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Original Article

Predictive Value of Faecal Calprotectin Level in the Diagnosis of Relapse in Moderate and Severe Ulcerative Colitis

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ABSTRACT

Background:
Ulcerative colitis is a chronic and recurrent inflammatory disease characterized by the inflammation of the colon mucous membrane, causing abdominal pain, diarrhea, and hematochezia. Colonoscopy is considered as the method of choice for the diagnosis of this disease. Furthermore, the severity of this condition in the relapse periods is determined based on clinical and laboratory criteria. Regarding this, the present study aimed to investigate the relationship between faecal calprotectin level, a cytosolic protein of neutrophils and macrophages, and the severity of disease in patients with ulcerative colitis relapse.

Materials and Methods:
This cross-sectional study was conducted on 65 patients (i.e., 35 men and 30 women) with ulcerative colitis relapse. The results of clinical, laboratory, and colonoscopy examinations were collected using a checklist. Data analysis was performed using MED Cal statistical software (version 8).

Results:
According to the results, the mean age of the participants was 36.31 ± 14.19 years. Out of the 65 patients, 26 (40%), 21 (32.3%), and 18 (27.7%) subjects had mild, moderate, and severe types of the disease, respectively. White blood cell count and erythrocyte sedimentation rate showed a significant decrease by the enhancement of disease severity and hemoglobin level ($p < 0.001$). Furthermore, the mean level of faecal calprotectin showed a significant elevation with the increase of the disease severity. The calprotectin level of > 387 µg/g with the sensitivity and specificity of 76.9% and 92.3%, respectively, was considered as indicating moderate and severe involvements.

Conclusion:
Faecal calprotectin level can be used as a non-invasive and reliable method to evaluate the severity of ulcerative colitis relapse.

Keywords: Inflammatory bowel disease, Ulcerative colitis, Calprotectin, Leukocyte L1 antigen complex

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INTRODUCTION

Inflammatory bowel disease (IBD) includes two diseases, namely ulcerative colitis and Crohn's disease, each of which has its own pathological and clinical features. Ulcerative colitis is a chronic inflammatory disease associated with the periods of remission and relapse. This disease is characterized by inflammation only in the mucus layer of the colon. The disease usually involves the rectum and extends proximally to the colon in a varying extent, and this involvement is continuous (1,2).

The clinical symptoms of the disease include diarrhea, hematochezia, mucus affection, abdominal pain, fever, and weight loss, which manifest with variable degrees of intensity. The standard diagnostic methods for ulcerative colitis include colonoscopy and biopsy of the intestine. Given the invasiveness of this procedure, it is difficult for some patients to accept it. Regarding this, recent studies have examined the diagnostic value of some biomarkers, including faecal calprotectin level in patients with IBD(3-5).

Calprotectin is a zinc- and calcium-binding protein found in neutrophil cytosols and human macrophages. The presence of this protein in the stool represents the migration of neutrophils into the lumen of the digestive tract. Based on some evidence, calprotectin is the best marker to confirm the presence of mucosal inflammation in patients with IBD(5).

In case of relapse, clinical and paraclinical findings are used to assess the severity of the disease and make clinical decisions. The Truelove and Witts Severity Index is used to determine the severity of the disease in cases with relapse. This index consists of several criteria, including stool frequency, hematochezia, body temperature, pulse rate, anemia, and erythrocyte sedimentation rate (ESR)(6,7).

Some factors including cytomegalovirus infection, pseudomembranous colitis, irritable bowel syndrome (IBS), malignancies, and treatment non-adherence are related to IBD flare up(8-12).

