Scientific Research Publishing

Open Journal of Gastroenterology, 2018, 8, 234-244
http://www.scirp.org/journal/ojgas
ISSN Online: 2163-9469
ISSN Print: 2163-9450

Diagnostic Value of Fecal Calprotectin and Serum MMP-9 in Diagnosing Disease Activity of Ulcerative Colitis

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How to cite this paper: Ghweil, A., Mounir, M., Khodeary, A. and Aziz, S.P. (2018) Diagnostic Value of Fecal Calprotectin and Serum MMP-9 in Diagnosing Disease Activity of Ulcerative Colitis. *Open Journal of Gastroenterology*, **8**, 234-244.
https://doi.org/10.4236/ojgas.2018.86026

Received: March 8, 2018
Accepted: June 26, 2018
Published: June 29, 2018

Abstract

Background and Study Aim: Ulcerative colitis (UC) is a chronic, idiopathic inflammatory bowel disease characterized by remission of disease activity. