

Faecal Calprotectin as Reliable Non-invasive Marker to Assess the Severity of Mucosal Inflammation in Patients with Ulcerative Colitis

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Background and study aim: We aimed to evaluate the validity and accuracy of the faecal calprotectin in differentiating patients with IBD from those with IBS and in the assessment of the severity of intestinal mucosal inflammation in patients with ulcerative colitis (UC) which may facilitate in the prognosis and follow.

Patients and Methods: We studied 60 Patients who came to endoscopy unit with lower gastroenterological symptoms. Patients with history of infections, malignancy, gastrointestinal surgery, pregnancy, alcohol abuse or taking non-steroidal anti-inflammatory drugs were excluded from study. All patients subjected to thorough medical history, simple clinical colitis activity index was determined with a score > 4 indicate active UC, complete blood picture, liver, kidney function tests, ESR, CRP, ANCA were done, a stool sample for FC levels determined by a highly sensitive enzyme-linked immunosorbent assay and total colonoscopy with histological examination of intestinal mucosa biopsy were done. The patients divided into 2 groups. Group A: patients with UC, group B: patients with manifestation of irritable bowel syndrome as a control group.

Results: There was a high significant difference between individuals with no pathological activity and other degree of mucosal inflammation as regard simple clinical colitis activity index, endoscopic appearance and faecal calprotectin ($p = 0.000$). The sensitivity, specificity, positive predictive value and negative predictive value of faecal calprotectin in diagnosis of UC were 93.5%, 89.7%, 90.6%, and 92.9% respectively. The positive predictive value and negative predictive value of simple clinical colitis activity index for diagnosis of UC were 76.5% and 80.8% respectively. The positive predictive value and negative predictive value of endoscopic appearance for diagnosis of UC were 100%, and 85.3% respectively. There was a high significant difference and positive correlation between faecal calprotectin, score of colonic pathological activity, endoscopic appearance and simple clinical colitis activity index.

Conclusion: Faecal calprotectin is highly useful for the diagnosis and disease monitoring of patients with UC as it is easy, non invasive, reliable tool.

INTRODUCTION

The cause of ulcerative colitis (UC) is currently under examination. It is believed that the 2 idiopathic forms of inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease

(CD), develop secondary to complex interactions among genetic predispositions, environmental risk factors, and the immune system.

Several genes likely play a role; their products, when combined with environmental factors and dysfunctional immunity, result in a disease spectrum with heterogeneous manifestations and many unique phenotypes [1].

The determination of inflammatory activity is crucial for patients with IBD for the diagnosis, monitoring and step up of therapy. Colonoscopy is the accepted gold standard for investigation of the colon, but is invasive and associated with risks [2]. Among objective clinical features; bloody stool frequency, body temperature and heart rate are good predictors of outcome. Laboratory markers have been studied intensively with varying degrees of success. The widely used acute phase protein C-reactive protein in this respect is a less good marker for assessing disease activity in UC than Crohn's disease [3].

More recently, faecal markers have demonstrated promising results. The most studied markers are faecal calprotectin and lactoferrin have shown accuracy at detecting colonic inflammation [4].

Calprotectin is a calcium-binding protein that is derived predominantly from neutrophils and, to a lesser extent, from monocytes and reactive macrophages [5].

It is worth noting that fecal calprotectin concentrations correlate more closely with histological than macroscopic (endoscopic) findings, suggesting that this biological marker is more sensible than endoscopy in evaluating IBDs activity [6].

The present study aimed at evaluation of the accuracy of faecal calprotectin and correlate it with clinical scores, common serum markers and endoscopy in the assessment of the severity of intestinal mucosal inflammation in patients with ulcerative colitis.

PATIENTS AND METHODS

This present study was conducted in the Tropical medicine department and gastrointestinal endoscopy unit, faculty of medicine, Zagazig University during the period from January 2011 to March 2012.

Our study included 31 patients with ulcerative colitis. The control group comprised 29 patients with manifestation of IBS matched for age and

sex with patient's group. Written informed consents were obtained prior to participation in this study.

Patients with history of infections (recent respiratory or urinary tract infections within 1 month), malignancy (current), gastrointestinal trauma or surgery (within 1 month), or regularly taking aspirin, anticoagulants, or non-steroidal anti-inflammatory drugs, pregnancy and history of alcohol abuse were excluded from this study.

