

# Irritable bowel syndrome–like symptoms in patients with inflammatory bowel disease in clinical remission phase are related to gut inflammation

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**BACKGROUND:** Symptoms consistent with irritable bowel syndrome (IBS) are common among patients with inflammatory bowel disease (IBD) in remission phase, and clinicians have difficulties in interpreting such symptoms as an ongoing disease activity or a coexistent IBS. We investigated if the assessment of fecal calprotectin (FC) could be helpful in this regard. **METHODS:** The study population consisted of 42 IBD patients in remission that fulfilled the IBS diagnostic criteria (Rome III), 24 IBS patients and 30 healthy controls. Clinical remission was determined based on physician's assessments, not using corticosteroids or biological agents within the preceding six months and activity indices. The FC and C-reactive protein (CRP) levels were investigated and compared among the groups. **RESULTS:** FC levels were significantly higher in patients with IBD ( $142.9 \pm 216.5 \mu\text{g/g}$ ) than those with IBS ( $24.9 \pm 27.8 \mu\text{g/g}$ ) and controls ( $17.9 \pm 14.8 \mu\text{g/g}$ ) ( $p < 0.001$ ). CRP levels were also higher in IBD than IBS patients [ $3.9$  (SE = 0.5) vs.  $2.1$  (SE = 0.5),  $p = 0.030$ ]. However, FC levels were not significantly correlated with CRP levels or with severity of symptoms in IBD and IBS patients ( $p > 0.05$ ). **CONCLUSIONS:** The presence of IBS-like symptoms in IBD patients in clinical remission may reflect an ongoing activity of IBD, which is undetectable by current activity indices. Serum CRP levels are not specific enough in such situation, and FC is a more accurate and specific test for investigating mucosal inflammation in this regard.

**KEYWORDS:** Inflammatory Bowel Disease, Crohn's Disease, Ulcerative Colitis, Remission, Inflammation, Irritable Bowel Syndrome

## BACKGROUND

There is a considerable overlap between symptoms of irritable bowel syndrome (IBS) and those of the inflammatory bowel diseases (IBD).<sup>[1]</sup> Some studies showed that in patients with IBD in the remission phase, IBS-like symptoms are present in more than 30% of the cases, two to three times higher than that in the general population.<sup>[2,3]</sup> On the other hand, there is evidence related to the possible role of microscopic inflammation and mucosal immune system activation in IBS patients, especially those with diarrhea-predominant subtype.<sup>[4,5]</sup> Considering the high prevalence of IBS (up to 25% to 50% of referrals to gastroenterology clinics)<sup>[6]</sup> and the role of mucosal immunity changes in the pathophysiology of IBS, clinicians are frequently challenged whether in an IBD patient in remission phase the presence of IBS-like symptoms is indicative of an actual IBS or reflects the ongoing activity of IBD. The optimal therapeutic target to modify the disease course in IBD patients should be the full mucosa healing, and not only the clinical remission.<sup>[7,8]</sup> Therefore, accu-

rate evaluation of the etiology of symptoms and diagnosing low-grade inflammation in IBD patients in remission phase is important to optimize and individualize the treatment.<sup>[7,9]</sup>

Conventional clinical activity indices, such as Crohn's Disease Activity Index (CDAI) and Colitis Activity Index (CAI) and also traditional laboratory markers of inflammation, such as C-reactive protein (CRP), lack the sensitivity and specificity to detect low-grade inflammation in IBD patients. Therefore, these indices and laboratory markers do not help the clinician to distinguish subclinical IBD with low-grade inflammation from coexisting IBS.<sup>[10-12]</sup> Fecal calprotectin (FC), a recently investigated biomarker of mucosal inflammation, is a calcium-binding protein found in neutrophils which is detectable in stool after mucosal inflammation and damage.<sup>[13,14]</sup> FC is highly correlated with clinical scores, serological markers, and more importantly with histologic grading of mucosal inflammation.<sup>[15,16]</sup> As neutrophils are not involved in

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inflammatory changes of the mucosa in IBS, the levels of FC are within the normal range in these patients.<sup>[17,18]</sup> and the specificity and sensitivity of FC in distinguishing IBD from IBS is reported about 100% and 97%, respectively.<sup>[19,12]</sup>

A recent study by Keohane and colleagues, evaluating FC in IBD patients in remission, showed that the mechanism behind IBS-like symptoms in most of the cases is occult inflammation rather than coexistent IBS.<sup>[9]</sup> Considering limited data in this regard and the importance of discriminating subclinical IBD from IBS, we evaluated mucosal inflammation using FC in IBD patients in remission with IBS symptoms and compared it between IBS patients and healthy controls.

