

Does faecal calprotectin differentiate between inflammatory bowel disease colitis and non-inflammatory bowel disease colitides?

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Abstract

Introduction: Chronic colitis is a major problem worldwide with high morbidity. Causes of chronic colitis are heterogeneous. A cut-off level of faecal calprotectin to predict inflammatory bowel disease (IBD) as a cause of chronic colitis is lacking.

Aim: To study the level of faecal calprotectin in different causes of colitis and to measure the cut-off level to differentiate between IBD and non-IBD colitides.

Material and methods: This prospective study was conducted from June 2018 to May 2019. The study included all patients aged 2 months up to 18 years who were confirmed to have chronic colitis endoscopically and histopathologically attending the Gastroenterology Clinic at Alexandria University Children's Hospital. Faecal calprotectin level was measured.

Results: We included 110 patients. Allergic colitis was the commonest cause followed by IBD followed by infectious colitis (50.9%, 38.1% and 6.3% respectively). Faecal calprotectin above 744 µg/g could predict IBD as a cause of chronic colitis with 86.8% specificity and 66.7% sensitivity. Significant elevation of faecal calprotectin was detected in IBD patients. Faecal calprotectin was significantly correlated with C-reactive protein level and erythrocyte sedimentation rate.

Conclusions: Faecal calprotectin could predict the cause of colitis and could aid the paediatrician for early referral of patients with chronic colitis.

Introduction

Colitis refers to inflammation of the mucosal lining of the colon. It is usually associated with mucoid or bloody stool, abdominal pain, tenesmus, fever and urgency. Chronic colitis in children comprises a heterogeneous group of disorders including chronic infections, allergic colitis, inflammatory bowel disease (IBD), colitis secondary to immune deficiency disorders or other rare causes such as Behcet's disease, ischaemic colitis, or microscopic colitis [1].

Faecal calprotectin is a 36.5 kDa calcium and zinc binding protein which is mainly exhibited in the cytoplasm of the neutrophils [2]. It is excreted in the intestinal lumen in inflammatory conditions of the gut and can be used to distinguish inflammatory bowel conditions such as colitis from non-inflammatory causes. It indicates gastrointestinal inflammation. Faecal calpro-

tecin is usually high in different types of colitis, but the degree of elevation is often related to the extent and severity of inflammation rather than the cause itself. It is a non-invasive initial test which may provide a clue to the anticipated type of colitis, guide the subsequent diagnostic steps and aid the paediatricians for early referral to gastroenterologists for patients presenting with chronic colitis [3, 4]. Moreover, the level of faecal calprotectin is a marker of mucosal healing and may reduce risk for endoscopic reassessment [5, 6].

Aim

The aim of this study was to assess the level of faecal calprotectin in different causes of chronic colitis, to measure the cut-off level to differentiate between IBD and non-IBD colitides and to study correlations of faecal calprotectin with different laboratory markers.

Material and methods

This work was conducted as a prospective study for 1 year, from June 2018 to the end of May 2019. The study included all patients aged 2 months up to 18 years who presented with symptoms suggestive of chronic colitis (mucooid or bloody diarrhoea for 14 days or more) attending the Gastroenterology Clinic at Alexandria University Children's Hospital. Infants with necrotizing enterocolitis or Hirschsprung-related enterocolitis were excluded. Formal informed written consent was obtained from the parents or the caregivers of all children before inclusion in the study. The study was conducted in accordance with the ethical standards of the institutional review board and with the Declaration of Helsinki (ZU-IRB#: 00012098). The study protocol was approved by the ethical committee of the Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Demographic data comprising age, gender and demographic area were recorded. The following laboratory investigations were conducted among all children: CBC, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum albumin, routine stool analysis including examination for ova, parasites or worms and faecal calprotectin. A stool specimen was obtained prior to administration of laxatives used to prepare patients for colonoscopy. It was collected by each subject using a disposable plastic bucket-type device to avoid contact with toilet water and simplify laboratory sampling. In children with diapers, stools were collected directly from the bottom into a test tube to avoid water absorption by the diapers, which may increase faecal calprotectin level [7]. Samples were stored at -20°C and thawed at room temperature before testing. The faecal calprotectin levels were analysed by a commercially available ELISA assay (Calprest, Eurospital Spa, Trieste, Italy) with the manufacturer recommended cut-off levels [8].

Results of faecal calprotectin were compared to the age-specific cut-off reference levels, with a higher cut-off level for children during the first year of life ($< 350 \mu\text{g/g}$) than for children aged from 1–4 years ($< 275 \mu\text{g/g}$) or older children ($< 50 \mu\text{g/g}$) [9–11]. However, a level of 200–400 $\mu\text{g/g}$ was considered mildly elevated for children below 4 years and a level above 400 $\mu\text{g/g}$ was considered significantly elevated while above 4 years a level of 50–200 $\mu\text{g/g}$ was considered mildly elevated while a level above 200 $\mu\text{g/g}$ was considered significantly elevated [8].

