INTRODUCTION

Inflammatory bowel disease (IBD), which includes both Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic idiopathic inflammatory disorder affecting the gastrointestinal tract.

In view of clinical management, it is essential to determine the disease activity and to achieve mucosal healing (MH) which is reported to be associated with sustained remission and a reduced risk of surgery in IBD. To evaluate activity in a given patient, doctors rely on a combination of clinical and endoscopic findings as well as levels of laboratory biomarkers. Nowadays, a gold standard for assessing the site, extension, and severity of the intestinal inflammation is the endoscopy with biopsy. However, endoscopic procedures are invasive, with a substantial risk of complications. Such procedures can be painful and frequently require use under general anesthesia, which may be dangerous for the patient. Bowel preparation for colonoscopy is unpleasant and could be hazardous in some situations. Moreover, the standard criteria for the evaluation of disease severity as well as the definitions of MH on colonoscopy are still unclear.

On the other hand, patient symptoms cannot reliably reflect the extent of disease and response to therapy, and their correlation with endoscopic activity is often limited.

ORIGINAL ARTICLE

Fecal Calprotectin for Assessment of Inflammatory Bowel Disease Activity

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ABSTRACT

Background: Fecal calprotectin (FCP) is frequently used for monitoring inflammatory activity and prediction of relapse in inflammatory bowel disease (IBD). We aimed to assess the usefulness of FCP as a marker of disease activity in patients with IBD. Methods: This prospective study enrolled 174 patients (84 with Crohn’s disease [CD] and 90 with ulcerative colitis [UC]) referred for colonoscopy to our center. FCP was analyzed in stool samples by means of a point-of-care Quantum Blue® method. Results: Mean FCP values in patients with colonic or ileocolonic CD were significantly higher than in patients with ileal CD (<0.001) and quiescent CD (<0.001). Mean FCP levels in quiescent CD were higher than those of controls (P = 0.001). Patients with active ileal CD had significantly higher FCP values than quiescent CD patients (<0.001). A cutoff value of 315 μg/g predicted mucosal inflammation in CD patients with 94% sensitivity and 98% specificity. There was no significant difference between mean FCP levels in controls and UC patients in remission (P = 0.205). Mean FCP in patients with active UC was significantly higher than in controls (P < 0.001) and in patients in remission (P < 0.001). Conclusion: FCP is a reliable surrogate marker of endoscopic activity in IBD. FC could be useful in the prediction of mucosal healing. Moreover, the rapid point-of-care method allows an easier assessment of FCP in clinical practice.

Key words: Fecal calprotectin, inflammatory bowel disease, mucosal healing

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Moreover, increased Calprotectin is a small calcium-binding protein consisting of two heavy and one light polypeptide chains. It is found in abundance in neutrophil granulocytes, in which it accounts for 60% of the cytosolic fraction, as well as in monocytes and macrophages.

Fecal markers, specifically, and calprotectin may be more specific for assessing intestinal disease activity. Calprotectin is used for monitoring IBD activity.

Fecal calprotectin (FCP) is a biomarker that is frequently used for monitoring IBD activity. Moreover, increased concentrations of FCP have been reported to be indicative of clinical relapse in IBD patients.

In the current study, we aimed to assess the usefulness of FCP as a marker of disease activity in patients with IBD.

MATERIALS AND METHODS

Study design, inclusion and exclusion criteria of enrolled subjects

This prospective observational case-control study enrolled 174 patients - 80 males and 94 females at an average age of 39.8 ± 9 (19–68) years with IBD (84 with CD and 90 with UC), referred for colonoscopy to the Clinic of Gastroenterology of “Tsaritsa Yoanna” University Hospital in Sofia between May 2015 and April 2017.

We included patients who responded to all inclusion and exclusion criteria. Inclusion criteria for were: (1) Age 18–85 years, (2) known UC or CD diagnosed according to the ECCO Guidelines and (3) completion of a written informed consent.