All patients should be subjected to the following:

- Thorough medical history taking.
- Simple clinical colitis activity index (SCCAI).
- Thorough clinical examination.
- Complete blood picture.
- Liver and kidney functions tests.
- Blood sample for estimation of ESR, and of CRP.
- Determination of ANCA in serum.
- Thorough stool examination.
- Quantitative measurement of faecal calprotectin levels were measured by a highly sensitive enzyme-linked immunosorbent assay (PhiCal™).
- Total colonoscopy with histological examination of intestinal biopsy specimens.

Statistical analysis:

Comparisons between means of several groups of mucosal inflammation were done by one way Anova (F test) and LSD when there was a significance difference between means. Comparison between median were done by non-parametric test (Kruskal wallis-H test) followed by Mann-Whitney u test. Receiver operating curve characters were used to develop best cut off value in estimating the validity of different parameter in diagnosis of ulcerative colitis. Kappa measurement of agreement was done to test agreement between studied parameters and degree of mucosal inflammation. P value was considered significant when P value is less than 0.05.

RESULTS

Table (1): Demographics distribution among the examined patients

Degree of colonic mucosal inflammation		Number	Sex (F/M)	Age		f	p
				Mean ± SD	Range		
Group A	No mucosal inflammation	29	15/14	32.2±9.6	19-49	0.32	0.81
	Mild	6	3/3	29.83±5.56	25-39		
	Moderate	9	7/2	29.89±9.97	19-44		
	Severe	16	9/7	29.63±10.52	19-49		

Table (2): Relation between degree of colonic mucosal inflammation SCCAI, endoscopic appearance and faecal calprotectin

	Degree of colonic mucosal inflammation			
	Group B		Group A	
	No mucosal inflammation	Mild	Moderate	Severe
	Median	Median	Median	Median
	Range	Range	Range	Range
SCCAI	3 (2-6)*	6 (6-11)	7 (6-11)	7 (4-12)
Endoscopic appearance	0 (0-1)*	1 (1-3)	2 (1-3)	3 (2-3)*
Faecal calprotectin (µg/g)	30 (10-176)*	108 (10-176)*	165 (149-190)	190 (170-215)

* Highly significant, SCCAI =Simple clinical colitis activity index

Table (3) Relation between degree of mucosal inflammation, CRP value and ESR

	Group B	Group A	t	p
CRP ± SD	6.76 ± 3.86	9.94 ± 5.09	2.711	0.009**
ESR ± SD	23.14 ± 13.23	26.94 ± 10.36	1.242	0.219

** Significant difference

Table (4): Validity of SCCAI, endoscopic appearance and faecal calprotectin in relation to pathology

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
SCCAI	83.9%	72.4%	76.5%	80.8%
Endoscopic appearance	83.9%	100%	100%	85.3%
Faecal calprotectin	93.5%	89.7%	90.6%	92.9%

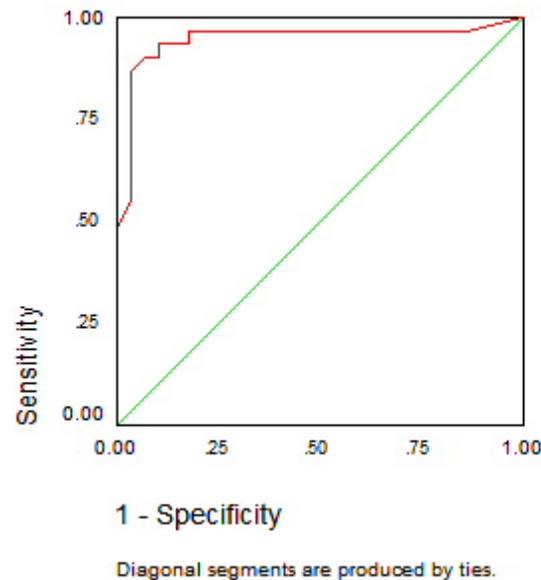


Fig (1): ROC curve of faecal calprotectin in predicting ulcerative colitis

DISCUSSION

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are common entities. Both conditions may present with similar clinical features such as diarrhea and abdominal pain. Patients with IBD oscillate between periods of active and inactive disease and may even present with concomitant functional IBS [7].

