Preliminary Evaluation of the Role of Faecal Calprotectin in the Diagnosis of Ulcerative Colitis Among Egyptian Patients

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Abstract

Introduction: Calprotectin, a member of the Ca2+-binding S100 family of proteins, makes up about 5% of the total protein content of the neutrophil. It is released upon activation and degranulation of neutrophils and correlates strongly with 111-indium-labeled leukocyte excretion. It was investigated as a promising tool in differentiating between Irritable Bowel Syndrome (IBS) and active Inflammatory Bowel Disease (IBD).

Aim of the Work: Is to compare faecal Calprotectin in patients known to have ulcerative colitis with normal healthy controls and to investigate possible correlation of Calprotectin with disease activity on clinical, laboratory and pathological bases.

Patients and Methods: Forty patients known to have UC were assessed. 19 were excluded: 10 due to non-steroidal anti-inflammatory (NSAID) intake, 2 due to pregnancy and 7 due to disease quiescence. So, 21 patients with active disease were studied, 7 males (33.3%) and 14 females (66.7%), mean age 37.5 (± 16.0) years. Ten healthy controls (8 females and 2 males), mean age 30.9 (± 16.1) years were included. Patients underwent clinical evaluation, determination of blood Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and faecal Calprotectin. Colonoscopy was done to confirm diagnosis, estimate disease extent and obtain colonoscopic biopsy specimens for histological grading of activity. An overall scoring of disease activity was done using the Mayo score.

Results: Faecal Calprotectin was significantly elevated among patients [mean: 12.6 µg/gm stools (±3.2)] in comparison to controls (9.4 µg/gm stools (±2.6), (p 0.01). At a cut off of 10.3 µg/gm stools it has a sensitivity of 86%, specificity of 70% p=0.004, positive predictive value of 86% and a negative predictive value of 70%. No correlation was found between faecal Calprotectin and ESR, histopathology and Mayo score. Calprotectin was significantly higher in cases with left sided colitis (14.1 ±2.7 µg/gm stools) than those with pancolitis (11.8±2.1 µg/gm stools), p 0.02.

Conclusion: Faecal Calprotectin is a good test in differentiating Egyptian patients with ulcerative colitis from healthy controls. Thus, its use as a screening test may be helpful in the selection of cases for endoscopic examination. It lacks specific correlation with the severity of ulcerative colitis. This leaves endoscopy and histopathologic examinations as the main diagnostic tools. Larger scale studies on Egyptian patients are strongly recommended with special reference to the local mucosal permeability and immune milieu of the Egyptian population.

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Key Words: Calprotectin – Ulcerative colitis.

Introduction

MANY aspects of the inflammatory bowel disease (IBD), [Crohn’s disease (CD) and Ulcerative colitis (UC)], still present challenges for physicians. Challenges include diagnosis, prognosis, assessment of disease activity, severity and outcome of therapy. For each of these aspects, there is no single “gold standard” test or examination. Instead, physicians apply a combination of indices: Clinical, laboratory, radiological and endoscopic with the histology to make the diagnosis, assess severity and predict outcome [1].

Several standard markers as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), acute-phase protein (albumin) and platelets are used to aid in diagnosing and monitoring the disease [2,3]. However, these markers lack specificity for gastrointestinal tract inflammation [4].

The symptoms of IBD are often subjective, many biochemical analytes have been studied as markers of IBD in a trial to gain an objective measurement of disease activity and avoid repeated endoscopic procedures. An ideal marker should be easy and rapid to perform, cheap, specific and reproducible. Furthermore, it should be able to identify individuals at risk for the disease, detect disease activity, monitor the effect of treatment
and have a prognostic value towards relapse or recurrence of the disease. Unfortunately, no single marker has proven to possess all the above listed qualities although some interesting markers have been identified [1].

Calprotectin is a heterocomplex of S 100A8/ S 100A9 and a member of the Ca2+-binding S 100 family of proteins [4]. It makes up about 5% of the total protein content of the neutrophil and about 60% of the cytosolic proteins [1]. Faecal Calprotectin is a sensitive, stable marker that is unaffected by dietary supplements, or enzymatic degradation [3,4]. Several properties of Calprotectin make it of significance in testing and monitoring patients with IBD. Being released upon activation and degranulation of neutrophils, it reflects the flux of leukocytes into the intestinal lumen and correlates strongly with 111-indium-labeled leukocyte excretion [5,6]. Based on these features, Calprotectin was investigated as a promising tool in differentiating between Irritable Bowel Syndrome (IBS) and active IBD and prediction of relapse and disease activity in patients with IBD [7,8].

