzyme-linked immunosorbent assay test in stool samples and the results were compared with the endoscopic and pathology findings.
Results: Of the 120 children included in this study, 71 (59.2%) were males and 49 (40.8%) females with a mean age of 93.6 months. Of the 112 patients in whom biopsies were taken, 16 had esophagitis, 89 chronic gastritis (79.5%) and 57 colonization with *Helicobacter pylori*.
Conclusions: There was a statistically significant correlation between fecal calprotectin and gastritis and severity of *H. pylori* infection. Fecal calprotectin level measurement can avoid unnecessary endoscopies and is also useful for evaluation of therapy response.

Keywords: Endoscopy, Feces, Calprotectin, Gastrointestinal Disease, Children

1. Background

Chronic abdominal pain or recurrent abdominal pain in children is common. Over 10% of people have experienced the abdominal pain in their childhood. The age of onset is between 7 to 12 years and in most cases associated with a nonorganic disease. Differentiation between organic and nonorganic (functional) abdominal pain is important because the treatment differs (1). The most common disorder that should be considered is a functional gastrointestinal disorder (FGID). The disease is usually diagnosed by the Rome III criteria. Organic diseases should be ruled out with paraclinical evaluations (2). Organic abdominal pain is associated with the following warning signs: fever, weight loss, bilious or bloody stained vomiting, jaundice, hepatosplenomegaly, back pain or pain farther from umbilicus, waking up with pain, pain referred to the shoulder, groin or back, increased erythrocyte sedimentation rate, white blood cells or C-reactive protein, anemia, edema, and a positive family history of inflammatory bowel disease (IBD) or celiac disease (2). If the alarm signs are positive, an organic disease should be considered and endoscopy performed.

Calprotectin is a protein released from endothelial cells after inflammation and activation of neutrophils or binding the monocytes to endothelial cells. Its plasma level is an important marker of inflammation. This protein is resistant against bacterial enzymes and intestinal protease and can be used as a screening marker for gastroin-

testinal inflammation (3). In the gastrointestinal tract, mucosal permeability is increased by any inflammation, leading to the release of large amounts of calprotectin in the stool. There is a good correlation between levels of fecal calprotectin and severity of the inflammation or infection (3). Endoscopy with biopsy is the gold standard diagnostic tool for the assessment of chronic gastrointestinal disorders, but it is an invasive procedure and does not show the entire digestive tract. Thus, a simple marker, with high sensitivity and specificity, inexpensive and noninvasive such as calprotectin is essential for diagnosis and monitoring any inflammation of the digestive system (4, 5). Some studies suggest that the level of fecal calprotectin is a good diagnostic marker to distinguish between organic and functional disorders (6). There are few papers dealing with the diagnostic role of fecal calprotectin in childhood GI diseases (7). Endoscopy and biopsy from gastric antrum is the standard method to diagnose *Helicobacter pylori* infection (1). The serum IgG antibody against *H. pylori* is not helpful in diagnosis of the infection and it only has epidemiological value (1). The urease breath test (UBT) is a valuable noninvasive method for follow-up of patients after eradication of *H. pylori*, but this method requires patient cooperation (1), which is not achievable in younger children.

In recent decades, calprotectin has been considered as a marker for diagnosis, treatment and monitoring of inflammatory bowel disease (IBD) and GI malignancies as well. The level of fecal calprotectin increases in these conditions and its amount varies depending on the type and severity of the disease (8, 9). Various studies in IBD have shown an increase in stool calprotectin (10). The measurement of fecal calprotectin is a completely noninvasive method; it could be used as an alternative diagnostic method.

2. Objectives

The aim of the present study was to examine the relationship between fecal calprotectin and other disorders of the upper GI tract such as esophagitis, gastritis, *H. pylori* infection, duodenitis and celiac disease, in which endoscopy is necessary for definitive diagnosis. Finally, the fecal calprotectin was compared with endoscopic findings.

3. Methods

This cross sectional study was conducted on children (age range, 1 - 14 years) with upper GI diseases referred to Be'sat hospital of Sanandaj city and children's medical center of Tehran during 2012 - 2013. We used a convenience sampling until the sample size of 120 was completed. This figure was calculated according to the type I error of 5%, and power of 90%, and the potential difference of the calprotectin level between the two groups was 150 ($\pm$ 200); the two groups, one with disorders and other group equal to 1 - 2, were compared based on endoscopic findings.

