Faecal calprotectin as a reliable screening biomarker in the patients with organic bowel diseases

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Abstract
Calprotectin is a calcium and zinc binding protein released from leucocytes, markedly elevated in organic bowel diseases (OBD). Faecal calprotectin (FC) is supposed to be a reliable biomarker to screen the suspected patients with OBD. This study was aimed to determine the role of FC level in screening the suspected OBD patients. It was carried out by measurement of FC using a commercially available ELISA kit among 50 patients with chronic diarrhoea for ≥ 6 weeks with or without other GIT symptoms who underwent colonoscopic evaluation (35 OBD patients and 15 disease control) and 12 healthy control. Significantly higher value (P<0.001) of FC level were observed among OBD patients (n=35) (479.5±133µg/g) than those in disease control (n=15) (82.17±75.64µg/g) and healthy control (n=12) (27±18.2µg/g). Measurement of FC in diagnosing OBD revealed the sensitivity 100%, specificity 67%, Positive Predictive Value (PPV) 88% and Negative Predictive Value (NPV) 100%. FC can be used as a reliable biomarker in screening of suspected OBD by selecting the patients who need colonoscopy.

Keywords: Organic bowel disease, Inflammation, Calprotectin.

Introduction
Organic bowel diseases (OBD) include inflammatory bowel disease (IBD), intestinal neoplasms, intestinal polyp, intestinal tuberculosis, NSAID induced enteropathy, celiac disease etc, in which there is marked inflammation or tissue damage. On the other hand, the term functional bowel disease (FBD) such as irritable bowel syndrome (IBS) used to describe a condition in which no such disease process is visible through standard diagnostic testing1,2,3. Gastroenterologists are often faced with the diagnostic difficulty of differentiating patients with OBD from those with FBD like IBS as many symptoms are common to both conditions4. For this reason, many patients with IBS are investigated extensively with invasive radiographic and endoscopic imaging to make a diagnosis of exclusion. This has significant implications for health care costs with various complications4.

Most clinicians proceed to and rely on routine serological and hematological parameters like erythrocyte sedimentation rate (ESR), blood count, C-reactive protein (CRP) to aid in the differential diagnosis of bowel pathology5. But these systemic markers have low sensitivity and specificity. Imaging studies such as CT and MRI scans, barium follow through and barium enemas can be useful in localizing intestinal inflammation, but these are expensive with suboptimal sensitivity and/or specificity and may expose the patient to ionizing radiation6. Endoscopy with biopsies is considered the gold standard for diagnosis of OBD and also for estimation of disease activity and efficacy of therapy7. But this is unsuitable for frequent use as it is an invasive and expensive procedure with risk of various complications6,7. In recent years, a non invasive, sensitive and specific intestinal biomarker have been tried to be used to select the patients who need endoscopic examination for confirmed bowel pathology6.

Calprotectin, an break down product of leucocytes, bind with calcium and zinc having molecular weight of 36.5 KD has been identified as a good biomarker that correlates more closely to histological than macroscopic intestinal inflammation5,7,8. It is derived predominantly from neutrophils and to a lesser extent, from monocytes and...
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It has both antimicrobial and anti proliferative properties and released extracellularly during neutrophil activation or during cell death and following endothelial adhesion of monocytes. As a result it can be detected and quantified in fluids with inflammation for example: serum, urine, cerebrospinal fluid and faeces. It is resistant to heat and enzymatic degradation in the gut lumen and remains remarkably stable within faeces at room temperature for at least 7 days. Elevated calprotectin concentrations have been found in recruitment of inflammatory cells because of infection, inflammation or malignant disorder. This protein can be measured by enzyme-linked immunosorbent assay (ELISA) method.

Faecal calprotectin (FC) can provide accurate non-invasive information regarding mucosal disease activity without necessarily the need for colonoscopy. Different studies showed FC is a specific, sensitive, non-invasive biomarker for gut inflammation. Meta-analysis from Netherland reported a significant reduction of colonoscopy in adult and children because of introducing FC as screening marker. This study has been undertaken to determine role of FC level in screening the suspected OBD patients who need colonoscopic evaluation.

Material and methods

This cross sectional study was conducted from January 2011 to December 2011 and received prior approval from Ethical Review Committee of Bangabandhu Sheikh Mujib Medical University (BSMMU) and all participants gave informed written consent. All collected data were checked, edited and analyzed by using computer based SPSS (Statistical Package of Social Science) software version 16.0. Data were presented by frequency distribution and percentage. P value < 0.05 was taken as minimum level of significance. The level of significance was calculated by t-test.

Stool samples were collected from 50 patients with chronic diarrhoea for ≥6 weeks with or without other symptoms e.g. abdominal pain, vomiting, weight loss, fever etc who underwent colonoscopic evaluation and 12 healthy controls without colonoscopic evaluation, attending the department of Gastroenterology of BSMMU, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM) and Dhaka Medical College Hospital (DMCH). Biopsy materials were taken for histopathological diagnosis from the patients with abnormal colonoscopic findings while not taken from the patients with normal colonoscopic findings. Stool samples were collected from all patients before bowel preparation for colonoscopy and from healthy controls. All laboratory works were performed in the department of Microbiology and Immunology, BSMMU, Dhaka.

Among 50 symptomatic patients who had definite bowel pathology detected by colonoscopy and biopsy examination were enrolled as OBD cases (n=35) and 15 had normal colonoscopic findings were enrolled as disease control group. 12 healthy persons were enrolled as healthy controls.