Aim of the work:

The aim of this work is to compare faecal Calprotectin in patients known to have ulcerative colitis with normal healthy controls and to investigate possible correlation of Calprotectin with disease activity on clinical, laboratory and pathological bases.

Patients and Methods

Initially 40 patients known to have ulcerative colitis disease were assessed in this study. Ten patients were excluded due to absence of clear negative history for non-steroidal anti-inflammatory (NSAID) intake. 2 were excluded due to pregnancy at the time of presentation and 7 cases were excluded as the cause of presentation was just for follow-up without evident disease activity. Only cases with active disease were included, this was done by either including newly diagnosed patients or patients who develop worsening of both symptoms and the endoscopic picture despite treatment. All cases had to have a positive histopathology confirming activity.

Accordingly, 21 patients known to have active ulcerative colitis (documented clinically, endoscopically and histopathologically) were included. They were 7 males (33.3%) and 14 females (66.7%) their mean ages was 37.5 (±16.0) years. Ten healthy controls (8 females and 2 males) with a mean age of 30.9 (±16.1) years were included.

Patients were evaluated clinically; blood samples were withdrawn to measure ESR and CRP as markers for disease activity. Stool samples were collected for analysis of faecal Calprotectin. Stool extracts were prepared using Sample preparation kit from Roche Diagnostics, Mannheim, Germany; cat # 745804. Supernatant of the extractions were diluted 1:50 with wash buffer. Feacal Calprotectin was measured using enzyme-linked immunsorbent assay (ELISA) according to the manufacturer's instruction (Immundiagnostik AG, Bensheim, Germany). Results were calculated using "4-Parameter-algorithm. The results were adjusted to the dilution factors of both the extract and the supernatant.

Colonoscopy was done after conventional bowel preparation with laxative and enemas. Full coloscopic examination was attempted whenever possible, but at least the extent of colonic lesions had to be determined in all patients. The aim of coloscopic examination was to confirm diagnosis, estimate disease extent and obtain colonscopic biopsy specimens using pentax videoscape Ec-3840L. Biopsies were immediately fixed in 10% neutral buffered formalin. Formalin-fixed paraffin embedded samples were prepared for histology and stained by hematoxylin and eosin for histological grading. The degree of inflammation was graded on a four point scale: normal (no significant inflammation); mild (elevated number of mucosal leukocytes but intact epithelium); moderate (aggregates of leucocytes with crypt abscesses and erosions but no ulceration of the epithelium) and severe (significant ulceration of the epithelium by mononuclear cell infiltrate) [9,10]. Histological grading was performed by the pathologist without knowledge of endoscopic or laboratory features.

An overall scoring of disease activity was done using the Mayo score which has three clinical variables (stool frequency, rectal bleeding, global physician assessment) and an endoscopy score. Each item is given a score of 0-3 and total score is calculated. A score of 2 to 5: Indicates mild disease and a score of 6 to 12: Indicates moderate to severe disease [11].

Exclusion criteria:

- History of non-steroidal anti-inflammatory drugs and/or antibiotics during the three months preceding enrolment.
- Concomitant malignancy, pregnancy, or alcohol abuse.

Statistical analysis:

Quantitative variables were expressed by mean and standard deviation (SD). They were compared
by t-student test for comparison between 2 groups and ANOVA when more than two groups are compared. Pearson correlation was used to correlate different quantitative parameters to Calprotectin levels. Receiver operator characteristic curve (ROC) was drawn to test for sensitivity and specificity of Calprotectin in the studied population. In all tests, p value was considered significant if less than 0.05.

Results

All patients presented with variable grades of diarrhea and rectal bleeding (mean number of motions per day was 5.6±1.9. Abdominal cramps were detected in 81%, anorexia in 7%, significant weight loss in 28.5%. Joint affection (ankylosing spondylitis and sacroiliitis) were detected in 14%. Two patients had concomitant liver cirrhosis (9%).

Left sided colitis was commonly detected (85.7% of cases: 33.4% confined to the rectosigmoid region and 52.3% extending to the proximal transverse colon). Variable sized colonic ulcerations were the commonest endoscopic finding (90%). Two patients had just diffuse mucosal hyperemia and easy bleeding on touch (9.5%), 3 had double lesions (ulcerations and polyp formation 14.2%), 2 presented with an additional colonic mass (negative for malignancy, 9.5%) and one case was complicated by stricture formation (4.7%).

