The diagnostic value of faecal calprotectin in differentiating inflammatory bowel diseases (IBD) from irritable bowel syndrome (IBS)


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Abstract: Background: Patients with inflammatory bowel disease and irritable bowel syndrome can have overlapping symptoms, yet a different management. Hence, a noninvasive biological marker is needed for the assessment of patients with lower bowel symptoms. Aim: This study aimed at evaluating the the diagnostic value of faecal calprotectin as a potential marker in differentiating patients with inflammatory bowel disease from those with a irritable bowel syndrome. Methods: twenty patients with IBD and twenty patients with IBS were recruited from Ain shams university outpatient clinic in the period between January 2008 to November 2009. In addition, a control group of 10 healthy individuals was included. Faecal calprotectin level using an ELISA technique (Calprest®) was measured in the stool of all groups. Also, atypical p-ANCA and ASCA were performed in the IBD group. Results: At a cut off value of 8.1 mg/L, fecal calprotectin had a negative predictive value (NPV) of 100% to exclude IBS patients with a sensitivity of 100% and a positive predictive value (PPV) to confirm IBD of 95.24% with a specificity of 95%. The diagnostic accuracy of faecal calprotectin in predicting IBD activity was 100% at a cut off value of 25.5 mg/L. Conclusion: fecal calprotectin appears to be a clinically useful noninvasive marker in differentiating IBD from IBS.

INTRODUCTION:

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), is a chronic, idiopathic inflammatory condition of the gut with a typically relapsing and remitting course. Exacerbations are characterized by symptoms of diarrhea, urgency of defecation and occasionally rectal bleeding and abdominal pain. The aim of treatment is to induce and maintain disease remission (Hanauer, 2006). Gastroenterologists are sometimes faced with the diagnostic difficulty of differentiating patients with irritable bowel syndrome (IBS) from those with organic intestinal pathology, in particular inflammatory bowel disease (IBD). They feel compelled to exclude all organic diseases using invasive diagnostic investigations as objective evidence for there being no other significant pathology. This has significant implications for health care costs as well as exposing patients to the inherent risks associated with invasive procedures (Tibble and Bjarnason, 2001).
enteric infection, would be more specific for IBD (Pardi and Sandborn, 2005). Although all faecal biomarkers studied provide a reliable and simple non invasive means in the differentiation of IBD and IBS, calprotectin appears to represent the most accurate marker to discriminate these two common causes of chronic diarrhea (Schröder et al., 2007).

Calprotectin, a 36 KDa calcium and zinc binding protein, is probably the most promising marker for various reasons. In contrast with other neutrophil markers, calprotectin represents 60% of cytosolic proteins in granulocytes. The presence of calprotectin in faeces can therefore be considered directly proportional to neutrophil migration to the gastrointestinal tract (Vermeire et al., 2006).

AIM OF THE STUDY:

The primary aim of the present study was to assess the diagnostic value of faecal calprotectin in differentiating IBD from IBS.

SUBJECTS AND METHODS:

Study subjects:

This study has been conducted on twenty patients with (IBD) inflammatory bowel diseases and twenty patients with IBS recruited from the outpatient clinic of gastroenterology in Ain Shams University Hospital in the period from January 2008 to November 2009 in addition to 10 healthy control subjects.

History and Clinical Examination: All patients were subjected to full history taking with special emphasis on abdominal pain, weight loss, rectal bleeding, diarrhea, constipation, malaise, lethargy, anorexia, nausea, tenesmus, abdominal distension, passage of mucous, vomiting and low-grade fever, along with full clinical examination.

Exclusion Criteria: Patients with positive stool culture, past history of any malignant condition, past history of major gastrointestinal surgical procedures, liver cell failure, chronic renal failure, congestive heart failure and/or bleeding tendency were excluded from the study, in addition to patients on non steroidal anti-inflammatory drugs.

Abdominal ultrasound: To exclude the presence of associated diseases or complications.