Collected stool samples were stored at -20°C. Mean time difference between specimen collection and analysis was 3 months. Analysis was performed using CALPROTECTIN ELISA kit (BÜHLMANN AG Co., Switzerland) following the manufacturer's instructions.

Results

A total of 62 subjects (50 patients and 12 healthy controls) were enrolled. Among 50 patients, 35 (70%) OBD cases were detected while 15 (30%) were the patients with gastrointestinal symptoms having normal colonoscopic findings (disease control group). Out of 35 OBD cases, 21 (60%) ulcerative colitis (UC), 4 (11%) Crohn's disease (CD), 6 (17%) colorectal carcinoma, 1 (3%) intestinal tuberculosis and 3 (9%) intestinal polyp were detected.

A variation in FC level between 50-100 μg/g has been observed among normal healthy population. According to kit manufacturer, 50 μg/g is labeled as cut-off value for FC. In some studies 50 μg/g of FC is showed significant for IBD patients while 100 μg/g cut-off value is observed in other studies. In this study, 100 μg/g has been considered as cut-off value, because mean FC was to be identified as 63.4 μg/g (mean ± 2 SD) in our healthy population/control. This study showed increased mean value of FC among OBD patients (n=35) (479.5±133 μg/g) than those in disease control (n=15) (82.17±75.64 μg/g) and healthy control (n=12) (27±18.2 μg/g). The differences were statistically significant (P<0.001). Among OBD, FC level showed higher value among UC (n=21) (500.58±136 μg/g) and lower value among the patients with intestinal polyp (n=3) (364.8±135.88 μg/g) (Table-I).

Measurement of FC in diagnosing OBD revealed the sensitivity 100%, specificity 67%, PPV 88% and NPV 100% by considering 100 μg/g as cut-off value. (Table-II)
Table I: Faecal calprotectin among the study population

<table>
<thead>
<tr>
<th>Study group</th>
<th>Faecal calprotectin (µg/g)</th>
</tr>
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<tbody>
<tr>
<td>Organic bowel diseases (n=35)</td>
<td>479.5 ±133 (230.7-748.8)</td>
</tr>
<tr>
<td>Ulcerative colitis (n=21)</td>
<td>500.58 ±136 (230.7-748.8)</td>
</tr>
<tr>
<td>Crohn’s disease (n=4)</td>
<td>476 ± 70 (392.7-546.1)</td>
</tr>
<tr>
<td>Colorectal carcinoma (n=6)</td>
<td>456 ± 156.70 (306.1-743.8)</td>
</tr>
<tr>
<td>Intestinal tuberculosis (n=1)</td>
<td>387.20</td>
</tr>
<tr>
<td>Intestinal polyp (n=3)</td>
<td>364.8 ± 135.88 (217.3-484.9)</td>
</tr>
<tr>
<td>Disease control (n=15)</td>
<td>82.16 ±75.64 (17.1-280.6)</td>
</tr>
<tr>
<td>Healthy control (n=12)</td>
<td>27±18.2 (9.25-74.8)</td>
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</tbody>
</table>

*: significant.
(Note: Figure within parenthesis indicates range)

Table II: Sensitivity, Specificity, PPV and NPV for faecal calprotectin assay in diagnosis of organic bowel diseases

<table>
<thead>
<tr>
<th>Faecal calprotectin cutoff value (µg/g)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>67</td>
<td>88</td>
<td>100</td>
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Discussion

Patients presenting to gastroenterology clinics with symptoms suggestive of bowel disorders often require extensive investigation to differentiate functional from organic disease. An important step in the primary assessment of the patients with OBD is measurement of bowel inflammation as it defines the extent and severity of involvement at the beginning of treatment and during monitoring in order to target medical therapies and manage different complications.

The present study showed that FC concentrations were significantly higher in patients with organic intestinal pathologies, colorectal carcinoma, intestinal tuberculosis and intestinal polyp than in disease control and healthy control. This finding confirms the results of previous studies. In this study, the measurement of FC revealed the sensitivity 100% by considering 100 µg/g as cut-off value. Carroccio et al. in 2003 reported 100% sensitivity and 95% specificity for FC detection in IBD cases (50µg/g as cut-off value), while Saadany et al. in 2008 reported 100% sensitivity and 92% specificity (50µg/g as cut-off value). These studies are in agreement with this present study results. In this study specificity of FC is low (67%) in comparison with previous studies. It may be due to lack of histopathological examination of biopsy materials from disease control group that can detect some organic bowel disease in spite of normal colonoscopic findings. So, biopsy materials should be taken from all patients who underwent colonoscopic evaluation for proper diagnosis of bowel pathology.

Various studies reported raised FC level among the patients with infective gastroenteritis. But it cannot distinguish infectious bowel diseases from OBD. So, in case of raised FC level, isolation and identification of infective pathogen or its marker should be done prior to colonoscopic examination. As has been discussed, calprotectin is released from infiltrated inflammatory cells predominantly from neutrophils during the inflammatory response. In OBD patients, there is release of calprotectin in the intestinal lumen by infiltrated inflammatory cells and FC can be used as a reliable biomarker for quantifying intestinal mucosal inflammation and as a first level test to screen the suspected patients with OBD who need colonoscopy in adjunct with other clinical and paraclinical examinations.

References:


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