Faecal Calprotectin was significantly elevated among cases in comparison to controls. Mean value among cases was 12.6 µgm/gm stools (±3.2) and a median of 11.9 µgm stools, compared to 9.4 µgm stools (±2.6) and a median of 9.0 µgm stools among controls, p (0.01). Using the ROC curve the best cut off was 10.3 µgm stools with a sensitivity of 86%, specificity of 70%; p=0.004. Calprotectin showed a positive predictive value (PPV) of 86% and a negative predictive value (NPPV) of 70%.

Calprotectin showed no statistically significant difference among the different grades of Mayo score, or patients with positive CRP. However, it was significantly higher in cases with colitis extending to the transverse colon rather than patients with rectosigmoiditis or pancolitis. Histopathologically, the patients were grouped into those having mild disease activity [4 patients (19%)]; moderate disease activity [12 patients (57%)] and those having severe disease activity [5 patients (24%)]. The mean faecal Calprotectin value in patients with mild disease activity was 13.5 (±3.4), while the mean Calprotectin value among patients with moderate disease activity was 12.6 (±3.3) and the mean value in patients with severe disease activity was 11.1 (±2.7). These results revealed an insignificant correlation between faecal Calprotectin values and the different grades of activity as determined by histologic criteria (p=0.55).

The mean fecal Calprotectin value in ulcerative colitis patients whose biopsy did not show evidence of atypia (81%), was 12.8 µgm (±2.9), while the mean value in ulcerative colitis patients (19%) whose biopsy showed evidence of atypia was found to be 11.4 (±4.3). Faecal Calprotectin values in relation to histologic atypia was also found to be insignificant (p=0.44).

Table (1): Laboratory, Histopathologic findings and Mayo score in patients with ulcerative colitis.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Mean/Number</th>
<th>STD/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory findings:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR 1st hour</td>
<td>41.5</td>
<td>27.8</td>
</tr>
<tr>
<td>ESR 2nd hour</td>
<td>68.0</td>
<td>35.3</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11.1</td>
<td>1.45</td>
</tr>
<tr>
<td>Leucocytic count</td>
<td>6.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Platelet</td>
<td>295</td>
<td>124</td>
</tr>
<tr>
<td>Positive CRP</td>
<td>15</td>
<td>71.4%</td>
</tr>
<tr>
<td>Histopathology:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild activity</td>
<td>4</td>
<td>19%</td>
</tr>
<tr>
<td>Moderate activity</td>
<td>12</td>
<td>57%</td>
</tr>
<tr>
<td>Severe activity</td>
<td>5</td>
<td>24%</td>
</tr>
<tr>
<td>Atypia</td>
<td>4</td>
<td>19%</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>1</td>
<td>4.7%</td>
</tr>
<tr>
<td>Mayo score:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>8</td>
<td>38.1%</td>
</tr>
<tr>
<td>Moderate-severe</td>
<td>13</td>
<td>61.9%</td>
</tr>
</tbody>
</table>

Table (2): Calprotectin correlation with different laboratory, histopathologic and endoscopic parameters.

<table>
<thead>
<tr>
<th>Mayo score CRP and calprotectin:</th>
<th>Number</th>
<th>Mean Calprotectin</th>
<th>STD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>8</td>
<td>11.3</td>
<td>2.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Moderate</td>
<td>13</td>
<td>13.4</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Positive CRP</td>
<td>15</td>
<td>13.4</td>
<td>3.0</td>
<td>0.07</td>
</tr>
<tr>
<td>Negative CRP</td>
<td>6</td>
<td>10.6</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Extent of colonic lesion and calprotectin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recto-sigmoid</td>
<td>7</td>
<td>10.6</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Transverse</td>
<td>11</td>
<td>14.1</td>
<td>2.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Pancolic</td>
<td>3</td>
<td>11.8</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Histopathology grade and calprotectin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>4</td>
<td>13.5</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>12</td>
<td>12.6</td>
<td>3.3</td>
<td>0.55</td>
</tr>
<tr>
<td>Severe</td>
<td>5</td>
<td>11.1</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Atypia and calprotectin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>12.8</td>
<td>2.9</td>
<td>0.44</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>11.4</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

N.B.: No correlation was done between Calprotectin and dysplasia there was only one patient in the entire study.

Picture (2): A case of ulcerative colitis with sessile polyp formation.

Picture (3): Large mucosal ulcers in a patient with ulcerative colitis.

Picture (4): A case of ulcerative colitis complicated by stricture formation.

Fig. (A): Colonic biopsy showing mild inflammatory infiltrate with intact epithelium (Hematoxylin and Eosin x200).

