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Research Article

Faecal Calprotectin in Inflammatory Bowel Diseases

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Abstract

Background: Inflammatory bowel diseases (IBD) are chronic intestinal disorders of unknown etiology and with a typically relapsing course. Faecal calprotectin (FC), an important granulocyte cytosolic protein, is closely correlated with faecal excretion of 111 indium labelled leucocytes, deemed to be the gold standard for measuring intestinal inflammation. Assessment of faecal calprotectin levels has been proposed as a non-invasive test for the direct evaluation of intestinal inflammation in patients with IBD. Since mucosal healing of ulcers reduces the need for surgical intervention and hospitalization in IBD, we examined the reliability of calprotectin levels in reflecting mucosal disease severity. The aim of the study was to compare faecal Calprotectin with the standard disease activity indices (UCAI and CDAI) of inflammatory bowel diseases (Ulcerative colitis and Crohn's disease).

Methods: Patients diagnosed to have IBD based on clinical, endoscopic and histological examination were included. Ulcerative colitis activity index (UCAI) and Crohn's diseases activity index (CDAI), were calculated. Faecal calprotectin was estimated by a commercially available quantitative ELISA test.

Results: Forty-three patients were included in the study, 20 patients with Ulcerative colitis (UC) and 23 with Crohn's disease (CD). Patients with active CD (CDAI > 150) were 18/23 (78%) and with active UC (UCAI > 2) were 17. Mean hemoglobin was not different in both the groups. Mean ESR was raised in both groups (37 in UC, 31 in CD; P = 0.361). Mean CRP was raised in both groups (UC 49 ± 60; CD 19 ± 19; P= 0.302). Mean UCAI was 7 (SD ± 3) and mean CDAI was 212 (SD ± 89). Mean faecal calprotectin was 890µg/g (SD ± 503) in UC patients and 641 µg/g (SD ± 739) in CD patients; P = 0.028. Faecal calprotectin was higher in active cases compared to those in remission but the difference did not achieve statistical significance. Correlation of faecal calprotectin with CDAI was strong (P = 0.0008) whereas correlation of faecal calprotectin with UCAI was weak (P = 0.274).

Conclusion: Faecal calprotectin correlated strongly with CDAI but weakly with UCAI. The difference in patients in remission vs active disease (as categorized by UCAI and CDAI) was not statistically significant.

Keywords: Inflammatory Bowel Disease; Crohn's Disease; Ulcerative Colitis; Faecal Calprotectin; Endoscopy; Diagnosis; Biomarker

Abbreviations

IBD: Inflammatory Bowel Disease; CD: Crohn's Disease; UCAI: Ulcerative Colitis Activity Index; CDAI: Crohn's Disease Activity Index; FC: Faecal Calprotectin; IBS: Irritable Bowel Syndrome; MIM: Mendelian Inheritance in Man

Introduction

Inflammatory bowel disease (IBD) mainly consists of Crohn's disease (CD) [MIM: 266600] and ulcerative colitis (UC) [MIM: 191390] both with uncertain etiology affecting the intestine. Chronic abdominal pain with diarrhea or constipation and rectal

bleeding are common clinical features [1]. It may result from unnatural immune reaction to gut micro flora which is triggered by certain environmental factors in genetically susceptible individuals. Researchers have been identified about 200 disease specific genetic loci for IBD. In these 110 loci were present in both inflammatory bowel diseases and the others were specific for any one of the disease [2]. High incidence was found in countries like North America and Europe. There is a significant increase in IBD incidence in countries like China, Japan, South Korea, and India. In 2010 India was second highest in total IBD incidence of 1.4 million, after USA [3]. Calprotectin is a cytosolic protein derived mainly from neutrophils and it is secreted during inflammation in the intestine. It has been used to detect intestinal inflammatory disease and to distinguish such diseases from non-inflammatory diseases like irritable bowel syndrome (IBS) [4]. Colonoscopy followed by mucosal biopsy is the standard of choice to diagnose inflammatory bowel disease (IBD) and to assess its severity. However, these techniques are costly and invasive in nature. In majority of people with suspected IBD the endoscopy findings may be negative. In such conditions simple and sensitive biomarker like calprotectin can be used as the best tool for disease screening [5].

Materials and Methods

A total of 43 adult outpatients and inpatients with a previously confirmed diagnosis of IBD referred for colonoscopy at the Departments of Gastroenterology of Amrita institute of medical sciences and research center during May 2008 to July 2009. They were diagnosed on the basis of clinical, endoscopic, and histologic criteria. Forty-three age and sex matched controls were included in the study. They were patients without any macroscopic or histopathological abnormalities and with no evidence for underlying intestinal pathology. Patient's signed informed consent forms and patient details were collected. Signed informed consent was obtained from all control subjects. The study protocol conforms to the ethical guidelines of the "World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, as revised in Tokyo 2004 [6,7]. The study was approved by the institutional ethical review committee. Patients who were using anti-inflammatory agents or patients with erosive/ulcerative upper gastrointestinal disease or a gastrointestinal infection within 60 days prior to endoscopy were excluded from the study, as these conditions cause elevated fecal calprotectin levels. Bowel preparation was done with electrolyte/polyethylene glycol solutions.