Given the reactivation of the inflammatory process in the cases with relapse, the achievement of positive results for IBD-related biomarkers including calprotectin is not out of expectation. In spite of the increasing research on the diagnostic value of calprotectin in IBD, no study has investigated the relationship between calprotectin level and disease severity in patients with ulcerative colitis relapse. With this background in mind, the present study was conducted to examine the relationship between the level of faecal calprotectin and the severity of the disease, as well as to determine the cutoff value of calprotectin level for the diagnosis of patients with severe ulcerative colitis relapse.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Gastrointestinal and Liver Disease Research Center affiliated to Qom University of Medical Sciences, Qom, Iran, on 65 patients with a definitive diagnosis of ulcerative colitis. The inclusion criteria were the relapse of ulcerative colitis and consent to participate in the study. The exclusion criteria were: 1) clostridium difficile infections (toxins A and B), 2) amyloid colitis (stool exam), 3) cytomegalovirus-induced enteritis, 4) infectious colitis caused by Shigella, Salmonella, and campylobacter jejuni, 5) inconsistency between the colonoscopy reports made by two gastroenterologists presented during the implementation of colonoscopy, and 6) long-term use of non-steroidal anti-inflammatory drugs.

Study population and sampling

Sampling was carried out successively until reaching the required sample size. The sample size was estimated as 65 cases using the formula for sensitivity and specificity studies in MED Cal software (version 8) by considering the results of a study performed by Voiosu and colleagues, with a sensitivity of 60%, specificity of 90%, and disease prevalence of 0.1%. The area under the curve was considered as 0.7 for the level of primary calprotectin in the diagnosis of disease relapse (13).

Methods

After obtaining informed consent from all patients, the questionnaires (data gathering form) were completed. The questionnaire consisted of information regarding age, sex, severity of the disease as determined by the Truelove and Witts criteria (i.e., stool frequency, hematochezia, body temperature, pulse rate, hemoglobin, and ESR), and underlying diseases. Additionally, a stool sample was obtained from each patient to measure the calprotectin level using a laboratory kit (Euro-Immune, Germany) by enzyme-linked immunosorbent assay. Subsequently, the patients were subjected to colonoscopy. Calprotectin levels of < 50, 50-120, and > 120 µg/g were considered as negative, borderline, and positive, respectively.

Statistical analysis

The sensitivity, specificity, and predictive value of faecal calprotectin level were calculated using the MED Cal statistical software (version 8). The receiver

Table 1: Details of pulmonary parameters according to types of UC and CD

Variables	Mild (Mean ± SD)	Moderate (Mean ± SD)	Severe (Mean ± SD)	*P* value
Age (years)	41.54 ± 14.20	33.19 ± 15.48	32.39 ± 10.50	0.050
Stool frequency (number per day)	2.04 ± 0.77	4.52 ± 0.81	6.44 ± 1.54	0.000
Body temperature (°C)	37.00 ± 0.00	37.05 ± 0.22	37.22 ± 0.43	0.019
Pulse rate (per minute)	79.31 ± 2.15	83.81 ± 4.91	86.67 ± 3.85	0.000
Hemoglobin (g/dL)	13.42 ± 0.95	12.05 ± 0.92	11.56 ± 1.15	0.000
ESR (cm/h)	14.00 ± 5.24	28.67 ± 3.67	36.44 ± 14.54	0.000
WBC (/µL)	6276 ± 634	9015 ± 676	11280 ± 1390	0.000
Faecal calprotectin (µg/g feces)	158.12 ± 171.67	866.14 ± 716.51	830.51 ± 838.72	0.001

ESR: Erythrocyte Sedimentation Rate, WBC: White Blood Cell

Table 2: The mean level of faecal calprotectin in various extents of colon involvement

Extent of involvement	Fecal calprotectin level Mean ± SD
Proctitis	132.66 ± 156.18
Left colitis	632.85 ± 711.81
Extensive colitis	1516.93 ± 696.68
Pancolitis	1645.57 ± 838.71

operating characteristic (ROC) curve was used to determine the cutoff value of faecal calprotectin level for the diagnosis of moderate and severe types of ulcerative colitis relapse. A 95% confidence interval was determined for the diagnostic indices of this condition. *P* value less than 0.05 was considered as statistically significant.

Ethical consideration

The present study was approved by the Research Ethics Committee of Qom University of Medical Sciences, Qom, Iran. In line with the research ethics principles, the patients' information remained confidential. Furthermore, informed consent was obtained from all the patients. Additionally, the patients were aware of all their rights and were informed about the possibility of withdrawal from the study.