## METHODS

### *Patients and settings*

This cross-sectional study was done in three outpatient clinics of gastroenterology in Isfahan (Iran) from May to December 2011. The study population consisted of three groups: (1) patients with Crohn's disease (CD) and ulcerative colitis (UC) in remission phase who fulfilled the IBS diagnostic criteria (Rome III), (2) IBS patients, and (3) healthy controls. IBD was diagnosed based on the Leonard Jones criteria.<sup>[20]</sup> Clinical remission was determined based on (1) physicians' assessments with history and physical examination, (2) absence of using corticosteroids or biological agents (including anti-TNF antibodies) within the preceding six months, and (3) simplified CDAI  $\leq 150$ <sup>[21]</sup> or an ACI  $\leq 3$ .<sup>[22]</sup> The Rome III criteria was used for definition of IBS symptom and IBS subtypes: diarrhea-predominant (D-IBS), constipation-predominant (C-IBS) and IBS with mixed bowel habit (M-IBS).<sup>[23]</sup> Patients with history of gastrointestinal surgery, presence of microscopic colitis, infectious ileocolitis, colorectal cancer or polyps, urinary incontinence (risk of contamination of fecal samples), and infection with HIV and/or hepatitis B or C were not included. IBS patients (Rome III) were selected from those referring with no alarm symptoms such as anemia or weight loss, and with normal endoscopic, histologic, and sonographic findings. Healthy symptom-free persons were selected for control group from the clinical and laboratory staff willing to provide blood and fecal samples. Informed consent was obtained from participants and the study was approved by the ethics committee of Isfahan University of Medical Sciences.

### *Assessments*

Clinical activity of IBD was assessed with CAI for UC patients and the CDAI for CD patients.<sup>[21,22]</sup> Severity of IBS symptoms was assessed using the IBS Symptom

Severity Scale (IBS-SSS) which scores abdominal pain and distension, bowel habits, and quality of life. The total score ranges from 0 to 500 and can be graded as mild ( $< 175$ ), moderate (175 to 299), or severe ( $> 300$ ).<sup>[24]</sup> Both groups of IBS and IBD patients completed the IBS-SSS. At the same time, a stool sample (50 to 100 mg) was collected from each patient using plain tubes without chemical additives and was stored at  $\leq -20$  °C. Quantitative enzyme-linked immunosorbent assay method (ELISA, EK-CAL, BUHLMANN, Schonenbuch, Switzerland) was applied for the measurement of FC as described by the manufacturer.<sup>[25]</sup> The serum CRP levels were measured by immunoturbidimetric method (PARS AZMOON, Tehran, Iran). All samples were analyzed in a blinded fashion.

### *Statistical analysis*

Descriptive statistics are expressed as mean  $\pm$  SD for quantitative and percentages for categorical data. Parametric and categorical data were compared using independent sample t-test (or ANOVA) and chi-square test, respectively. When data was not normally distributed, appropriate nonparametric tests were applied. A P-value of  $< 0.05$  was considered to be significant. All calculations were analyzed with SPSS software for windows version 16.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

During the study period, 24 cases with IBS, 42 with IBD (24 UC, 18 CD), and 30 controls were evaluated. IBD patients were included 25 UC and 21 CD cases with mean age of  $41.2 \pm 14.1$  years (21 male and 25 female). IBS patients were 7 males and 24 females with the mean age of  $46.0 \pm 11.3$  years. IBS-subtypes included IBS-C (29.0%), IBS-D (25.8%) and IBS-M (45.1%). Controls were 17 male and 13 female cases with mean age of  $37.4 \pm 14.0$  years. Disease duration was  $63.1 \pm 39.0$  months in IBD and  $83.0 \pm 97.2$  months in IBS patients.