Colonoscopy, biopsy and histopathology: Total colonoscopy with ileoscopy and biopsy sampling was performed in all patients to differentiate UC from other IBDs, asses the severity and extent of endoscopic findings if present, take biopsy samples from diseased and healthy mucosa to give idea about the histopathological criteria if present giving confirmation to the diagnosis.

Laboratory investigations: Eight milliliters of whole blood were collected onto 3 tubes; 3 ml on EDTA for CBC and ESR; 2 ml of blood were collected onto citrate for immediate assay of P.T and P.T.T and lastly 3 ml blood were collected into plan tube, prompt separation of serum was done for assay of fasting blood glucose, kidney & liver function tests, electrolytes and CRP. Part of serum was stoted at – 200 C until assay of ASCA and P-ANCA. Another sample was withdrawn after two hours for assay of postprandial blood glucose.

Complete blood count was performed using coulter B66, Miami, Florida, USA, Liver function tests including total protein, serum albumin, AST, ALT, total and direct serum bilirubin and kiney function tests including serum creatinine, blood urea nitrogen as well as electrolytes (Na & K) and glucose were all done on Synchron CX9 autoanalyzer (Brea, California, USA), P.T. and P.T.T. using coagulometer. Quantitative CRP was done using nephelometry.

Complete stool analysis and stool culture: To exclude the presence of infection.

Faecal calprotectin: A single stool sample (about 5 gm weight) placed in a suitable disposable container is sent to the laboratory on the same day under temperature < 30ºC. About 100 mg of the faecal sample is added to 4.9 ml of diluted extraction solution in a screw cap tube which is then shaken vigorously for 30 seconds by means of a vortex mixer then homogenized 30 minutes on a shaker or roller. 1 ml of the homogenate is transferred to an Eppendorf tube and centrifuged at 10 000 rpm for 20 minutes. Then 0.5 ml of the clear extract supernatant is transferred to another Eppendorf tube and tested immediately by an ELISA technique named Calprest® (Eurospital SpA, 34147 Trieste. Via Flavia 122) which uses a polyclonal antibody against calprotectin in an enzyme linked immune-sorbent assay system. Calprotectin presented in the diluted sample is bound by the antibody adsorbed to the surface of the plastic well. The enzyme conjugated antibody binds to the captured antigen and subsequently the enzyme catalyses the conversion of the substrate to a coloured product. The intensity of the colour is proportional to the amount of conjugate bound, and thus to the amount of captured calprotectin. Concentration of calprotectin in the samples is calculated using the provided samples. (Normal values < 15 mg/L or < 37.5 mg/kg).

ASCA and P-ANCA detection for the first group only:

ASCA IgA and IgG detection was done by the QUANTA-Lite ELISA assay (Inova Diagnostics, San Diego, CA), while atypical P-ANCA detection was done by Indirect immunoflourescence (Inova Diagnostics, San Diego, CA/EurolImmun, Germany).
Statistical analysis of the results (Data management):

Data were collected, revised, verified then edited on PC. Data were then analyzed statistically using SPSS statistical package version (16). Descriptive statistics were presented as mean ± SD and frequency according to the nature of the data. Comparative analysis was performed by ANOVA test with calculation of the least significant difference and Chi square test. Pearson correlation coefficient (r) was calculated for variables correlation. ROC curve (Receiver operating characteristic curve) was presented along with calculation of diagnostic performance of studied parameters. P value was considered significant if less than 0.05, non significant if more than 0.05 and highly significant if less than 0.01.

RESULTS:

This study has been conducted on 20 patients with (IBD) inflammatory bowel diseases versus 20 patients with (IBS) irritable bowel syndrome in addition to 10 healthy persons as control. IBD group was further sub classified according to nature of the disease into 15 patients with ulcerative colitis and 5 patients with Crohn’s disease. According to UC activity index (Truelove and Witts Severity Index), 7 UC patients were inactive (mild activity index) and 8 patients were active (3 patients had moderate activity index and 5 patients had severe activity index). Moreover, according to Crohn’s disease activity index, 3 patients had inactive disease while 2 patients were active. The three studied groups were age- and sex-matched.

