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Original Article

Fecal calprotectin role in diagnosis of ulcerative colitis and treatment follow-up

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ABSTRACT

Objective: Ulcerative colitis usually affects the rectum and potentially can involve the whole colon. Noninvasive methods such as fecal calprotectin measurement may be considered as a reliable and inexpensive approach in assessing disease severity or treatment change strategy.

Methods: In this retrospective cross-sectional study, records of 56 ulcerative colitis patients who hospitalized with exacerbation between May 2016 and April 2017 were assessed based on IBD Data Bank Software in Gastrointestinal and Liver Diseases and Research Center (GLDRC), Guilan province, Iran between. A questionnaire of demographic characteristics, clinical findings and fecal calprotectin level was completed. Montreal classification severity of ulcerative colitis and Mayo disease activity index were scored. Data were analyzed for descriptive and analytical analysis.

Results: Fecal calprotectin was significantly different in terms of disease severity based on both Mayo score ($p = 0.007$) and Montreal classification ($p = 0.001$). In patients with mild symptoms, no increase in fecal calprotectin was observed, but in patients with moderate and severe elevations in fecal calprotectin levels was significant. Also, C-Reactive Protein surge was related to disease severity ($p = 0.02$). Furthermore, regression comparison among high-chance patients based on fecal calprotectin was significantly related to higher Erythrocyte Sedimentation Rate levels and smoking, $p = 0.01$ and $p = 0.05$, respectively.

Conclusion: It seems fecal calprotectin levels are related to the disease severity. Non-invasive methods, such as fecal calprotectin assay, may seem to be an alternative to aggressive, costly and time-consuming methods, such as colonoscopy and biopsy, to reduce the suffering of patients and ultimately help improve the patients' life quality.

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Papel da calprotectina fecal no diagnóstico de colite ulcerativa e no acompanhamento do tratamento

Palavras-chave:
Calprotectina
Doença inflamatória intestinal
Colite ulcerativa
Inflamação

RESUMO

Objetivo: A colite ulcerativa geralmente afeta o reto, podendo acometer todo o cólon. Métodos não invasivos, como a dosagem de calprotectina fecal, podem ser uma abordagem confiável e barata para a avaliação da gravidade da doença ou da estratégia de mudança de tratamento.

Métodos: Neste estudo transversal retrospectivo, os registros de 56 pacientes com colite ulcerativa que foram hospitalizados devido a exacerbação entre maio de 2016 e abril de 2017 foram avaliados usando o software IBD Data Bank no Centro de Pesquisa e Doenças Gastrointestinais e Hepáticas (GLDRC), na província de Guilan, Irã. Foi aplicado um questionário de características demográficas, achados clínicos e nível de calprotectina fecal. Foram usados o escore de Mayo de atividade da doença e a classificação de Montreal da gravidade da colite ulcerativa. Os dados foram analisados de forma descritiva e analítica.

Resultados: A calprotectina fecal apresentou diferença significativa em termos de gravidade da doença com base no escore de Mayo (p = 0,007) e na classificação de Montreal (p = 0,001). Em pacientes com sintomas leves, nenhum aumento na calprotectina fecal foi observado. Entretanto, em pacientes com sintomas moderados e severos, o aumento nos níveis de calprotectina fecal foi significativo. Além disso, o aumento nos níveis de proteína C reativa foi associado à gravidade da doença (p = 0,02). A análise da regressão entre pacientes considerados de alto risco com base na calprotectina fecal indicou uma associação significativa com níveis elevados da taxa de sedimentação de eritrócitos e tabagismo (p = 0,01 e p = 0,05, respectivamente).

Conclusão: Os níveis de calprotectina fecal parecem estar relacionados com a gravidade da doença. Métodos não invasivos, como o estudo de calprotectina fecal, podem ser uma alternativa a métodos agressivos, caros e demorados, tais como colonoscopia e biópsia, reduzindo o sofrimento e ajudando a melhorar a qualidade de vida dos pacientes.

