Original Article

Faecal Calprotectin in Differentiating Inflammatory Bowel Disease (IBD) from Irritable Bowel Syndrome (IBS)

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Abstract

Inflammatory bowel disease (IBD) is a chronic idiopathic inflammatory disorder of the gastrointestinal tract with relapsing and remitting course. Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by altered bowel habit in association with abdominal discomfort and pain. Faecal biomarker may be used an accurate tool in the differentiation of IBD and IBS. The aim of this study was to measure faecal calprotectin (FC) level in patients with IBD and IBS and compare between them. This cross-sectional observational study conducted at the department of Gastroenterology, BSMMU, Dhaka, Bangladesh. Patients with IBD were diagnosed on the basis of compatible history, clinical examination, laboratory, radiological and endoscopic findings, where IBS patients were selected by using the Rome IV criteria. Quantitative faecal calprotectin enzyme-linked immune sorbent assay (ELISA), BÜHLMANN Quantum Blue® test was done and compared between IBD and IBS patients. In this study, Ninety (90) patients were enrolled, 45 patients with IBD and 45 patients with IBS. Mean age of the IBD patients was 32.24±9.76 years and IBS patients was 33.80±9.70 years. There were 28 (62.2%) male and 17 (37.8%) female patients with IBD and 30 (66.7%) male and 15 (33.3%) female patients with IBS. We found faecal calprotectin (FC) level was 445.68 ± 237.35µg/g in IBD patients and 39.16 ± 17.31µg/g in IBS patients. There was a significant difference of faecal calprotectin level between IBD and IBS patients (p-value < 0.001). The sensitivity and specificity of faecal calprotectin to differentiate IBD from IBS was 91.1% and 86.7% respectively. The test accuracy was 88.9%. Area under ROC was 0.959 (95% CI, 0.909 to 1.0). This study showed that faecal calprotectin appears to be clinically useful, non-invasive, rapid and reliable marker to differentiate IBD from IBS.

Keywords: Inflammatory bowel disease, irritable bowel syndrome, faecal calprotectin.

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INTRODUCTION

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn'sdisease (CD) are chronic idiopathic inflammatory disorders of the gastrointestinal tract with a typically relapsing and remitting course. Peak incidence of UC and CD occurs in second to fourth decade of life. Genetic susceptibility and a number of environmental factors such as smoking, drugs, diets and infectious gastroenteritis are related to IBD.[1] Crohn's disease (CD) is a chronic inflammatory disorder that may involve any part of the alimentary tract from mouth to anus. It can involve all layers of intestine from mucosa to serosa. Patients usually present with diarrhoea, abdominal pain and weight loss. Common complications include stricture and fistula. Numerous extra intestinal manifestations also may occu.[2] Ulcerative colitis (UC) is a chronic relapsing amd remitting disease characterized by diffuse mucosal inflammation of the colon.[3] The exact

etiology of UC is unknown however, it is thought to be caused by inflammatory response to the gut contents in genetically predisposed individuals.[4] The cardinal symptoms of UC are rectal bleeding with passage of mucous and bloody diarrhoea. In severe or extensive UC, acute complications such as severe bleeding , toxic megacolon and perforation may occur. Colorectal cancer is common in UC patients compared to the general population; risk factors include long duration of disease, extensive colonic involvement, severe inflammation and epithelial dysplasia, and childhood-onset disease.[5] There is no single test which allows the diagnosis of IBD. Diagnosis of IBD and differentiation between CD and UC which can be made accurately in most patients based on the patient's history and physical examination, ileocolonoscopic examination, biopsy, double contrast barium enema examination and microbiology.[6] Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by altered bowel habit in association with abdominal discomfort and pain in the absence of detectable structural and biochemical abnormalities.[7] Visceral hypersensitivity, altered gastrointestinal motility , post infectious reactivity, brain-gut interactions, alteration of faecal microflora, bacterial overgrowth, food sensitivity, carbohydrate malabsorption, and intestinal inflammation all have been implicated in the pathogenesis of IBS.[8] IBS is diagnosed solely on the basis of patient-reported symptoms when obvious biochemical and anatomical pathology have been excluded since no biomarkers have been identified to date.[9] The use of recognized diagnostic criteria does allow for a certain degree of standardization in patient characteristics and Rome III are useful resources for this purpose.[10] Rome III criteria cannot exclude IBD before the diagnosis of IBS. Till date there are only few researches about Rome III criteria for diagnosing IBS which is the most commonly used criteria in the world. It has modest value to diagnose IBS. Recently, it showed that Rome IV criteria is much superior to the Rome III criteria, although the clinical relevance of this is uncertain.[11]