Comparison between the studied groups as regard laboratory parameters revealed that active IBD patients had statistically significant hyponatremia, hypokalemia, hypoproteinemia and hypoalbuminemia with higher serum BUN values in comparison to IBS patients and control group (P value<0.05). Creatinine was the only laboratory parameter that showed non-significant difference between studied groups (P value>0.05). On the other hand, regarding hematological parameters, active IBD patients had a statistically highly significant higher TLC, PLT count and lower HB values in comparison to other groups (P value<0.01).

All IBS and inactive IBD patients had normal colonoscopy while positive findings were present in active UC patients in the form of mucosal ulceration, oedema, erythema and pseudopolyps, and in active CD patients in the form of deep ulcers with cobble stone appearance with patchy distribution.

Histopathological examination was free in all IBS patients however positive findings were present in all active IBD patients, in addition to 2 (28%) out of 7 inactive UC and 2 (66.7%) out of 3 inactive CD patients.

All CD patients had negative atypical P-ANCA while it was positive in 57.1% of inactive UC and 62.5% of active UC patients (P value<0.05), so it might be helpful in differentiating UC from CD however it was not related to disease activity. On the other hand, all UC patients had negative ASCA IgA and IgG while active CD patients had a statistically highly significant higher IgA and IgG values compared to inactive patients (P value<0.01), therefore ASCA could differentiate UC from CD, moreover it might be helpful in differentiating active from inactive CD patients (Table 1).

Patients with IBD had a statistically highly significant higher faecal calprotectin values (28.86±17.32) in comparison to IBS patients (5.45±2.3) and control (1.9±1.85) (P value<0.01), moreover IBS patients had higher values than control but that was statistically non significant (P value<0.05). Accordingly, faecal calprotectin could be used in differentiation between IBD from IBS patients (Table 2).

Active IBD patients had statistically significant higher values of ESR (active UC=80±19; active CD= 80±14) than IBS patients and control group (IBS-D=17±9; IBS-C= 21±7; control= 12±3) (P value<0.01). Same pattern was found with significant higher values of CRP in active IBD (active UC=9.1±3.1; active CD=10.0±5.6) in comparison with IBS patients and control group (IBS-D=0.6±1; IBS-C= 0.6±1; control= 0) (P value<0.01). On the other hand, inactive patients had nearly similar results to IBS patients and control (P value>0.05), so ESR and CRP might be used as markers for disease activity (Table 3).

Faecal calprotectin showed highly significant positive correlation with TLC (r=0.858, p<0.01), PLT (r=0.688, p<0.01), ESR (r=0.887, p<0.01), CRP (r=0.893, p<0.01) and UC disease activity index (r=0.815, p<0.01) and highly significant negative correlation with HB (r=-0.774, p<0.01), while there was no correlation with CD activity index (r= 0.819, p>0.05). Also, UC disease activity index showed highly significant positive correlation with TLC (r=0.760, p<0.01), PLT (0.720, p<0.01), ESR (r=0.821, p<0.01), CRP (r=0.578, p<0.01) and highly significant negative correlation with HB (r=-0.681, p<0.01). On the other hand, CD disease activity index showed significant positive correlation with TLC (r=0.891, p<0.05) and ESR (r=0.929, p<0.05), significant negative correlation with HB (r=-0.916, p<0.05), and non significant correlation with PLT (r=0.471, p<0.05) and CPR (r=0.801, p>0.05) (Table 4).
Table (1): Comparison between IBD patients as regard atypical P-ANCA antibody and ASCA IgA and IgG antibodies

<table>
<thead>
<tr>
<th></th>
<th>P-ANCA ANTIBODIES</th>
<th>ASCA ANTIBODIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Inactive UC</td>
<td>n=7</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>Active UC</td>
<td>n=8</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Inactive CD</td>
<td>n=3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Active CD</td>
<td>n=2</td>
<td>2 (100%)</td>
</tr>
</tbody>
</table>