Fig. (B): Colonic biopsy showing moderate inflammatory infiltrate and aggression against crypt epithelium (Hematoxylin and Eosin x200).

Fig. (C): Colonic biopsy showing intense inflammatory infiltrate with surface ulceration (Hematoxylin and Eosin x200).
ESR showed 60% sensitivity and 100% specificity, while CRP showed 70% sensitivity and specificity in the detection of IBD cases. There was no linear correlation between each of numeric Mayo score and ESR and Calprotectin values in the 21 studied patients (p=0.23, 0.8 respectively, r=0.044, 0.271 respectively).

Discussion

Inflammatory bowel disease is a common cause of chronic diarrhea and yet still challenges physicians. Because of the absence of a single "gold standard" test, physicians apply a combination of indices: clinical, laboratory, radiological, endoscopic together with histology to make the diagnosis, assess severity and predict outcome [1].

Several laboratory markers have evolved in the diagnosis and follow-up of IBD patients. These include blood leucocytic counts, platelet counts and serum albumin. These have proved their low specificity and sensitivity due to various reasons. On the other hand, ESR and CRP have gained relative interest.

After the better understanding of the immunopathogenesis of IBD, it became more evident that active gut inflammation is associated with an acute phase reaction and migration of leucocytes to the gut, an action that is translated into the release of several proteins, which may be detected in serum or stools [7,12,13,14]. The search for faecal markers seems tempting since stools are easily accessible and more specific than serum markers that may be increased by conditions other than gut inflammation. Hypothetically, if faecal markers are representative of mucosal inflammation of the bowel in IBD patients, endoscopic examinations could be potentially spared [1].

Out of numerous neutrophil derived proteins present in stools, Calprotectin, a 36kDa calcium and zinc binding protein, is probably the most promising. It represents 60% of cytosolic proteins in granulocytes and therefore can be seen as directly proportional to neutrophil migration to the gastrointestinal tract [1]. It was mainly studied in the differentiation between irritable bowel syndrome and IBD patients in patients presenting with diarrhea. Later on other studies investigated its role in predicting response to treatment, predicting relapse and even in screening relatives of IBD patients. Some studies have incorporated pooled IBD patients without segregating ulcerative colitis (UC) from Crohn’s disease (CD) cases. However, recent data suggest that laboratory markers showed better performance for UC than for CD [1,15].

This study is considered a pilot study that investigates the role of faecal Calprotectin in ulcerative colitis among Egyptian patients. Restricting the inclusion of patients to only active disease (as based on clinical, endoscopic and histopathologic levels) has relatively influenced the number of included patients. In addition to Calprotectin, other laboratory markers were studied. The mean blood leucocytes was not elevated among cases. Costa and colleagues stated that white cell count is neither sufficiently sensitive nor specific as a diagnostic tool for IBD because it doesn't directly reflect the level of local inflammation. Leucocytosis can be influenced by other inflammatory conditions, or steroid therapy, while azathioprine can cause leucopenia [16].

In this study, both ESR and CRP showed lower sensitivities 60% and 70% respectively in comparison to Calprotectin which showed 86% sensitivity in detection of cases of IBD. An early study from St Mark’s Hospital, London, found that CRP was elevated in 50% of patients diagnosed with UC but in none of patients with functional bowel symptoms [17]. According to Beattie and colleagues, the best laboratory marker in differentiating IBD from normal controls was CRP being reported in 60% of UC patients [18]. Vermeire and colleagues suggested that the sensitivity of CRP may increase in UC patients with the use of highly specific assays [1]. Other studies have considered ESR to
be the second best marker, with 23% positivity in UC patients compared to none of the controls.

The most important findings that can be reported from this work are: First, a statistically significant elevation in Calprotectin in IBD cases in comparison to controls and second an acceptable incidence of faecal Calprotectin positivity among cases. On the other hand, no correlation was found between faecal Calprotectin and ESR, histopathology and Mayo score. Generally, it should be noted that this is a preliminary work and observations in this situation should be interpreted with great caution.

It is important to highlight the significant difference between cases and control in terms of Calprotectin detection among cases (p 0.01) and the acceptable sensitivity (86%), specificity (70%), PPV (86%) and NPPV (70%) at a cut off of 10.3 γ gm/gm stools. In a study by Carroccio and co-workers [19] that investigated the diagnostic accuracy of faecal Calprotectin, they reported a 64% sensitivity and 80% specificity with 70% positive and 74% negative predictive values in adult IBD cases. Furthermore, Garcia et al., detected sensitivity of 85% and specificity of 81% in adults [20]. In contrast, Canani et al., reported sensitivity of 73% and specificity of 98% in children [21].