IBS is a chronic functional gastrointestinal disorder that affects about 23% of the population across the world. Patients who are seeking health care related to IBS is by far the largest subgroup seen in gastroenterology clinics in primary health care settings.[12] In Bangladesh, its prevalence is reported at 20.6% in men & 27.7% in women.[13] Women present with IBS more commonly than men with a ratio of 2:1.[14] Though, this syndrome is not life threatening, it can significantly impair quality of life resulting in high health care costs.[15] This economic burden adds to the importance of accurately diagnosing and managing IBS in both primary and secondary healthcare.Lower GI endoscopy was done in most of the patients presented with chronic diarrhoea. IBS is a chronic functional GI disorder. But, if IBD is missed then, several life threatening complications may occur such as, toxic megacolon, intestinal perforation, intestinal obstruction and colonic malignancy. Due to lack of simple clinical or laboratory mean, we do colonoscopy for all. Most of colonoscopic findings are negative, but we do it to ensure that IBD is not missed.[16] We routinely use the inflammatory marker, C-reactive protein (CRP), to track inflammation in our IBD patients, but in our experience, it lacks sufficient sensitivity to make the diagnosis.[17] In several conditions serum markers of intestinal inflammation can be raised. Faecal markers of inflammation in the absence of enteric infection would be more specific for IBD.[18]

Usually faecal biomarkers provide a reliable and simple noninvasive means in the differentiation of IBD and IBS, calprotectin appears to represent the most accurate marker to differentiate between IBD and IBS.[19] Calprotectin is probably the most promising markers for various reasons. Most of the cytosolic proteins in neutrophils is calprotectin. Calprotectin in faeces can therefore be considered directly proportional to neutrophil migration to the gastrointestinal tract. [20]

Calprotectin is stable in stool samples for up to seven days at room temperature and one sample of less than 5 gm is sufficient for a reliable measurement.[21] It is difficult to distinguish IBD from IBS using symptoms and signs only. Most patients with IBS are evaluated by endoscopy and radiographic imaging to exclude a diagnosis of IBD as clinical differentiation remains challenging and may delay effective treatment. This not only exposes patients to the inheretent risks associated with this procedure, but also increases their economic burden.[19] Endoscopic evaluation is often not comfortable but also expensive and has some significant risk such as perforation.[22] Radiological imaging also has drawbacks with observer variability and does not allow histological sampling.[23] Therefore, in this situation a simple, rapid, non-invasive and inexpensive test in discriminating IBD from IBS is of great importance. Environmental and genetic factors are implicated in IBD pathogenesis. Several studies were done in western population regarding faecal calprotectin. But, only limited data are available in Bangladesh in this regard. So, this

cross-sectional observational study was done in Bangladeshi population to see the value of faecal calprotectin level to differentiate IBD from IBS.

MATERIALS AND METHODS

This was a cross-sectional study done in the department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from May 2017 to August 2018. IBD patients were selected on the basis of compatible history, clinical examination, and laboratory, radiological and endoscopic findings.[24,25] IBS patients were selected using the Rome IV criteria. [26] A total of 45 IBD and 45 IBS patients were enrolled purposively aged 18-50 years. IBD and IBS patients diagnosed based on aforementioned criteria were enrolled. Patients with microscopic colitis, indeterminate colitis, infectious colitis, colorectal carcinoma, intestinal tuberculosis, intestinal lymphoma, colonic polyp, history of taking NSAIDs, pregnancy were excluded. IBS patients having alarm features such as anemia, fever, weight loss, melena, family history of colon cancer, thyroid disease, and Diabetes mellitus were excluded. Complete blood count, erythrocyte sedimentation rate (ESR), CRP, RBS, serum albumin, stool R/M/E, stool culture, celiac serology, thyroid function test, abdominal ultrasound and ileocolonoscopy was done. Barium follow through or enteroscopy was also done case by case basis where needed to confirm the diagnosis. Quantitative faecal calprotectin ELISA test was performed. The study was performed after taking ethical clearance from Institutional Review Board (IRB) of BSMMU. Data obtained from the study was used only for the research purpose and the confidentiality of all study information was maintained strictly.

Estimation of faecal calprotectin

For estimation of faecal calprotectin less than 1 gram of native stool was collected in plain tubes without any chemical or biological additives. Samples were stored in refrigerator at 2-8 degree Celsius. Quantitative measurement of faecal calprotectin was done in the department of microbiology, BSMMU. A cut off value ≥50 microgram/gram was considered positive as per manufacturer's guide.