The colonoscopic examination was performed for all children included in the study. Gross macroscopic findings and extent of the inflammation were recorded. All the biopsy specimens were fixed in neutral buffered formalin and they were processed and stained with haematoxylin and eosin. Histopathological findings

were reported. Final diagnosis was reached based on endoscopic and histopathologic findings.

Statistical analysis

Data were fed to the computer using IBM SPSS software package version 20.0. Quantitative data were expressed as the mean \pm SD and/or as median (range). Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using the χ^2 test. When more than 20% of the cells had an expected count less than 5, correction for χ^2 was conducted using Monte Carlo correction. Quantitative data were described using median, minimum and maximum for non-normally distributed data. For non-normally distributed data, the Kruskal-Wallis test was used to compare between different groups and the Mann-Whitney test was used to compare between two groups. Spearman's coefficient was used to correlate between quantitative variables. The cut-off level of faecal calprotectin was determined using a receiver operating characteristic (ROC) curve to differentiate IBD from non-IBD colitides; a level of significance of 0.05 was used, equal/below which the results considered to be statistically significant [12].

Results

The study included 110 patients. Fifty-eight (52.7%) patients of the whole group were male. Seventy-two (65.5%) patients were from rural areas. The most common causes for colitis in our cohort were allergic colitis (50.9%), followed by IBD (38.1%) and infectious colitis (6.3%). These three main causes compromised 95.4% of the whole group. Immunodeficiency related colitis (2 cases with chronic granulomatous disease related colitis and 1 case with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked)), Behcet's colitis and microscopic lymphocytic colitis accounted for 2.7%, 0.9% and 0.9% respectively. Allergic colitis was the most common cause of chronic colitis in children aged less than 4 years (80.3%) while IBD was the most common cause of chronic colitis in children aged more than 4 years (83.3%) ($p < 0.001^*$).

The most common findings during laboratory evaluations were elevated CRP, hypoalbuminaemia followed by anaemia (50.9%, 47.3% and 45.5% respectively). Patients with IBD had more leukocytosis, thrombocytosis, anaemia and hypoalbuminaemia. Patients with infectious colitis had the highest frequency of elevated levels of CRP followed by patients with IBD. Eighty percent of the patients had an elevated faecal calprotectin level (16.4% had mild elevation, 63.6% had significant elevation). The majority of IBD patients had significant elevation (92.7%). The levels of faecal calprotectin were

Table I. Comparison between the three main groups according to laboratory investigations

Laboratory investigations	Total group (n = 110)		Allergic colitis (n = 56)		Infectious colitis (n = 7)		IBD (n = 42)		Test of significance	P-value
	N	%	N	%	N	%	N	%		
Leukocytosis	33	30.0	12	21.4	1	14.3	16	38.1	$\chi^2 = 3.693$	0.158
Anaemia	50	45.5	21	37.5	1	14.3	24	57.1	$\chi^2 = 6.177^*$	^{MC} p = 0.046*
Thrombocytosis	30	27.3	11	19.6	0	0.0	18	42.9	$\chi^2 = 8.721^*$	0.008*
Hypoalbuminaemia	52	47.3	20	35.7	3	42.9	25	59.5	$\chi^2 = 5.507$	^{MC} p = 0.059
Elevated CRP	56	50.9	12	21.4	6	85.7	34	81.0	$\chi^2 = 39.322^*$	^{MC} p < 0.001*
Elevated ESR	39	35.5	1	1.8	5	71.4	29	69.0	$\chi^2 = 60.887^*$	^{MC} p < 0.001*
Stool analysis:										
RBCs	13	11.9	4	7.3	0	0.0	8	19.0	$\chi^2 = 3.362$	0.175
Pus cells	52	47.3	4	7.1	7	100.0	40	95.2	$\chi^2 = 95.265$	< 0.001*
Positive faecal calprotectin	88	80.0	35	62.5	7	100.0	41	97.6	$\chi^2 = 19.860^*$	< 0.001*
Mild elevation	18	16.4	12	34.3	2	28.6	3	7.3	$\chi^2 = 8.739^*$	0.013*
High elevation	70	63.6	23	65.7	5	71.4	38	92.7		
Min.–max.	10.0–3000.0		10.0–1800.0		200.0–2611.0		36.0–3000.0		H = 27.365*	< 0.001*
Mean ± SD	742.99 ± 746.61		402.9 ± 389.9		725.9 ± 861.4		1230.6 ± 868.0			
Median	406.0		300.0		300.0		995.0			

χ^2 – chi square test, MC – Monte Carlo, *statistically significant at $p \leq 0.05$, H – Kruskal-Wallis test, CRP – C-reactive protein, ESR – erythrocyte sedimentation rate, RBCs – red blood cells, min. – minimum, max. – maximum, SD – standard deviation.