X²/F* value 7.38  
P value <0.05

Table (2): Comparison between IBS patients, IBD patients and controls as regard faecal Calprotectin

<table>
<thead>
<tr>
<th></th>
<th>IBD (no=20)</th>
<th>IBS (no=20)</th>
<th>Controls (no=10)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal Calprotectin</td>
<td>28.86±17.32 (8.2 - 62)</td>
<td>5.45±2.3 (2 - 10)</td>
<td>1.9±1.85 (0 - 5)</td>
<td>29.561</td>
<td>&lt;0.01 (H.S)</td>
</tr>
</tbody>
</table>

Table (3): Comparison between the studied groups as regards ESR, CRP and faecal Calprotectin values

<table>
<thead>
<tr>
<th></th>
<th>ESR</th>
<th>CRP</th>
<th>Calprotectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive UC</td>
<td>7</td>
<td>16±4 (10 – 21)</td>
<td>2.1±2 (0 – 5)</td>
</tr>
<tr>
<td>Active UC</td>
<td>8</td>
<td>80±19 (55 – 110)</td>
<td>9.1±3.1 (6 – 15)</td>
</tr>
<tr>
<td>Inactive CD</td>
<td>3</td>
<td>26±9 (17 – 35)</td>
<td>1.3±1.5 (0 – 3)</td>
</tr>
<tr>
<td>Active CD</td>
<td>2</td>
<td>80±14 (70- 90)</td>
<td>10.0±5.6 (6 – 14)</td>
</tr>
<tr>
<td>IBS-D</td>
<td>10</td>
<td>17±9 (8 – 30)</td>
<td>0.6±1 (0 – 2)</td>
</tr>
<tr>
<td>IBS-C</td>
<td>10</td>
<td>21±7 (10 – 35)</td>
<td>0.6±1 (0 – 3)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>12±3 (8 – 15)</td>
<td>0</td>
</tr>
</tbody>
</table>

F value 53.236  
P value <0.01 (HS)

Table (4): Correlation between UC activity index, CD activity index, fecal calprotectin and other studied parameters

<table>
<thead>
<tr>
<th></th>
<th>UC activity Index</th>
<th>CD activity index</th>
<th>Fecal Calprotectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>P value</td>
<td>r value</td>
</tr>
<tr>
<td>TLC</td>
<td>0.760</td>
<td>H.S</td>
<td>0.891</td>
</tr>
<tr>
<td>HB</td>
<td>-0.681</td>
<td>H.S</td>
<td>-0.916</td>
</tr>
<tr>
<td>PLT</td>
<td>0.720</td>
<td>H.S</td>
<td>0.471</td>
</tr>
<tr>
<td>ESR</td>
<td>0.821</td>
<td>H.S</td>
<td>0.929</td>
</tr>
<tr>
<td>CRP</td>
<td>0.578</td>
<td>S</td>
<td>0.801</td>
</tr>
<tr>
<td>Fecal Calprotectin</td>
<td>0.815</td>
<td>H.S</td>
<td>0.819</td>
</tr>
</tbody>
</table>

The diagnostic accuracy of faecal calprotectin was better than CRP as at a cut off value of 8.1 mg/L, it had a negative predictive value (NPV) of 100% to exclude IBS patients with a sensitivity of 100% and a positive predictive value (PPV) to confirm IBD of 95.24% with a specificity of 95% in comparison to CRP diagnostic values which were lower at its best cut off value of 2.5 mg/L. The diagnostic accuracy of faecal calprotectin and CRP in predicting IBD activity was 100%, cut off value of faecal calprotectin of 25.5 mg/L and of CRP of 5.5 mg/L had 100% specificity, sensitivity, PPV and NPV with an AUC = 1 (Table 5, Figure 1).
Table (5): Diagnostic validity of faecal calprotectin and CRP in discriminating IBD from IBS patients