In the different studies [22-25] carried out to measure the diagnostic performance of Calprotectin in IBD, an obvious variation in the mean values of Calprotectin in IBD cases were detected. Also, the cut off values calculated by ROC curve to differentiate cases from the controls varied from one study to another. Faecal Calprotectin is a very stable marker and is resistant to degradation (up to 1 week at room temperature, therefore the difference can not be explained by lability of the marker [1]). Alternatively, there are variable assays for the detection of Calprotectin. Some assays were developed to be more sensitive than others. A number of authors have asserted that results obtained from one assay method may be directly compared with results obtained from the other method through simply multiplying the former by factor of 5 [26,27]. An optimal cut off point for distinguishing IBD from other diagnosis has not been identified. The cut off value differs accordingly to the specific assay used and direct comparisons would only be valid if the same assay was being used [28]. Also, Calprotectin was proven to be dependent on the geographic distribution of different populations [1] that is why different manufacturers recommend that each lab has to establish its own normal concentration. Schroder et al., reported cut off of value 15 γ gm/gm stool and sensitivity 93% versus 18 γ gm/gm stool with sensitivity 61% detected by Silberer et al. [25,29]. In our study the mean value of Calprotectin measured in IBD cases was 12.6 γ gm/gm ± 3.2 with a cut off value of 10.3 γ gm/gm stool. In contrast, Xiang and co-workers found the mean value of faecal Calprotectin in UC to be 402.16 ± 48.0 γ gm/gm stools [30]. In another study by Tibble a cut off of 30 γ gm/gm stools had 100% sensitivity in discriminating active IBD from IBS [7] and in another by Costa et al., a faecal concentration of 150 γ gm/gm stools was a strong predictor of relapse [16].

From a clinical and histopathologic aspect, all recruited patients in our study had active disease confirmed by endoscopy and histopathology and the majority of them had moderate (57%) or severe (24%) grading by histopathology as well as moderate-severe grade by Mayo score (61.9%). In spite of this we reported a mean value of Calprotectin among cases 12.6 γ gm/gm stools (± 3.2). Also, no correlation was found between Calprotectin and the histopathological findings. Calprotectin is a very sensitive marker for detection of inflammation in the gastrointestinal tract, yet, there are numerous conditions associated with increased gut permeability, these may be as simple as fasting and total parenteral nutrition or more pronounced as Diabetes mellitus and bacterial overgrowth [7,22], all of these are possible associations with IBD. To our knowledge the exclusion criteria in the previous studies performed on faecal Calprotectin have not clearly excluded these common associations. Although, we didn’t emphasize as well these exclusion criteria; yet our study included smaller number of patients.

An appreciate proportion of earlier studies were primarily conducted on children [4,19,21,24,31]. According to Carroccio and co-workers [19], adults generally have lower normal range of Calprotectin as compared to children probably because of the higher frequency of associated systemic diseases and the more use of drugs that could alter the intestinal mucosal permeability. They also added that the lack of NSAIDs discontinuation at least 3 weeks before running the test is a common cause of Calprotectin false positivity. Actually, NSAIDs therapy causes increased influx of Calprotectin from the gastrointestinal mucosa in up to 44% [32]. In our study all of our patients were adults who were strictly off NSAIDs at least 3 weeks before testing.

Some issues in this work raise considerable concerns like the lack of correlation between Calprotectin and each of ESR, Mayo score and
histopathology. This might be acceptable for ESR which originally correlates poorly with the disease and for Mayo score which is liable to subjective variation. An opposite situation applies for histopathology which strongly reflects the disease severity specially that most of our IBD cases were graded histopathologically as moderate to severe. This may be collectively explained by the small number of studied cases and possible different gut permeability among the Egyptian patients. With respect to endoscopy, Calprotectin was significantly higher among cases with left sided colitis than proctitis and pancolitis without a possible explanation other than that the number of cases with left sided colitis was higher among cases (11 patients 52%).

It is concluded that Calprotectin is a good test in differentiating Egyptian patients with ulcerative colitis from healthy controls. Thus, its use as a screening test may be helpful in the selection of cases for endoscopic examination. It lacks specific correlation with the severity of ulcerative colitis. This leaves endoscopy and histopathologic examinations as the main diagnostic tools. Larger scale studies on Egyptian patients are strongly recommended with special reference to the local mucosal permeability and immune milieu of the Egyptian population.

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