RESULT

Of the 65 patients included in the study, 35 (53.8%) were men. The mean age of the patients was 36.31 ± 14.19 years (age range: 15-84 years). With regard to the severity of the disease, 26 (40%), 21 (32.3%), and 18 (27.7%) patients had mild, moderate, and severe types of the disease. The severity of the disease was determined based on the Truelove and Witts criteria. Table 1 presents the mean values of the demographic and laboratory variables.

The examination of the relationship between the severity of the disease (i.e., mild, moderate, and severe) and the level of faecal calprotectin in patients with ulcerative colitis was accomplished using one-way ANOVA. The patients with mild, moderate, and severe symptoms had the mean age of 41.54, 33.19, and 32.39 years, respectively. The results demonstrated a significant relationship between the severity of the disease and age ($p = 0.05$). In this regard, aging was accompanied by an increase in disease severity. However, there was no significant relationship between sex and severity of the disease relapse ($p = 0.317$).

Among the clinical criteria, the severity of the disease, body temperature, pulse rate, and stool frequency per day showed a significant increase with the enhancement of disease severity. Regarding the laboratory criteria, the severity of anemia and ESR elevation were associated with an increase in the severity of the disease. Furthermore, the mean number of white blood cells enhanced significantly with the increase in the severity of the disease. Additionally, there was a significant elevation in the rate of faecal calprotectin with the enhancement of disease severity.

Table 2 tabulates the mean level of faecal calprotectin with regard to the extent of colon involvement. According to the findings of the current study, the mean level of faecal calprotectin significantly enhanced with an increase in the extent

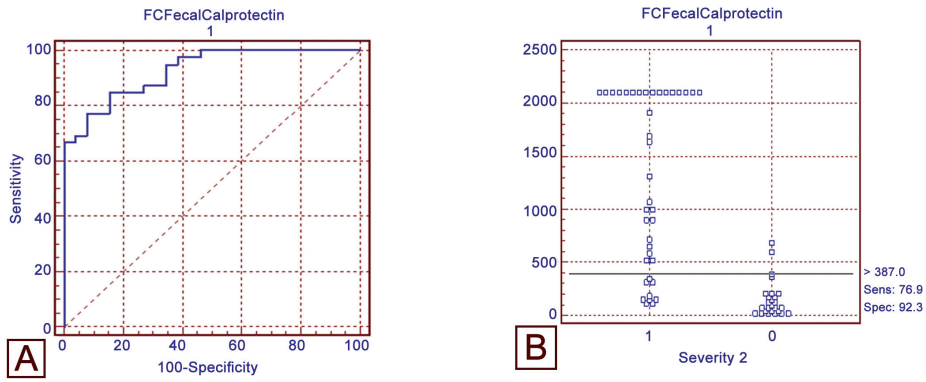

Fig. A and B: **ROC (Receiver Operating Characteristic) curve for detecting calprotectin cut-off level**

of colon involvement ($p = 0.001$). The ROC curve was used to find the best cutoff value for the determination of calprotectin level with the aim of diagnosing the moderate and severe types of the disease relapse.

The cutoff value of faecal calprotectin level for the diagnosis of moderate and severe ulcerative colitis was obtained as 387 µg/g with the sensitivity and specificity of 76.9% and 92.3%, respectively. Furthermore, the positive and negative predictive values of faecal calprotectin level for the diagnosis of moderate and severe types of ulcerative colitis were estimated as 90.8% and 80%, respectively. Figures A and B illustrate the cutoff value with maximum sensitivity and specificity.

DISCUSSION

Colonoscopy and intestinal biopsy are currently the standard methods for the diagnosis of IBD. It is not reliable to schedule patients for endoscopy based on clinical symptoms. Many suspected patients with IBD have negative endoscopic findings(14). Moreover, this diagnostic method is invasive, costly, time-consuming, and uncomfortable for many patients. Regarding this, it is required to consider non-invasive diagnostic methods that are cheaper and faster.