Sample collection:

For estimation of faecal calprotectin less than 1 gram of native stool was collected in plain tubes without any chemical or biological additives. Samples were stored in refrigerator at 2-8 degree celsius. All patients were requested to provide a stool sample in a container supplied to them.

Test Procedure:

The test was designed for the selective measurement of calprotectin antigen by sandwich immunoassay. A monoclonal capture antibody (mAb) being highly specific for calprotectin is coated onto the test membrane. A second monoclonal detection antibody conjugated to gold colloids is deposited onto the conjugate release pad and released into the reaction system after addition of the extracted diluted stool sample. The Calprotectin/anti-calprotectin gold conjugate bind to the anti-calprotectin antibody coated on the test membrane and the remaining free anti-calprotectin gold conjugate binds to the goat anti-mouse antibody coated on the test membrane (control line: control band). The signal intensities of the test line and control line are measured quantitatively by the BUHLMANN Quantum Blue Reader. The color intensity is directly proportional to the concentration of calprotectin in the test sample.

Statistical analysis: Numerical variables were presented as mean ± SD. Categorical variables were expressed in percentage. A p-value ≤ 0.05 were considered statistically significant. Only age was normally distributed. All other numerical variables were non-normally distributed. During comparison of two independent numerical variable, student's t test and Mann Whitney U test were used for normally and non-normally distributed data respectively. Two set of categorical variables were tested using Chi-Square test. Sensitivity and specificity was calculated for each test by 95% confidence interval.

Ethical consideration:

Before starting this study, the research protocol was submitted to the institutional review board (IRB) of BSMMU, Dhaka and IRB clearance was obtained. All participants were informed about the objectives, methodology and purpose of the study in easily understandable way. Verbal and written consents were obtained from all participants without any influences prior to sample collection. Data obtained from the study was used only for the research purpose and the confidentiality of all study information was maintained strictly.

RESULTS

A total of 90 patients were enrolled, among them 45 were IBD patients and 45 were IBS patients. Mean age of the IBD patients was 32.24±9.76 years and IBS patients was 33.80±9.70 years. Twenty eight (62.2%) male and 17 (37.8%) female patients with IBD and 30 (66.7%) male

and 15 (33.3%) female patients with IBS. Demographic variables are shown in Table 1. Hemoglobin level was significantly lower in IBD patients than IBS patients (p ≤ 0.001). ESR and CRP was significantly higher in IBD group (Table 1).Faecal calprotectin level was 445.68 ± 237.35µg/g in IBD patients and 39.16 ± 17.31µg/g in IBS patients (p ≤ 0.001)(Table 2). Distribution of the patients according to level of faecal calprotectin at a cut off value 50 µg/g of stool is shown in table 3.No significant difference of faecal calprotectin was found between ulcerative colitis (UC) and crohn's disease (CD) (Table 4). The sensitivity and specificity of faecal calprotectin to distinguish between IBD and IBS using a cut-off value 50 µg/g was 91.1% and 86.7% respectively, with a negative predictive value 90.7% and positive predictive value 87.2%.The test accuracy was 88.9%. Area under ROC was 0.959 (95% CI, 0.909 to 1.0) which is close to 1. It indicates that classifier was very good and difference between the test results of the IBD and IBS was highly significant (p-value <0.001); shown in table 5 and figure 1.

Table I Shows the demographic and biochemical factors in IBD and IBS patients. There were 28 (62.2%) male and 17 (37.8%) female patients with IBD and 30 (66.7%) male and 15 (33.3%) female patients with IBS .The mean difference of hemoglobin level, ESR and CRP level between the patients of IBD and IBS patients were statistically significant (p value < 0.001).