Exclusion criteria were: (1) Colorectal cancer or colon polyps, (2) indeterminate colitis, (3) history of colorectal surgery, (4) urinary incontinence (due to the risk of contamination of fecal samples), (5) pregnancy, (6) history of active nonsteroidal anti-inflammatory drugs intake (2 tablets/week), (7) oral steroids or steroid enemas intake in the past 3 months, or initiation of azathioprine treatment within the past 3 months, (8) infectious colitis, and (9) primary immunodeficiency.

We defined clinical remission in UC as a Lichtiger Clinical Activity Index of 3 points or less and endoscopic remission - as UC Endoscopic Index of Severity of 0 or 1 if the descriptor was limited to vascular pattern. Clinical remission in CD was determined as CD activity index <150, and endoscopic remission - as lack of mucosal lesions (erosions, ulcers, and aphthous lesions) on ileocolonoscopy.

All enrolled patients underwent a full medical assessment including a detailed medical history and physical examination. All therapies taken by the patients before enrollment were recorded. All patients underwent ileocolonoscopy. Indications for colonoscopy were clinically active disease (flare), evaluation of disease activity after treatment, having unexplained symptoms, and dysplasia surveillance for the longstanding disease.

Fecal samples were collected within 1–2 days before the colonoscopy. FCP levels were assessed in all the patients and 18 subjects, taken as a control group.

Fecal calprotectin

Calprotectin was analyzed in stool samples by means of point-of-care desktop Quantum Blue Reader® (POC Reader) method. It is a lateral flow technology based on ELISA techniques. We performed the test according to the manufacturer’s instructions (Quantum Blue® Calprotectin, Bühlmann Laboratories AG, Switzerland). The POC device uses internal standards within a range of 30–300 μg/g and sensitivity of <10 μg/g, thus, guarantying consistency in results. When we received results >300 μg/g, we performed additional 1:10 dilution with extraction buffer according to the manufacturer’s instructions, allowing us to receive FCP levels up to 3000 μg/g. FCP values above the upper limit of the measurement ranges were registered as 3000 μg/g, and FCP values below the lower limit were accordingly registered as 30 μg/g.

Statistical analysis

The statistical analysis was performed using SPSS for Windows, Version 25.0. (SPSS Inc., Chicago, USA). For data analysis, the following statistical methods were used: Descriptive statistics for the tabular and graphical presentation of results, Kolmogorov-Smirnov test, Shapiro-Wilk test, Mann-Whitney test, and receiver operating characteristic (ROC) curve analysis - to determine the cutoff of the quantitative variables. The obtained results were assessed as statistically reliable in the threshold level of significance $P < 0.05$.

Ethics approval

The study was approved by the Ethics Committee of “Tsaritsa Yoanna” University Hospital in Sofia, Bulgaria. Before initiating this study, written informed consent was obtained from all patients and healthy controls included in the study.
The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution’s human research committee.

RESULTS

A total of 84 patients with CD, 90 with UC and 18 controls (Group 1) were enrolled in this study. 41 of CD patients were with quiescent disease (Group 2), 20 with active ileal disease (Group 3), and 23 with active colonic or ileocolonic CD (Group 4). 40 of UC patients were with quiescent disease and 45 with active.

An overview of the demographic patient characteristics at baseline is provided in Table 1.

Mean FCP levels of patients with quiescent CD (97.12 ± 85.48 µg/g) were significantly higher than those of controls (34.72 ± 7.43 µg/g) (P = 0.001). There was a statistical significance between mean FCP levels of controls and patients with active ileal CD (958.44 ± 694.25 µg/g) (P < 0.001), patients with active colonic or ileocolonic CD (2143.35 ± 882.29 µg/g) (<0.001), and patients with active CD irrespective of location (Group 3 and 4) - (1533.97 ± 984.05 µg/g) (<0.001). There was a significant difference between mean FCP levels of quiescent CD and active ileal CD (<0.001), colonic or ileocolonic CD (<0.001), and active CD irrespective of location (<0.001). Furthermore, FCP concentrations of patients with active ileal CD were significantly lower than those with active colonic or ileocolonic CD (<0.001) [Table 2 and Figure 1].