A faeces sample was collected before the endoscopy. Blood samples were collected and used to estimate heamoglobin, ESR and CRP. CDAI (Crohn's disease activity index) provides a simple and practical measure of clinical activity of Crohn's disease which should be reproducible, and independent of ESR, CRP or hs CRP. The CDAI scores were collected in CD patients [8,9]. The simple index CDAI is calculated based on only five items: (1) General well-being [grade 0-4]; (2) Abdominal pain [grade 0-3]; (3) Number of liquid stools per day; (4) Abdominal mass [grade 03]; (5) Complications: [arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, anal fissures, new fistula, abscess (score 1 per item)]. In UC patient's disease activity was assessed by using modified Mayo scoring system [10]. This index was calculated by adding the grades of factors like stool frequency, rectal bleeding, physician's assessment and endoscopy determinations. The grade is from 0 to 3 and range from 0- 12. Based on these grades the patients are classified in to four groups via remission 0-2, mild 3-5, moderate 6-10, and severe 11-12. Patients with grades above 6 were only included in this study. Fecal samples were stored at 8°C before the assay. After weighing extraction buffer was added and centrifuged. The supernatant was used for the ELISA test.

Results

Forty-three patients were included in the study; 20 patients with ulcerative colitis (UC) and 23 patients with Crohn's disease. Male to female ratio was 30:13. Mean age of patients 1n UC was higher than in CD (49.55 versus 32.43). The duration of disease in months was 33 and 56 in UC and CD respectively. Patients with active CD (CDAl > 150) were 18/23 (78%) and with active UC (UCAI>2) were 17/20 (85%). 72% patients had diarrhea (15 in UC and 16 in CD). 40% had bleeding (16 in UC and 1 in CD) (table 1). Abdominal pain was present in 53% cases (13 in UC and l0 in CD). Extra intestinal symptoms were present in 16 cases of CD and none with UC. Mean hemoglobin was not different in both the groups. Mean ESR was raised in both groups (37mm/hr in UC, 31mm/hr in CD). Mean CRP was raised in both groups; 49 (SD ± 60) mg/L in UC, 19 (SD ± 19) mg/L in CD. CRP positively correlated with the severity of disease (correlation coefficients from 0.26 to 0.73 for UC and from 0.21 to 0.61 for CD). Mean UCAI was 7 (SD ± 3) and mean CDAI was 212 (SD ± 89). Mean faecal calprotectin (FC) was 890µg/g (SD ± 503) in UC patients and 641µg/g (SD ± 739) in CD patients. In normal con-

trol subjects FC level was 1060µg/g. Fecal catprotectin was higher in active cases compared to those in remission but the difference did not achieve statistical significance. There was significant positive correlation between FC and CDAl (r=0.436. p=0.0008) but not between FC and UCAI(r = 0.280, p = 0.274).

Variables	Ulcerative colitis (n = 23)	Crohn's disease (n = 20)
Age	49.55 (SD ± 16.26)	32.43 (SD ± 11.35)
Gender	M 15, F 5	M 15, F 8
Duration (in months)	33.72 (SD ± 33.88)	56.60 (SD ± 56.36)
Symptoms		
Diarrhea (72%)	15 (75%)	16 (70%)
Bleeding (40%)	16 (80%)	1 (4%)
Abdominal pain (53%)	13 (65%)	10 (43.5%)
Extra intestinal symptoms (37%)	0 (0%)	16 (37%)
Lab parameters		
Hemoglobin	11.11 (SD ± 2.13)	11.68 (SD ± 2.43)
ESR	37 (SD ± 19.23)	31 (SD ± 15.2)
CRP	49 (SD ± 60)	19 (SD ± 19)
Disease activity index	7 (SD ± 3)	212 (SD ± 89)
Fecal calprotectin	890 (SD ± 503)	641 (SD ± 739)

Table 1: Variables in patients with Inflammatory Bowel diseases (UC/ CD) (n = 43).

Correlation of faecal calprotectin with CDAI was strong (P = 0.0008) whereas correlation of faecal calprotectin with UCAI was weak (P = 0.274).

Discussion

Faecal calprotectin levels are elevated in patients with IBD and this helps to identify the disease. Based on our analysis it can be concluded that calprotectin is the best available marker for the presence of intestinal inflammation. Our results are in line with these previously published data and with the conclusion that faecal calprotectin is the best available marker for intestinal inflammation. However, based on our data we cannot confirm that faecal calprotectin tests are less reliable in patients with UC, since the results were only marginally different from those of the whole cohort. Several studies have been published for the diagnostic value of calprotectin in IBD [11,12]. Costa, F., *et al*. (2003) found a positive correlation between clinical activity scores in CD and UC with FC (r = 0.44, p < 0.01 in CD; r = 0.60, p < 0.001 in UC) [13].

We propose the cut off value of faecal calprotectin level 250 µg/g. This is the best predictive value for sensitivity and specificity for UC and CD mucosal inflammation or presence of large ulcers in intestine. In addition to the measurement of calprotectin in confirmed IBD cases, it is also has a diagnostic value in patients with chronic abdominal symptoms of unknown origin. Diagnostic accuracy was high, in younger patients with lower risk as well as in older patients at higher risk for IBD. It should be taken care that calprotectin can be increased in patients using NSAIDs; intercurrently occurring gastrointestinal infection/malignancies can also lead to increased fecal calprotectin values.

Conclusion

Faecal calprotectin is a best marker of mucosal inflammation in IBD and it can also useful in differentiating IBD from IBS. It measures the disease severity and monitors the treatment response. Together with the disease activity indexes (UCAI, CDAI) or alone it is effective as a biomarker in IBD.

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