The method of sample collection: children aged 1 to 14 years with the upper GI disease and indications for endoscopy were enrolled in the study. These patients were referred to the Be'sat Hospital of Sanandaj city and children's medical center of Tehran City, Iran, from January 2012 to November 2013. Initial demographic information was recorded in a questionnaire, which included detailed history, physical examination, past medical and family history. The children with lower GI symptoms including diarrhea, tenesmus, or any symptom of infection were excluded from the study. One stool sample was obtained before the endoscopy and maintained at -20°C for analysis. All samples were analyzed using the ELISA kit (Buhlmann Company of Switzerland) for calprotectin by one technician in a laboratory center in Sanandaj. The study was conducted in a double-blind fashion. Functional GI disorders were ruled out according to the Rome III criteria. Then, endoscopy was performed by a pediatric gastroenterologist with a pediatric endoscope (the Pentax model, Japan) in Sanandaj and Tehran. The endoscopic findings were classified as follows: esophageal mucosal erythema, gastric mucosal erythema, esophageal ulcer, stomach ulcer, duodenal ulcer, nodularity in the stomach, nodularity in duodenum, duodenal mucosal atrophy. Samples were taken from the esophagus, stomach and duodenum and sent to the department of pathology of the same center. Data were analyzed by the SPSS software. The mean and median of quantitative data were calculated and compared between the two groups. The chi-square test was used for normally distributed data and the Mann-Whitney test for others to compare the calprotectin levels between the two groups.

3.1. Ethical Considerations

The study protocol was approved by the ethics committee of Kurdistan University of Medical Sciences.

4. Results

From a total of 120 children enrolled in the study, 71 (59.2%) were males and 49 (40.8%) females with the mean age of 93.6 months (standard deviation 36.8). the results of endoscopy showed that gastric mucosa was normal in 22 cases and abnormal in 98 cases. Almost all children (97.5%) had abdominal pain. The findings of the upper GI endoscopy revealed abnormalities in the esophagus, stomach and the second section of the duodenum in 83.3%, 81.7%

and 11.7% of the subjects, respectively. A total of 112 (93.3%) children underwent biopsy of the GI tract. Esophagitis was found in 16 patients (14.3%) and gastritis in 89 (79.5%). Fifty-eight percent of the patients with gastritis had active gastritis and 21.5% of them had inactive gastritis. The colonization of *H. pylori* was observed in 57 patients (50.9%).

There was no significant association between sex, urban and rural areas, erythema of the distal esophagus, hiatal hernia, esophagitis and displacement of Z line with calprotectin (P = 0.438, P = 0.591 and P < 0.05, respectively) (Table 1). Calprotectin levels were significantly higher in the group with abnormal stomach and nodularity of antrum than in the group with normal stomach and without nodularity (P < 0.001, and P = 0.009, respectively) (Table 2). There was no significant correlation between duodenal mucosal atrophy and the average levels of calprotectin (P = 0.111), but this level was higher in patients with erythema and duodenal nodularity than in the others (Table 3). In the study, there was no case of the celiac disease.

Calprotectin levels were significantly higher in the group with a positive serologic test of *H. pylori* (IgG) than in the group with a negative serologic test (P = 0.003). There was a significant correlation between the mean levels of fecal calprotectin, gastritis (degree and intensity of inflammation) and colonization of *H. pylori* (P = 0.004, P < 0.001 and P < 0.001, respectively). The levels of fecal calprotectin in patients with colonization of *H. pylori* were higher than in those without colonization.

5. Discussion

There are few studies evaluating the relationship between upper GI disorders and fecal Calprotectin. Many researchers have investigated the association between inflammatory bowel disease (IBD) with the marker, the severity of intestinal involvement and response to treatment. The results of the present study showed that there was no correlation between the calprotectin level and abnormalities of the esophagus. The calprotectin level was not increased by inflammation, erosions of the esophagus, displacement of Z line, hiatus hernia and relaxation of the lower sphincter of the esophagus. There are no similar studies that can be compared with our current findings. With confirmation of these findings by further research, it can be concluded that calprotectin is not a suitable marker for the evaluation of esophageal pathology. We also found that inflammation of the stomach or gastritis (based on pathologic findings) and nodularity of antrum can increase the level of fecal calprotectin. However, erythema of the stomach in the macroscopic view was not associated with a marked increase in fecal calprotectin. There was a significant relationship between the fecal calprotectin levels and grade and severity of gastritis. The results showed that infection with *H. pylori* leads to increase in the level of fecal calprotectin. The severity of *H. pylori* colonization has a direct correlation with levels of fecal calprotectin. Also, the level of fecal calprotectin was significantly higher in the group with a positive serologic test for *H. pylori*. Pathological changes in the duodenal bulb, such as erythema, ulceration and nodularity do not increase the level of fecal calprotectin. In our study, there was a significant difference in the concentration of fecal calprotectin between chronic active gastritis and chronic nonactive gastritis (P = 0.004). It can also be seen a significant difference in chronic gastritis with different severity (mild, moderate, severe) (P < 0.001). In the Manz et al. study (2012), it was seen that the level of stool calprotectin was lower in normal patients than in the ones with erosive gastritis (P < 0.001). The number of patients in this study was 147 (11). Another study conducted by Montalto et al. measured the calprotectin level in 61 patients with gastritis (based on histopathological findings) and then compared it with 74 healthy children (12). Finally, no significant difference was found between the groups. The number of patients in our study and the two other studies (Manz et al. and Montalto et al.) were not equal and this issue influenced the results of the investigation. *H. pylori* infection led to significantly increased levels of calprotectin in stool (P < 0.001). However, Montalto et al. did not find a significant correlation between them. This could be due to the larger number of patients with *H. pylori* infection in our study than in his (57 and 24 patients, respectively). The results of our study showed a significant association between *H. pylori* infection and the concentration of calprotectin in stool (P < 0.001).