The ROC analysis found that a cutoff FCP level of 315 µg/g differentiates quiescent CD from active ileal disease with 94% sensitivity, 98% specificity, and area under the curve (AUC) 0.984 [Figure 2]. The same cutoff FCP level (315 µg/g) differentiates quiescent CD from active CD irrespective of disease location with the same sensitivity (84%), and specificity (98%), and AUC 0.989 [Figure 3].

In UC patients, we did not found statistically significant difference between mean FCP levels of controls (34.72 ± 7.43 µg/g) and quiescent UC (47.10 ± 26.91 µg/g) (P = 0.205). However, patients with active UC had significantly higher mean FCP levels (1933.08 ± 940.98 µg/g) than controls (P < 0.001) and patients with quiescent UC (P < 0.001) [Table 3 and Figure 4].

DISCUSSION

At present, there are sufficient data in the literature demonstrating relationship between FCP concentrations and severity of

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td>Females (%)</td>
<td>45 (53.6)</td>
<td>49 (54.4)</td>
</tr>
<tr>
<td>Age</td>
<td>40.2±9 (19–60)</td>
<td>39.4±9 (18–63)</td>
</tr>
<tr>
<td>Disease duration, mean±SD (years)</td>
<td>4.78±3.8</td>
<td>4.2±3.5</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>20 (23.8)</td>
<td>17 (18.8)</td>
</tr>
<tr>
<td>Location of disease (%)</td>
<td>L1 (ileal) - 26 (30.9)</td>
<td>E1 (proctitis) - 13 (14.4)</td>
</tr>
<tr>
<td></td>
<td>L2 (colonic) - 25 (29.8)</td>
<td>E2 (left-sided colitis) - 42 (46.7)</td>
</tr>
<tr>
<td></td>
<td>L3 (ileocolonic) - 33 (39.3)</td>
<td>E3 (extensive colitis) - 35 (38.9)</td>
</tr>
<tr>
<td>Disease phenotype</td>
<td>B1 (inflammatory) - 71 (84.5)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B2 (stricturing) - 8 (9.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B3 (penetrating) - 5 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Fecal calprotectin (FCP) values in controls (Group 1), quiescent Crohn’s disease - CD (Group 2), active ileal CD (Group 3), active colonic or ileocolonic CD (Group 4), and in all active CD patients (Groups 3 and 4)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean FCP</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (controls)</td>
<td>18</td>
<td>34.72</td>
<td>7.43</td>
<td>31.00</td>
<td>30.00</td>
<td>58.00</td>
</tr>
<tr>
<td>Group 2 (quiescent CD)</td>
<td>41</td>
<td>97.12</td>
<td>85.48</td>
<td>60.00</td>
<td>30.00</td>
<td>360.00</td>
</tr>
<tr>
<td>Group 3 (ileal CD)</td>
<td>20</td>
<td>958.44</td>
<td>694.25</td>
<td>800.00</td>
<td>136.00</td>
<td>3000.00</td>
</tr>
<tr>
<td>Group 4 (colonic or ileocolonic CD)</td>
<td>23</td>
<td>2143.35</td>
<td>882.29</td>
<td>2150.00</td>
<td>247.00</td>
<td>3000.00</td>
</tr>
<tr>
<td>Group 3 and Group 4</td>
<td>43</td>
<td>1533.97</td>
<td>984.05</td>
<td>1460.00</td>
<td>136.00</td>
<td>3000.00</td>
</tr>
</tbody>
</table>

CD: Crohn’s disease
intestinal inflammation in IBD. Therefore, FCP assessment is essential for the determination of remission and activity in these patients. The main goal of the modern IBD treatment is not only symptom amelioration but also MH achievement.