Table I: Demographic and biochemical factors (n=90)

		IBD group	IBS group	P
Age in years (mean ± SD)		32.2 ± 9.8	33.8 ± 9.7	0.450[a]
Gender n (%)	Male	28 (62.2)	30 (66.7)	0.660[b]
	Female	17 (37.8)	15 (33.3)	
Hb (gm/dl)		9.9 ± 0.9	13.4 ± 1.1	<0.001[c]
ESR (mm in 1st hour)		47.5 ± 16.8	15.9 ± 9.9	<0.001[c]
CRP(mg/L)		21.1 ± 11.8	4.2 ± 1.1	<0.001[c]

n= number, %= percentage

P < 0.05 considered significant

a-Students t-test, b- Chi-square test, c- Mann-Whitney U test

Table II shows the faecal calprotectin was 445.68 ± 237.35µg/g in IBD patients and 39.16 ± 17.31µg/g in IBS patients. The p-value was <0.001. The mean difference of faecal calprotectin level was statistically significant between IBD and IBS patients

Table II: Faecal Calprotectin level in IBD and IBS patients (n=90)

Parameter	Groups		p-value
	IBD (n=45)	IBS (n=45)	
Faecal Calprotectin (µg/g) (Mean±SD)	445.68±237.35	39.16±17.31	<0.001c

P < 0.05 considered significant

c- Mann-Whitney U test

Table III shows the distribution of patients of IBD and IBS at a cut off value of 50µg/g faecal calprotectin level. There were 41 (91.1%) patients of IBD and 6 (13.3%) patients of IBS with faecal calprotectin level ≥ 50µg/g of stool and 4 (8.9%) patients of IBD and 39 (86.7%) patients of IBS had faecal calprotectin level < 50 µg/g of stool. The p-value was <0.001.

Table III: Distribution of the patients according to level of faecal calprotectin level at a cut off value 50 µg/g of stool (n=90)

Faecal Calprotectin (µg/g of stool)	Groups		Total	p-value
	IBD (45)	IBS (45)		
≥50	41 (91.1)	6 (13.3)	47 (52.2)	<0.001b
<50	4 (8.9)	39 (86.7)	43 (47.8)	
Total	45 (100.0)	45 (100.0)	90 (100.0)	

P < 0.05 considered significant

b- Chi-square test

Table IV Shows mean±SD of faecal calprotectin level in patients of IBD. In Crohn's disease patients the faecal calprotectin level was 413.91 ± 230.24µg/gm and in patients of Ulcerative colitis was 478.90 ± 245.43µg/gm. The mean difference among the patients of CD and UC was not statistically significant.

Table IV: Faecal Calprotectin level in CD and UC patients (n=45)

Parameter	Groups		p-value
	CD (n=23)	UC (n=22)	
Faecal Calprotectin (µg/gm) [Mean±SD]	413.91 ± 230.24	478.90 ± 245.43	< 0.358[c]

< 0.05 considered significant

c- Mann-Whitney U test

Table V Shows the performance of diagnostic test of faecal calprotectin at a cut-off value 50 µg/g. The sensitivity was 91.1%, Specificity was 86.7% in differentiating IBD from IBS. The PPV was 87.2%, NPV was 90.7% and test accuracy was 88.9%.

Table V: Performance of diagnostic test of faecal calprotectin at a cut off value 50 µg/g

Performance of diagnostic test	%	95%CI	
		Min	Max
Sensitivity	91.1	81.7	96.6
Specificity	86.7	77.3	92.2
PPV	87.2	78.2	92.5
NPV	90.7	80.8	96.5
Accuracy	88.9	79.5	94.4

PPV-positive predictive value,

NPV-Negative predictive value

Performance of diagnostic test of faecal calprotectin at a cut-off value 50 µg/gm to differentiate IBD from IBS.

ROC (Receiver Operator Characteristic) Curve:

The curve was generated by plotting the relationship of true positive versus false positive rate as the threshold value for classifying an item as 0 or is increased from 0 to 1.

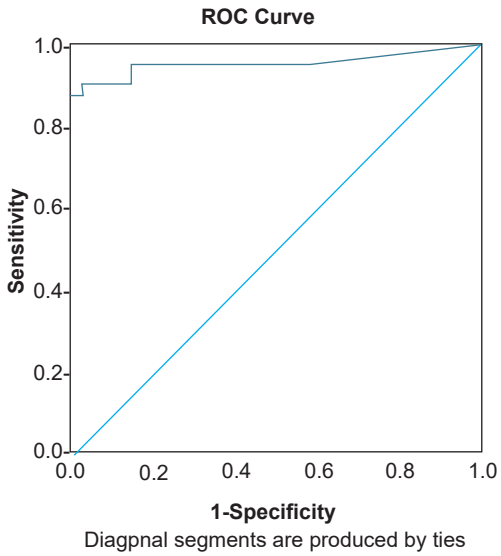

Figure 1: The figure shows AUC score of 0.959 (0.909-1.000) with 95% CI which is close to 1. It indicates that classifier was very good and difference between the test results of the IBD and IBS were highly significant (p-value <0.001).