<table>
<thead>
<tr>
<th></th>
<th>Differentiation between IBD and IBS</th>
<th>Differentiation between active IBD and inactive IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calprotectin (8.1mg/L)</td>
<td>CRP (2.5mg/L)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>70%</td>
</tr>
<tr>
<td>Specificity</td>
<td>95.0%</td>
<td>95%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>95.24%</td>
<td>93.33%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>100%</td>
<td>76%</td>
</tr>
<tr>
<td>Diagnostic Accuracy</td>
<td>97.5%</td>
<td>82.5%</td>
</tr>
<tr>
<td>AUC</td>
<td>0.996</td>
<td>0.863</td>
</tr>
</tbody>
</table>

Figure 1: Diagnostic performance of fecal calprotectin and CRP in differentiation between IBD and IBS (p<0.01)

DISCUSSION:

As serum markers of inflammation can be elevated in a variety of conditions, it seems likely that faecal markers of inflammation, in the absence of enteric infection, would be more specific for IBD (Pardi and Sandborn, 2005). Although all faecal biomarkers studied provide a reliable and simple non-invasive means in the differentiation of IBD and IBS, calprotectin appears to represent the most accurate marker to discriminate these two common causes of chronic diarrhea (Schröder et al., 2007). Faecal calprotectin is easy to measure, resistant to proteolysis and stable in stool for 7 days, and thus has been proposed as a simple non-invasive investigative tool, which may help distinguish inflammatory from functional disorders (Fagerberg et al., 2005). The present study was conducted on 40 patients (20 with IBD and 20 with IBS) and 10 healthy persons to reveal the diagnostic value of faecal calprotectin in differentiating IBD from IBS.

In the current study, it was found that active IBD patients had statistically significant
hyponatremia, hypokalemia, hypoalbuminemia and hypoproteinemia in comparison to IBS patients and control. This goes with Cucino and Sonnenberg (2001) study which revealed that severe cases of UC and CD were found to be associated with protein/calorie malnutrition, hypoalbuminemia, hypoproteinemia and electrolyte disturbances.

Active IBD patients had a statistically highly significant higher TLC, PLT count and lower HB values in comparison to IBS and inactive IBD. This was in agreement with Tibble et al. (2000) who found higher TLC and PLT count in active CD patients in comparison to patients with quiescent disease and IBS patients. This could be explained by the fact that these parameters are increased in inflammatory conditions as acute phase reactants.

When studying atypical p-ANCA, it was found that it was helpful in differentiating UC from CD however it was not related to disease activity. Similar results were found in the study conducted by Papp et al. (2007) who found that atypical p-ANCA was present in the sera of 40% to 80% of patients with UC and to a lesser extent in CD (5%-25%), also no correlation was found between the presence of atypical p-ANCA and the activity of the disease. Regarding ASCA, It could also differentiate UC from CD; moreover it might be helpful in differentiating active from inactive CD patients. Papp et al. (2007) found that ASCA was more frequently detected in patients with CD (50%-80%) compared to patients with UC (2%-14%). These findings agreed with that detected in the present study. However, as regards the correlation with the disease activity, Papp et al. (2007) found that the antibody titers were relatively stable and didn’t correlate with the disease activity. This could be explained by the small sample size of CD patients.

In the current study, it was found that ESR and CRP were helpful in differentiating active IBD from IBS and inactive IBD so they might be used as markers of disease activity. Similarly Xiang et al. (2008) found that the patients with active UC had higher levels of CRP and ESR than patients with inactive UC and control group. Also, Tibble et al. (2000) found that the median ESR and CRP values in patients with active CD were significantly higher compared to patients with inactive CD and IBS.

As regard faecal calprotectin, it appeared to be clinically useful in differentiating IBD from IBS and that was highly significant statistically. Similarly, Tibble et al. (2000), Carroccio et al. (2003) and Schoepfer et al. (2008) found that faecal calprotectin was significantly elevated in IBD patients, or the CD subgroup when compared to IBS patients.

In various studies conducted on IBD patients with different activity patterns, Loftus et al. (2007), Langhorst (2008) and Xiang et al. (2008) found that the faecal calprotectin concentrations were significantly higher in the active group in comparison to inactive patients group. Similarly, in the present study, faecal calprotectin was helpful in differentiating active from inactive IBD patients with a high statistical significance.