Even though endoscopy with biopsies is the best way to evaluate MH, repetitive endoscopic examinations cannot be an integral part of any management strategy for IBD because of the well-known drawbacks of this technique. Therefore, FCP is increasingly being used as an alternative for MH assessment.

Several studies have previously suggested FCP as a marker for endoscopic disease activity in IBD patients. However,
some studies have shown contrary results.[7,21,22] Several studies showed a significant correlation between FCP levels and endoscopic activity in both UC and CD.[22-24] Røseth et al. performed colonoscopy with biopsies on 45 patients with IBD in clinical remission and found that 38 of them had both normal FCP values and normal histology.[21] Lamb et al. reported that patients with UC in endoscopic and histological remission had similar FCP values to controls.[25] This proved the presence of MH and therefore suggested that FCP could be considered as a surrogate marker of MH.

In the current study, we did not find a statistically significant difference between FCP levels in controls and UC patients in remission and thus confirmed the presence of MH. In a large study by Schoepfer et al., the mean FCP value in UC patients in remission was 42.0 μg/g, which was very close to the results in the present study - 47.10 μg/g.[26] In some patients with inactive UC, low-grade intestinal inflammation might occur, with higher than normal FCP values, but usually not exceeding 150 μg/g.[26] In line with that, in our group of patients with UC in remission, the highest FCP value was 136 μg/g. On the other hand, FCP concentrations in patients with active UC were so significantly higher than those of controls and patients in remission that we could not even detect a cutoff level between activity and remission in UC patients.

In our study, we demonstrated that a cutoff value of 315 μg/g predicts mucosal inflammation in CD patients with 94% sensitivity and 98% specificity. Similar results are shown by other authors. D’Haens et al. reported that a cutoff of 250 μg/g could predict mucosal inflammation with 94% sensitivity and 62% specificity in CD patients.[27] Vázquez-Morón et al. showed that a cutoff concentration of ≥170 μg/g (sensitivity 77.6% and specificity 95.5%) predicts a high probability of endoscopic activity.[28]

Some authors believe that FCP levels may depend on the location of inflammatory changes in CD.[7,29] However, the data are currently insufficient and controversial. In the present study, we demonstrated that FCP values in patients with the colonic or ileocolonic location were significantly higher than those with ileal disease and the latter were significantly higher than those with quiescent disease.

Although one of the most commonly reported drawbacks of FCP is its decreased diagnostic value in patients with ileal disease, in our study FCP values of all patients with active ileal CD were increased. Furthermore, a cutoff value of 315 μg/g distinguishes with the same sensitivity (94%) and specificity (98%) quiescent CD from active ileal disease and active CD regardless of location.

Probably, the biggest strength of the study is that this is one of the first observations of a large cohort of patients with IBD examined with point-of-care Quantum Blue® method. We find this test really useful, because it is simple to use, can be done in doctor’s office and is quite fast (results can be obtained in <30 min including protein extraction). Another major advantage is the simplicity of sample preparation and analysis. No more than 80 mg stool sample is required for the assessment and sample preparation and analysis is user-friendly and does not involve the need of special equipment, which makes it ideally suited for every lab and even doctor’s office. Moreover, the point-of-care test can serve as a reliable alternative to ELISA.[30,31] It has been shown that Quantum Blue® is the instrument of choice for fast and reliable determination of FCP levels.[24,30,31]

CONCLUSIONS

FCP is a reliable surrogate marker of endoscopic activity in IBD. FCP could be useful in the prediction of MH. Therefore, FCP has the potential to replace colonoscopy for the serial assessment of mucosal inflammation in IBD patients. Moreover, the rapid point-of-care method allows an easier assessment of FCP in clinical practice.

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**How to cite this article:** Nakov RV, Nakov VN, Tankova LT, Gerova VA, Kandrzhiev TG. Fecal Calprotectin for Assessment of Inflammatory Bowel Disease Activity. Clin Res Immunol 2018;1(1):1-6.