Introduction

Inflammatory bowel disease (IBD) has two forms of ulcerative colitis (UC) and Crohn's Disease (CD).[1] Crohn's disease is an inflammatory disease, with the early signs of more subtle patterns because of variable and distributed anatomical localization.[2–4] In each age group, the classic symptoms of CD include abdominal pain, diarrhea, anorexia and weight loss.[5] About 80% of people experience the complex symptoms with or without extra-intestinal manifestations. CD is a pancentric inflammatory disease which is segmental in comparison with UC and commonly associated with the gastrointestinal tract.[6–8] In UC, inflammation is classically limited to the large intestine with typical and continuous essence begins with rectum.[9] CD and UC are mainly diseases of young age, with a maximum incidence of 15–30 years of age.[10] Epidemiological studies have been declared different prevalence and incidence of IBD, based on the geographical location of life and racial or ethnic background.[11,12] The rate of CD has risen in almost all Western countries over the past three decades.[13,14] The IBD incident rate in Iran shows similar trend to Asian countries and lower than western countries.[15] Besides, in one study in Guilan, among the 756 patients with IBD, 87.1% had UC. The mean age of the patients was about 40–45 years old, and patients with coronal disease were younger than patients with UC.[16] Today, the most sensitive and specific diagnostic method is colonoscopy and biopsy of intestinal inflammatory diseases. However, this diagnostic method is aggressive, time-consuming and costly as well as unpleasant among many patients. Patients mostly are reluctant to repeat colonoscopy for evaluation, therefore, researchers' inclination is considered towards less invasive diagnostic methods.[17] It is also important for disease and patients' follow up. Inflammation of the intestine in patients with CD or UC is associated with activation of acute phase proteins and migration of leukocytes to the intestine.[18] Erythrocyte Sedimentation (ESR) and the level of CRP-Reactive Protein (CRP) are considered as two general markers of inflammation biomarkers[19] which have not enough sensitivity and not the necessary qualities. Over the past several decades, several leukocyte products released in the stool have been identified as inflammatory bowel markers.[20] Among the proteins derived from neutrophil, calprotectin and lactoferrin have been introduced as the best parameters.[21,22] Calprotectin is a heterodimeric protein that has the ability to bind to calcium and zinc.[23,24] This protein exists in neutrophil cytosol and in monocyte membranes.[25] Following the activation of neutrophils or binding of monocytes to endothelial, calprotectin is released and its level in the serum or body fluids is an important indicator of

inflammation.[26] Calprotectin has both a bacteriostatic effect and a cytokine-like effect on the local environment.[27] The physiological roles of this protein are not well-known and extensive research in this area is underway in the world's labs.[17,28,29] Studies have shown that increased levels of Fecal Calprotectin (FCP) in IBD can be explained through raising the leukocytes regeneration and increasing the neutrophil migration to the intestinal lumen.[30] Therefore, we decided to compare the FCP level in UC patients with different disease severity, so that FCP may be considered as a suitable marker for patients' following up.

Methods

Study design and patients

In this retrospective cross-sectional study the records of UC hospitalized patients with exacerbation between May 2016 and April 2017 were collected from IBD database in the Gastrointestinal and Research Center (GLDRC), Razi Hospital, Guilan province. The inclusion criterion was all UC patients who were diagnosed based on clinical, colonoscopy and histopathological standards by gastroenterologist and liver diseases specialists. Patients with the following features were excluded: (i) documents which were not based on clinical, endoscopic or histological evidence; (ii) Presence of infectious enterocolitis (positive stool samples for *Salmonella, Shigella, Campylobacter* and other bacteria); (iii) simultaneous malignancy, particularly gastrointestinal malignancies; (iv) patients with urinary or fecal incontinence, risk of contamination of the stool sample; (v) patients without stool samples; (vi) pregnant patients; (vii) history of extensive intestinal resection (ileosigmoidostomy and ilorectostomy); (viii) patients taking regular aspirin or non-steroidal anti-inflammatory drugs over 2 weeks. Also, as patients were asked to complete a written consent form in order to use their registered information in future studies, the records of patients who did not consent were excluded. Finally, 56 patients were included out of 66 UC patients. This study was approved by the ethical committee of Guilan University of Medical Sciences (GUMS). The demographic characteristics of the patients included age, sex, UC duration, smoking, and location of intestinal involvement, medication history, ongoing treatments, colonoscopy report, and FCP based on registered records in the IBD Data Bank Software. In order to have a uniform report of FCP, just the samples which were experimented by Razi Hospital laboratory using ELISA Kit CalproLab (Lysaker, Norway) were assessed. FCP values were expressed in mg/kg of wet stool. Based on this kit, values of less than 50 μg/g were considered normal.