FC level was  $142.9 \pm 216.5$   $\mu\text{g/g}$  (range: 11.0 to 983.0  $\mu\text{g/g}$ ) in the IBD,  $24.9 \pm 27.8$   $\mu\text{g/g}$  (range: 3.0 to 89.0  $\mu\text{g/g}$ ) in the IBS, and  $17.9 \pm 14.8$   $\mu\text{g/g}$  (2.1 to 54.0  $\mu\text{g/g}$ ) in the control groups ( $p < 0.001$ ). FC was significantly higher in IBD than IBS patients and controls ( $p < 0.001$ ), but IBS group and controls were similar in this regard ( $p = 0.983$ ) (Figure 1). There was no difference between UC and CD patients in FC level ( $65.2 \pm 42.7$  vs.  $60.5 \pm 35.0$   $\mu\text{g/g}$ ,  $p = 0.722$ ). Among IBS patients, FC level was slightly higher in IBS-M ( $35.1 \pm 32.1$   $\mu\text{g/g}$ ) than IBS-C ( $5.6 \pm 3.0$   $\mu\text{g/g}$ ) and IBS-D ( $10.5 \pm 9.1$   $\mu\text{g/g}$ ) subtypes ( $p = 0.065$ ). However, this difference was not observed

among IBD patients with regards to the predominant bowel habit (IBD with IBS-M:  $165.3 \pm 273.5$   $\mu\text{g/g}$ , IBS-C:  $90.8 \pm 106.2$   $\mu\text{g/g}$ , and IBS-D:  $143.4 \pm 189.2$   $\mu\text{g/g}$ ,  $p = 0.609$ ) (Figure 2). CRP levels ranged from 0.10 to 12.8 mg/l in IBD and 0.1 to 6.9 mg/l in IBS patients, and there was a significant difference between IBD and IBS patients ( $3.9 \pm 0.5$  [SE] vs.  $2.1 \pm 0.5$  [SE],  $p = 0.030$ ) in this regard.

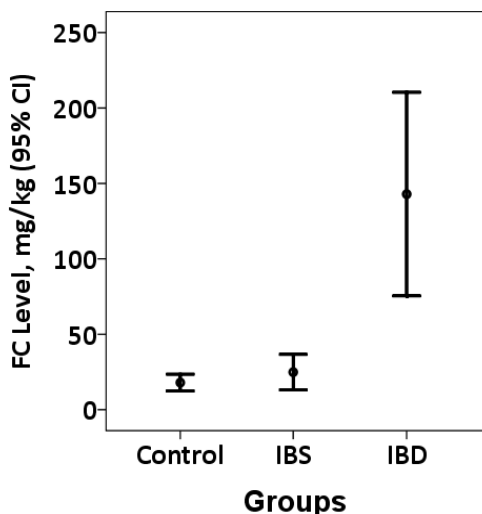


Figure 1. Fecal calprotectin in IBD and IBS patients and controls

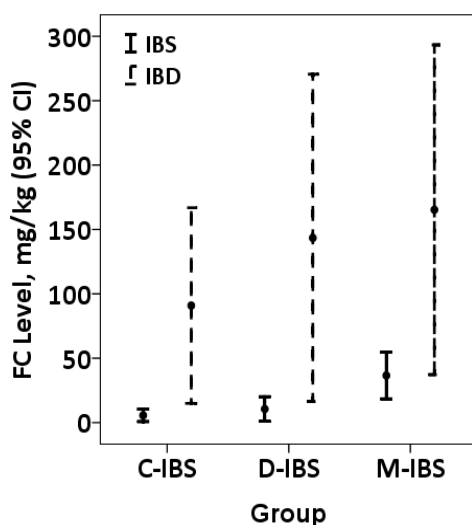


Figure 2. Fecal calprotectin among subtypes of IBS in patients with IBS and IBD

FC level was not correlated with symptom severity (based on IBS-SSS scores) in IBS patients ( $r = -0.024$ ,  $p = 0.913$ ). Also, FC was not correlated with CAI scores in UC patients ( $r = -0.015$ ,  $p = 0.946$ ), or CDAI scores in CD patients ( $r = 0.088$ ,  $p = 0.727$ ). When using IBS-SSS in IBD patients, FC was not correlated with the severity of IBS-like symptoms ( $r = -0.168$ ,  $p = 0.293$ ). There were no correlation between FC and CRP levels in patients

with UC ( $r = -0.170$ ,  $p = 0.438$ ) or those with CD ( $r = -0.364$ ,  $p = 0.137$ ).