Biomarkers, such as faecal calprotectin, can be helpful in the diagnosis of IBD and its differentiation from IBS, as well as the prediction of IBD relapse. The findings of this study demonstrated a significant relationship between faecal calprotectin level and severity of disease in patients with ulcerative colitis relapse. Accordingly, with an increase in the extent of the involvement, the mean level of faecal calprotectin significantly elevated (15,16).

The investigation of the patients' laboratory variables revealed that the subjects with a higher disease severity had higher levels of ESR and white blood cells and a lower level of hemoglobin. The calprotectin level of > 387 µg/g with a sensitivity of 76.9% and specificity of 92.3% was indicative of moderate and severe involvements.

In a study conducted by Zittan and colleagues on 58 patients with ulcerative colitis and Crohn's disease, it was shown that faecal calprotectin level was significantly lower in the remission phase of the disease than in the active phase. Furthermore, the researchers reported a significant relationship between disease activity based on colonoscopy and histology and the level of faecal calprotectin (17).

In another study, Krzesiek and co-workers examined the level of faecal calprotectin as a marker of disease activity in children with IBD. The researchers concluded that patients with IBD, including ulcerative colitis and Crohn's disease, had a higher level of faecal calprotectin in comparison with the controls. In addition, an increase in faecal calprotectin levels was observed with the elevation of inflammatory markers, such as ESR and C-reactive protein (CRP), and reduction of hemoglobin level in patients with moderate and severe IBD (18).

In a study carried out by Mousavi and others in Hormozgan, Iran, patients with IBD (i.e., Crohn's disease and ulcerative colitis) had a higher level of ESR and CRP and lower level of hemoglobin,

compared with those with IBS. Furthermore, the level of faecal calprotectin was significantly higher in patients with IBD than in those with IBS (19).

The best cutoff value for the level of faecal calprotectin to differentiate IBD from IBS was obtained as 77.5 µg/g with the sensitivity and specificity of 96.7% and 91.7%, respectively. In the present study, the calprotectin cutoff value was obtained as 387 µg/g feces. This value was for the diagnose of the type of moderate and severe ulcerative colitis rather than its differentiation from IBS. Other results reported by Mousavi are in line with our findings.

Voiosu and colleagues showed that faecal calprotectin is associated with the endoscopic view of the disease in its active phase. They also reported that while other inflammatory markers were indicative of acute phase, they were not consistent with the endoscopic view. In this regard, faecal calprotectin level of > 300 µg/g had the sensitivity of 60% and specificity of 90% for the diagnosis of endoscopic active phase, as well as the sensitivity of 61% and specificity of 89% for the prediction of mucosal repair. These values are in line with our study findings (13).

Lin and co-workers found a significant correlation between the colonoscopic view and the low level of faecal calprotectin among patients with ulcerative colitis in the remission phase. The present study revealed a significant relationship between the extent of colon involvement in the active phase of the disease and calprotectin elevation (20).

CONCLUSION

Based on the findings of the present study and their comparison with those of the previous studies, it can be concluded that the measurement of calprotectin levels is helpful in the diagnosis of moderate and severe types of ulcerative colitis relapse. Given the fact that this test is cheaper, faster, more comfortable, and less invasive than colonoscopy, it can be used to detect the relapse of ulcerative colitis with different intensities, thereby eliminating unnecessary colonoscopy procedures as far as possible. It is suggested to perform a similar study using a higher number of patients with IBD, multiple centers, and other laboratory kits (for the evaluation of faecal calprotectin level) and considering other factors, such as disease duration and different age groups. Further studies could focus on the comparison of pathological and endoscopic findings with non-invasive tests.

Authors contributions:

AH and SJE contributed to study conception and design, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MR contributed to study conception and design, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AK contributed to acquisition the data, preparation of article draft and agreed for all aspects of the work. FA contributed to acquisition the data, drafting and revising the article, approval of the final version of the manuscript, and agreed for all aspects of the work. AM contributed to study design, analysis and interpretation of the data, preparation of article draft and revising the final version and agreed for all aspects of the work. SS contributed to study conception and design, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

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