In the future and by more detailed studies, fecal calprotectin can be used to follow up patients with *H. pylori* infection. We found a significant correlation between nodularity of antrum (based on endoscopic finding) and level of fecal calprotectin (P = 0.009), that other studies had not detected. Also, the results of the current study showed that there was no association between the peptic ulcer disease (PUD) and elevated levels of fecal calprotectin (P = 0.111). These differences can be secondary to the small number (9 cases) of patients with PUD. We also found no association between gastric erythema seen at the endoscopy and fecal calprotectin (P = 0.564), an issue that was not examined in previous articles. It can be concluded that gastric erythema is operator-dependent and the mucosal inflammation should be evaluated only based on pathological findings. We discovered a significant relationship between serological tests (IgG) of *H. pylori* and the level of fecal calprotectin (P = 0.003). Measurement of the fecal cal-

Table 1. Comparison of Fecal Calprotection in Both Sexes and in Different Situations of the Esophagus

Group	Number	Mean ± SD	Statistic t (Degrees of Freedom)	P Value
Male	71	138.84 ± 145.19	0.779 (118)	0.438
Female	49	118.28 ± 137.70		
Normal esophagus	20	136.38 ± 155.57	0.204 (118)	0.839
Abnormal esophagus	100	129.26 ± 139.89		
With erythema in distal esophagus	54	110.56 ± 94.36	1.470 (118)	0.144
Without erythema in distal Esophagus	66	146.72 ± 170.40		
With displacement of Z line	43	145.04 ± 144.03	-0.841 (118)	0.402
Without displacement of Z line	77	122.30 ± 141.07		
With hiatal hernia	64	130.33 ± 143.38	0.010 (118)	0.992
Without hiatal hernia	56	130.59 ± 141.60		
With LES relaxation	51	146.80 ± 148.90	-1.086 (118)	0.280
Without LES relaxation	69	118.36 ± 136.43		

Abbreviations: LES, lower esophageal sphincter; SD, standard deviation.

Table 2. Comparison of Fecal Calprotectin in Different Situations of the Stomach

Group	Number	Mean ± SD	Statistic t (Degrees of Freedom)	P Value
Abnormal stomach	98	144.25 ± 152.85	-4.32 (117)	< 0.001
Normal stomach	22	68.97 ± 37.71		
Stomach with generalized erythema	38	141.49 ± 155.14	-0.578 (118)	0.564
Stomach without generalized erythema	82	125.33 ± 136.11		
Stomach with patchy erythema	49	128.92 ± 130.85	0.098 (118)	0.922
Stomach without patchy erythema	71	131.50 ± 150.05		
With nodularity of the antrum	55	169.20 ± 181.42	-2.68 (74.01)	0.009
Without nodularity of the antrum	65	97.66 ± 85.53		

Abbreviation: SD, standard deviation.

Table 3. Comparison of Fecal Calprotectin in Different Situations of Duodenum

Group	Number	Mean ± SD	Statistic t (Degrees of Freedom)	P Value
Abnormal bulb	40	173.24 ± 184.05	-2.03 (53.50)	0.047
Normal bulb	80	109.06 ± 110.64		
Bulb with nodularity	19	207.11 ± 205.27	-1.872 (20.487)	0.076
Bulb without nodularity	101	116.03 ± 122.76		
Bulb with ulcer	19	153.21 ± 173.66	(118) -0.760	0.449
Bulb without ulcer	101	126.17 ± 135.78		

Abbreviation: SD, standard deviation.

protectin in the future can be useful to follow up patients with *H. pylori* after its eradication and to identify patients with gastritis as well. One of our limitations was that endoscopies were performed at two centers so that commentaries may not match. We tried at the beginning to make these endoscopists consonant. The second limitation was

the possibility of bias in the selection of the subjects, as many patients refused endoscopy.

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