Most patients with quiescent IBD have low-grade inflammation and it is possible that symptomatic relapse occurs only when the inflammatory process reaches a critical intensity. Furthermore, because inflammation is a continuous process, direct assessment of the level of inflammatory activity may provide a quantitative presymptomatic measure of impending disease relapse [8].

Calprotectin is a valuable marker at the very early stage of inflammatory reactions in human beings [9].

Faecal calprotectin assessment is that it is a measure of mucosal inflammatory activity that may be detected at a level insufficient to cause an increase in ESR and CRP [10].

In the current study, more intense levels of inflammations are associated with elevated level of faecal calprotectin value, demonstrating a significant correlation between calprotectin and the severity of inflammation. Furthermore, faecal calprotectin had a high correlation with the histologic grading as that observed for endoscopy. Its sensitivity was 93.3%, specificity

was 89.7%, and also it had a high negative predictive value. The results were the same as those obtained by Bunn et al., [6] who claimed that faecal calprotectin concentrations predicted the severity of colorectal inflammation, with advanced histological grades of colorectal inflammation.

Inflammation is the basis for many signs and symptoms of IBD, making its detection and monitoring fundamental to clinical management [5].

One means to assess inflammation that has been discussed in recent years is the analysis of the infiltration of neutrophil in the intestinal mucosa and their transmigration to the lumen [11].

Calprotectin is derived predominantly from neutrophils and, to a lesser extent, from monocytes and reactive macrophages [5].

Therefore the presence of calprotectin in faeces is directly proportional to neutrophil migration towards the intestinal tract [1].

When intestinal inflammation occurs, the calprotectin levels correlate closely with histological evaluation than macroscopic findings, suggesting that this biological marker is more sensible than endoscopy in evaluating IBDs activity [12].

Our study revealed that level of faecal calprotectin was higher in IBD patients than in non-IBD patients (by 205 $\mu\text{g/g}$), which is matched by a study conducted by von Roon et al., [13] who stated that fecal calprotectin was

higher in IBD patients than in non-IBD patients (by 219 $\mu\text{g/g}$), and showed excellent pool sensitivity and specificity rates in distinguishing between these groups (95% and 91%, respectively).

In our study faecal calprotectin resulted the most accurate tool to assess the presence of active mucosal inflammation when compared to C-reactive protein, erythrocyte sedimentation rate. These results had matched with Tibble et al. [10].

Our study showed that faecal calprotectin concentration above 72 $\mu\text{g/g}$, gave a sensitivity of 93.5%, a specificity of 89.7%, a positive predictive value (PPV) of 90.6%, and a negative predictive value (NPV) of 92.9% in predicting UC.

The data obtained by our study revealed that there is a good agreement between faecal calprotectin, and endoscopic appearance. These results showed that fecal calprotectin at a concentration above 72 $\mu\text{g/g}$ was in agreement with Simple clinical colitis activity index when it was above 4 of about 46%, while with endoscopy when the score above 1 the agreement was about 80%.

Faecal calprotectin allows a non-invasive monitoring of disease activity, especially when the repeated measurements are considered, among UC patients, as better identifying controlled disease activity.

In most clinically quiescent IBD, residual mucosal inflammation is still present to some extent. When disease activity increases, clinical symptoms are usually not present during the early relapse stage. Faecal calprotectin seems to be able to detect subclinical mucosal inflammation, and thus might earlier identify those patients at risk for IBD relapse [14].

We can conclude that measurement of faecal calprotectin is highly useful for the diagnosis and disease monitoring of patients with ulcerative colitis, and might additionally predict disease outcome. It is a sensitive and direct biomarker of intestinal inflammation with a better performance than the traditional non-invasive tests. It is both easily carried out and reliable, which makes it suitable for use as a first-level test for the diagnosis of organic ulcerative colitis as well as for the activity monitoring of UC.

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Conflicts of interest: None.

Ethical approval: Informed consents were routinely obtained from patients. The study was performed in accordance with the ethical standards on human experimentation and with the Helsinki Declaration of 1964.

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