## DISCUSSION

IBS-like symptoms are common among IBD patients in remission phase, and clinicians have difficulties in distinguishing such symptoms between an IBD with low-grade inflammation state and a simple IBS scenario.<sup>[1]</sup> Prompt and complete treatment of mucosal damage in IBD patients is of great importance to prevent long-term complications. On the other hand, unnecessary use of corticosteroids or other drugs have some risk. In practice, the absence of symptoms in IBD patients does not necessarily indicate a clinical remission. Symptoms might be of insufficient severity and regarded as an inactive disease by current activity indices (with insufficient sensitivity), and thus not needing treatment change.<sup>[26]</sup> Moreover, presence of symptoms can be attributed to coexisting IBS or an irritable bowel caused by low-grade inflammation or immune dysfunction.<sup>[27]</sup> Therefore, investigators have tried to find more sensitive and specific biomarkers of the mucosal inflammation. Calprotectin, a calcium-bound protein, is a marker of neutrophil turnover which is released into the intestine and indicates colonic inflammation and can be easily measured in the stool by means of an ELIZA assay.<sup>[28]</sup> FC is a simple and noninvasive marker that is useful to predict relapse in IBD patients in remission.<sup>[29]</sup> It could be also helpful in discriminating IBD patients with apparent clinical and laboratory remission from IBS patients.<sup>[30]</sup>

The results of our study showed that IBS-like symptoms in IBD patients while in clinical remission are mostly attributed to mucosal inflammation which is not detectable by activity indices. Although IBD patients had higher levels of CRP than IBS patients, almost all of our in-remission IBD cases had CRP levels of  $< 10$  mg/l and there was a significant overlap with IBS cases in this regard. Furthermore, there was not a significant correlation between FC and CRP levels in IBD patients. Other studies also showed that CRP is not specific enough for distinguishing IBD from IBS<sup>[12,31]</sup> and thus, with regards to our findings, it is not helpful in attributing the IBS-like symptoms in IBD patients to a low-grade inflammation. The results of our study were similar to the study by Keohane et al. that found significantly elevated FC levels above the upper limit of normal in IBD patient with and without IBS like symptoms, and significantly higher levels of FC in those with compared to those without IBS-like symptoms.<sup>[9]</sup> In addition, in their study, FC levels in

IBD patients with IBS symptoms were similar to the levels that can predict a relapse.<sup>[29]</sup> Therefore, the presence of IBS-like symptoms in IBD patients in remission may reflect ongoing activity of their disease and confirms the value of FC in distinguishing an inflammatory state, needing appropriate treatment, from an irritable bowel. Of course, considering the effects of gut inflammation on visceral sensory functions, there is the likelihood that post-inflammatory sensory changes is responsible for IBS-like symptoms in a subset of IBD patients and not all symptoms in IBD patients in remission are due to occult inflammation, which needs further investigation.<sup>[32]</sup>

Our study also provided further evidence that intestinal inflammation can result in symptoms just like to that of functional bowel disorders and underlines the need for caution in the interpretation of such symptoms. Although some evidence indicated the role of mucosal immune changes in the etiology of IBS, and one can suspect that IBS patients with diarrhea-predominant subtype must have higher levels of FC.<sup>[4,5]</sup> Our results did not show such association. We also did not find an association between symptoms severity and FC levels in IBS patients. These results highlight the distinctive nature of mucosal and systemic immunology between IBD and IBS.

There are some limitations about our study for sure. First, our study was small in sample size. The absence of a group of IBD patients without IBS-like symptoms could enhance the accuracy of our findings about the nature of the disease, though this defect is somehow interpretable by results of Keohane et al. study.<sup>[9]</sup> Finally, in future, it is better to include IBD patients who are in remission phase based on endoscopic and histologic assessments. Therefore investigation of the frequency and nature of IBS-like symptoms in these patients would be more accurate.

## CONCLUSIONS

In summary, the presence of IBS-like symptoms in IBD patients in remission phase may reflect an ongoing activity of IBD, which is undetectable by current activity indices or serum markers. These symptoms may also predict a relapse as high level of FC has a suitable accuracy in predicting relapse in IBD patients. Therefore, clinicians must investigate mucosal inflammation, which might be subclinical, first before assuming a diagnosis of coexisting IBS in an IBD patient in remission phase. FC is a non-invasive test for this approach.

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