Efficacy assessments

We used two known disease activity instrument for UC, Montreal classification severity of ulcerative colitis in order to evaluate qualitative disease activity and Mayo disease activity index,[31] which is scored on a scale from 0 to 12 and includes stool frequency, rectal bleeding, a physician's global assessment, and a sigmoidoscopic evaluation[32] based on Up-to-date Software. Also, we arranged the following intervals conventionally in this study as Mild: stool frequency < 4, mild rectal bleeding, no fiver and tachycardia, mild anemia, ESR < 30 mm/h, colonoscopy findings such as erythema and decrease in vascularity; Moderate: stool frequency 4–5, moderate rectal bleeding, fever < 37.5, tachycardia < 75, moderate anemia, colonoscopy findings such as loss of vascularity and Severe: stool frequency > 6, severe rectal bleeding, fever > 37.5, tachycardia > 75, moderate anemia, colonoscopy findings such as incontinence bleeding.

Table 1 – Clinical and laboratory symptoms according to the Mayo Table.

Mayo table features		*n* (%)
Tachycardia	No	50 (89.3)
	Yes	6 (10.7)
Fever	No	50 (89.3)
	Yes	6 (10.7)
Hemoglobin	Normal	16 (28.6)
	Abnormal	40 (71.4)
ESR	>0	31 (55.4)
	<30	25 (44.6)
Bowel frequency	<4	28 (50)
	4–6	19 (33.9)
	>6	9 (16.1)
Occult blood	Mild	37 (66.1)
	Moderate	17 (30.4)
	Severe	2 (3.6)

Statistical analyses

All data were entered into SPSS-21 software and analyzed for descriptive and analytical analysis. To describe the data, mean, standard deviation, percentage of frequency of tables and charts were used. Also, for analytical statistics, *t*-test and two ways ANOVA were used.

Results

In this study substantially most of the UC patients (85.7%) were between 20 and 60 years old with the mean age of 35.6 ± 13.2. Almost half of them had one year disease duration while slightly above one out of 10 (10.7%) had ten year disease duration. Surprisingly, near three of 4 (69.6) patients were non-smoker. Also, 73.2% had positive CRP in their laboratory reports. The distribution of clinical and laboratory symptoms according to the Mayo scale is stated in Table 1. Furthermore, the distribution of colonoscopy findings in subjects based on the Mayo scale in depicted in Table 2. Approximately three quarters (67.9%) were in severe and moderate phases based on Montreal classification. However, quantities estimations in terms of mayo classification presented a higher percent (85.7%) of severe and moderate phases. Positive FCP was shown in 66.1% of patients. Although there was a significant difference between CRP and the quality of disease in patients ($p = 0.02$), this difference was not significant between CRP and Mayo score. In terms of both quality and quantity of disease FCP showed a significant difference $p = 0.001$ and $p = 0.007$, respectively. The comparison of regression of high-chance individuals and demographic characteristics according to FCP

Table 2 – Colonoscopy findings according to Mayo Table.