DISCUSSION

The mean age of the patients with IBD was 32.24 ± 9.76 years and IBS patients was 33.80 ± 9.70 years. Mehrjardi et al.[27] conducted a similar study and found the mean age of the IBD patients was 35.4±8.6 years and the mean age of the IBS patients was 32.3±6.8 years. The mean age of the patients of this study was close to our study. Considering gender distribution, among the IBD patients28 (62.2%) were male and 17 (37.8%) were female. On the other hand 30 (66.7%) were male and 15 (33.3%) were female in IBS patients. Male patients were predominant in both groups in our study. Mehrjardiet al.[27] showed majority of patients in both groups were female. This dissimilarity may be due to easy health care access for male is in our country.

For estimation of faecal calprotectin we used the BUHLMANN Quantum Blue Reader in this study. Same method was used by other authors like Sharbatdaran M. *et al*[28], Dhaliwal *et al.*[29], Chang *et al.*[30]and Kotze et al.[31]. In this current study, low hemoglobin and high ESR and CRP was found in IBD patients. On the other hand, normal hemoglobin and low ESR and CRP was found in IBS patients (p<0.001). This finding was similar as studied by said *et al.*[32], whereas no difference of ESR was found in two groups of patients as studied by Chang *et a*[30]

In our study, we found mean faecal calprotectin level was 445.68±237.35 µg/g in IBD patients and 39.16 ± 17.31 µg/g in IBS patients. Significant difference of faecal calprotectin level between IBD and IBS patients was found (p-value <0.001). Faecal calprotectin at a cut-off value 50µg/g showed sensitivity 91.1%, specificity 86.7% to differentiate IBD from IBS, PPV 87.2%, NPV 90.7% and test accuracy was 88.9%. Consistent result was found in another study done by Kotze et al.[31]in 2015 .They found faecal calprotectin level in Crohn`s disease was 405µg/g and in Ulcerative colitis was 457µg/g and in IBS patients was 50.5µg/g. A significant difference of faecal calprotectin level was found between IBD and IBS patients with p value <0.001. Another study done by Dhaliwal *et a*[29]in 2015 had shown that faecal calprotectin level was 674.0±480.0µ g/g in active IBD patients and 34.0±69µg/gin IBS patients. Sensitivity and specificity of faecal calprotectin to differentiate IBD from IBS using a cut off value of 50µg/g was 88% and 78% respectively. A study in Tiawan by Chang et al[30]. in 2014 found mean faecal calprotectin level in IBD patients was 694.8±685.0µg/g and 85.8±136.1µ g/g in IBS patients (p <0.001).In comparison of our study, close results were seen regarding difference of faecal calprotectin level in IBD and IBS patients in previous two studies. But faecal calprotectin level was higher in IBD patients in comparison to our study. This could be explained by most patients in our study were previously diagnosed and most of them getting treatment for long time. But, in above mentioned studies all patients were newly diagnosed and faecal calprotectin level was measured before treatment started. Langhorstet al.[33]and Xiang et al.[34]showed significant difference of faecal calprotectin between IBD in relapse and IBD in remission.

There were 6 patients with elevated faecal calprotectin level in IBS which may be due to subtle inflammation or post-infectious IBS as explained by David *et al.*[35] whereaspatients of IBD had <50µg/g of faecal calprotectin level which may be due to IBD in remission as shown by Said*et al.*[32],Erik *et al.*[36]and Dhaliwal et *al*[29].In our study, faecal calprotectin level was significantly higher in IBD patients in comparison to IBS patient, which is similar that of other studies conducted by Tibble *et al.*[37], Antonio et *al.*[38]and Schoepfer*et al*[39].Schoepferet al.[39]measured faecal calprotectin by using another kit and their findings were similar with our study results. C-reactive protein and ESR are commonly used inflammatory markers used by clinicians to discriminate organic gastrointestinal diseases from functional GI disorder. Sensitivity and specificity of ESR and CRP is much lower than faecal calprotectin. Faecal calprotectin is more superior to CRP and ESR to differentiate IBD from IBS[40].

CONCLUSIONS

In conclusion, based on our study findings and previous study results, estimation of faecal calprotectin is a simple, rapid, accurate and noninvasive test to differentiate inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS).

Limitation

Healthy controls were not taken in the study population.

All patients were recruited in this study from a single tertiary level hospital which does not reflect the whole country.

Clinical Significance

Faecal calprotectin can be used as a screening tool before selection of patients for colonoscopy in adjunct with other clinical examinations to differentiate IBD from IBS.

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