Moreover, faecal calprotectin values were higher in inactive IBD patients compared to IBS patients and control. Likewise Xiang et al. results showed that faecal calprotectin concentration was higher in the patients with inactive UC than in the controls (Xiang et al., 2008). Also, Tibble et al. (2000) results showed that inactive IBD patients had higher faecal calprotectin compared to IBS patients and control.

Focusing on the evaluation of relationship that might exist between the mucosal neutrophil infiltrations represented by fecal calprotectin and TLC, PLT count, ESR, CRP and disease activity indices, the present study revealed that faecal calprotectin correlated significantly with the TLC, PLT count, ESR, CRP and UC activity index, however non-significantly with CD activity index. Similar findings were found in various studies. Xiang et al. (2008) found a good correlation between the concentrations of faecal calprotectin, ESR, CRP and UC activity index in UC patients. Also, Tibble et al. (2000) found a good correlation between faecal calprotectin, TLC, PLT count, ESR and CRP. However, the controversy was detected on correlating fecal calprotectin with CD activity index. As detected in the present study, there was non-significant correlation between both parameters. Similar findings were encountered by Tibble et al. (2000) and Gaya et al. (2005). On the other hand, Vermiere et al. (2004) found a good correlation between both parameters.

Regarding hemoglobin level, in agreement with Eder et al. (2008), it was found that fecal calprotectin inversely correlate with hemoglobin level.

On calculating the diagnostic performance, calprotectin, at its best cut-off as a marker for differentiation of IBD from IBS was much better in comparison to CRP. Calprotectin has an area under the curve (AUC) of 0.996, while CRP was 0.863. Faecal calprotectin had sensitivity, specificity, PPV and NPV of 100%, 95%, 95.24% and 100%, respectively when measured at 8.1 mg/L, while CRP had values of 70%, 95%, 93.33% and 76%.

Close results were extracted from data collected from other studies. Tibble et al. (2000) found that at a cutoff point of 30 mg/l, faecal calprotectin had 100% sensitivity and 97% specificity in discriminating between active Crohn's disease and
the irritable bowel syndrome. Tibble et al. (2002) found that faecal calprotectin at cut off value of 10 mg/L had maximal sensitivity and specificity of 89% and 79% respectively with a PPV of 76% and a NPV of 89% in differentiating patients with organic and non organic intestinal diseases. Carroccio et al. (2003) found that the calprotectin value with the highest diagnostic accuracy was 170 µg/g: it was 100% sensitive and 95% specific in differentiating CD from IBS adult patients. Schoepfer et al. (2008) used the PhiCal test (another method) in the measurement of faecal calprotectin and found that it had specificity, sensitivity, PPV and NPV of 83%, 100%, 100% and 74% at a cut off value of 50 µg/ml faeces (the cut off value provided by the manufacturer) in differentiating IBD from IBS patients. Also, ROC curve analysis showed a sensitivity of 95%, specificity of 91%, and an area under the curve (AUC) of 0.95 for the diagnosis of IBD (von Roon et al., 2007).

Finally, both faecal calprotectin and CRP showed a 100% diagnostic accuracy in discriminating active from inactive IBD at values of 25.5 mg/L and 5.5 mg/L respectively. However, Xiang et al. (2008) found that the faecal calprotectin at a cut off value of 50 µg/g and CRP at a cut off value of 5 mg/L had a specificity of 79.4%, 69% and a sensitivity of 91.9%, 62.2% respectively in differentiating active from inactive UC patients. Also, Gaya et al. (2005) in their study on CD patients, found that faecal calprotectin at levels >100 µg/g gave a sensitivity of 80%, specificity of 67%, PPV of 87%, NPV of 64% and an accuracy of 87% in identifying those with and without any inflammation.

In conclusion, faecal calprotectin appear to be a clinically useful marker in differentiating IBD from IBS as well as active from inactive IBD.

REFERENCES:


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