Symptoms in Mayo Table		*n* (%)
Mild	Erythema	4 (7.1)
	Vascularity reduction	14 (25)
	Delicate granulation	0 (0)
Moderate	Obvious erythema	2 (3.6)
	Rough granulation	6 (10.7)
	Loss of vascularity	3 (5.4)
Sever	Incontinent bleeding	9 (16.1)
	Ulcer	15 (26.8)

Table 3 – Regression comparison of high-chance patients and demographic characteristics according to FCP.

Characteristics		Exp (95% CI)	*p*-Value
Age	<20	Ref	
	20–40	0.001 (0.4–1.7)	0.9
	40–60	0.001 (0.3–1.5)	0.7
	>60	0.001 (0.6–1.9)	0.8
Smoking	No	Ref	
	Yes	0.1 (0.02–1.1)	0.05[a]
ESR	>30	Ref	
	<30	6.7 (1.4–30.1)	0.01[a]
CRP	Negative	Ref	
	Positive	1.2 (0.2–5.8)	0.8
Bowel frequency	<4	Ref	
	4–6	0.1 (0.4–2.9)	0.09
	>6	0.3 (0.6–2.3)	0.3
Occult blood	Mild	Ref	
	Moderate	0.001 (0.5–2.8)	0.8
	Severe	0.001 (0.3–2.6)	0.9

[a] $P < 0.05$ significance level.

is shown in Table 3. Fig. 1 represents the distribution of FCP based on both quantitative intensity for evaluation of UC activity and qualitative criteria.

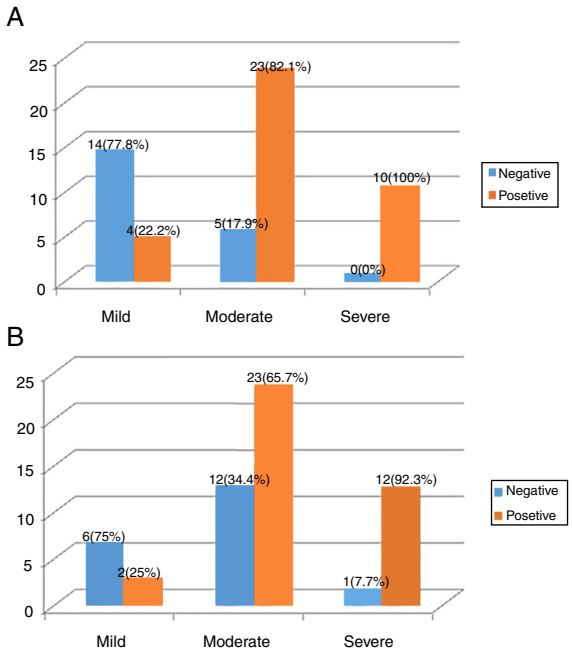

Fig. 1 – Distribution of calprotectin based on (A) quantitative intensity for evaluation of ulcerative colitis activity (Mayo score) and (B) qualitative criteria (Montreal Score).