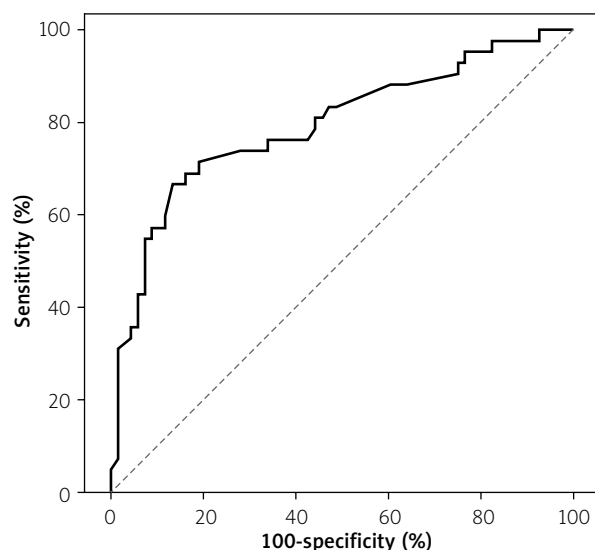
Table II. Correlation of faecal calprotectin with different parameters

Faecal calprotectin	r_s	P-value
CRP	0.436*	< 0.001*
ESR	0.558*	< 0.001*
Number of loose stools	0.098	0.306
Duration of the disease [months]	0.151	0.115
WBCs	0.178	0.063

CRP – C-reactive protein, WBC – white blood cells, ESR – erythrocyte sedimentation rate, r_s – Spearman coefficient, *statistically significant at $p \leq 0.05$.

significantly higher in children with IBD (Table I). Patients with pancolitis had significantly higher faecal calprotectin levels than those with left sided colitis ($p < 0.001^*$).

In the total group, faecal calprotectin was significantly correlated with the laboratory parameters CRP and ESR (Table II). The cut-off level of faecal calprotectin to differentiate IBD from non-IBD causes of colitis was 744 $\mu\text{g/g}$ with 86.8% specificity and 66.7% sensitivity with the 95% confidence interval 0.703–0.885 (Figure 1).

**Figure 1.** ROC curve of faecal calprotectin for prediction of IBD versus non-IBD cases

Discussion

Chronic colitis is a major problem worldwide in different paediatric age groups. It has different presentations and aetiologies. An approach for differentiation

between different types of chronic colitis is lacking. The epidemiology and management of this condition are different in the developing world [13]. Paediatricians often treat all cases with chronic colitis as infectious colitis, leading to delay in the diagnosis of other diseases.

Elevated inflammatory markers are associated with active colitis and usually correlate well with the severity of colitis, especially in IBD [14, 15]. In the present study, patients with IBD had the highest frequency of elevated inflammatory markers, especially leukocytosis and thrombocytosis. This corresponds to the laboratory abnormalities reported in multiple studies, which proved that elevated inflammatory markers have a diagnostic value in patients with IBD [14, 15].

Faecal calprotectin level is a sensitive, but not a disease-specific marker, to detect gastrointestinal inflammation and so can be used to distinguish colitis from other causes of non-inflammatory diarrhoea. It may also be used in primary care to aid decision making for early referrals to gastroenterologists [4]. The presenting symptoms of IBD and other causes of chronic colitis can be similar. Distinguishing them by clinical signs and symptoms can be sometimes difficult. Higher levels are usually reported in IBD than other causes of chronic inflammatory diarrhoea, which may help the gastroenterologist to distinguish IBD from other less urgent causes [4, 16]. This may help to prioritize patients with higher possibilities of IBD for earlier endoscopy especially in busy centres or in countries with limited resources. Similarly, in our study, we observed significant elevation of faecal calprotectin in IBD cases compared to other non-IBD causes. We also found that a cut-off level of 744 µg/g for faecal calprotectin may differentiate IBD from non-IBD cases with 86.8% specificity and 66.7% sensitivity. A cut-off level to distinguish between IBD and non-IBD is still lacking in the literature. A meta-analysis (9 studies, describing 853 patients) demonstrated that faecal calprotectin has a high overall sensitivity of 0.97 (95% confidence interval: 0.92–0.99) and a specificity of 0.70 (0.59–0.79) for diagnosing IBD [17].

In our study, the level of faecal calprotectin was significantly correlated with CRP and ESR. In a study in India, Samant *et al.* reported that faecal calprotectin correlates well with CRP but not ESR in patients with ulcerative colitis [18]. Another study reported the correlation of faecal calprotectin to CRP in Crohn's patients, ulcerative colitis and IBD in total [19]. The literature is lacking studies about correlations of faecal calprotectin with CRP and ESR in chronic colitis with all its different types. These correlations emphasize the value of faecal calprotectin in assessing severity of IBD and possibly other causes of non-IBD colitides.

Data about faecal calprotectin level and cut-off levels are lacking. Few studies have compared faecal calprotectin level in IBD versus non-IBD as causes of chronic colitis. Moreover, few studies have correlated faecal calprotectin with other laboratory markers. This work highlighted the value of the level of faecal calprotectin in predicting the cause of chronic colitis even before definitive diagnosis by endoscopic and histopathological assessment and so early referral to the gastroenterologist.

Conclusions

From this study, it can be concluded that faecal calprotectin level can categorize patients with chronic colitis into IBD colitis and non-IBD colitides. It can aid the paediatrician for early referral to the gastroenterologist.

Conflict of interest

The authors declare no conflict of interest.

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