Discussion

IBD is currently the common cause of gastrointestinal tract involvements. Although death is not commonly related to death, it still causes disability and mortality, especially in adults, which has the potential for growth, education and employment, subsequently leads to a lot of social and economic burden. Colonoscopy and biopsy are the only current approaches to IBD. These patients have abdominal pain and chronic or recurrent diarrhea after undergoing colonoscopy and histopathological sampling. However, electing patients for colonoscopy just based on clinical symptoms is not reliable as well as most suspected IBD patients would have negative colonoscopy findings.[33] Since this is an aggressive, time-consuming and costly method that is uncomfortable in many patients, an easy, non-invasive and inexpensive method should be first considered for the evaluation of patients.[30] The use of markers such as calprotectin in the diagnosis and differentiation of the disease can be helpful as the level of Fecal Calprotectin (FCP) represents neutrophils migration to the intestinal lumen in conditions of acute inflammation. In fact, calprotectin is released after the activation of neutrophils or the binding of monocytes to the endothelial, and its level in the serum or body fluids is an important indicator of inflammation.[29] Increased concentrations of calprotectin can be measured in plasma, cerebrospinal fluid, synovial fluid, urine or stool in inflammatory or malignant conditions.[30] Calprotectin is a peptide with unique properties and is known as the biomarker and the main actor of pathophysiological processes in IBD such as Ulcerative Colitis (UC) and Crohn's Disease (CD).[22] Calprotectin stimulates inflammatory response by increasing the production of certain inflammatory chemokines and increasing the expression of sticky molecules.[34] It has been shown that serum calprotectin in the active phase of CD was much more than its inactive phase. In addition, its serum concentrations were directly related to the concentration of radioactive protein c and the disease activity index.[35] In this study, FCP level among most patients was strikingly high. Erbayrak and colleagues also reported a significant surge.[30] In Kawashima and colleagues study the association between FCP level and clinical severity in UC patients witnessed that its importance is a potential inflammation marker.[36] In Alike Molodecky et al. study,[37] the severity levels of UC patients was not linked to demographic variables such as age, sex, UC duration and smoking. Smoking was associated with a 95% decrease in the incidence of ulcerative colitis. As smoking is not a risk factor for ulcerative colitis[38]; it does not mean that smoking would be recommended but it reflects that smoking cessation program should be supervised by physicians to prevent the probable flare ups in accordance with an abrupt crackdown. It is deduced that smoking does not affect the both qualitative and quantitative disease severity[39] which also was not seen in this study. On the other hand, ESR soared in patients by 99%, this factor as one of the most important determinants of inflammation in the acute state of ulcerative colitis. In the study of Erbayrak

et al., the ESR rate in IBD patients was higher than that of IBS patients as control group.[30] ZareMehrjardi and colleagues study showed higher ESR levels depict an increased inflammation in acute conditions.[40] In the present study, patients with a moderate disease activity had a 5 fold increase in the level of calprotectin as well as a 100% increase among patients with severe activity. However, in one study there was no abnormal calprotectin among three difference groups in terms of disease activity and there was no obvious relationship between the severity of inflammation and the measured calprotectin[41] despite the fact that, the association of FCP with tissue inflammation is well documented in comparison with colonoscopy biopsy.[33] It has also been shown that calprotectin levels between IBD and non-organic diseases provide a reliable differentiation in symptomatic patients. Also, increased levels of FCP in patients with clinical improvement indicate an increased risk of relapse in the next 12 months.[42] It has been concluded that of FCP concentration has an acceptable diagnostic accuracy for differentiating the organic disease from functional intestinal diseases and has a strong association with the rate of IBD activity.[33] In Wang et al. study, calprotectin was suggested as a non-invasive diagnostic method for IBD.[34] Furthermore, Casta and colleagues reported calprotectin as a strong predictor of UC than CD, suggesting this test as a non-invasive tool for monitoring and optimal treatment.[35] Meanwhile, it would predict the clinical recurrence of disease activity in patients with CD and UC[43] as well as a valuable tool for IBD suspected patients to determine the need for colonoscopy.[44] In this study we were not able to include all eligible patients due to some limitations such as lack of access to some patients records, inadequate bowel preparation for colonoscopy, failure to perform colonoscopy due to lack of patients' affordability or its aggressive essence. Also, as patients were undergone colonoscopy by different gastroenterologist specialists, the accuracy of subsequent colonoscopy would be increased by one expert gastroenterologist.

Conclusion

Measurement of FCP can be a non-invasive technique along with colonoscopy to IBD detection and follow-up. In summary, colonoscopy and biopsy are currently used to evaluate the disease's activity, but calprotectin assessment is also thought to be well correlated with disease activity. It does not lead to removal of colonoscopy from the diagnosis process and disease follow-up, however raising this marker points to the severity of the disease, and we require taking diagnostic measures quickly to find the cause of the escalation.

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Conflicts of interest

The authors declare no conflicts of interest.

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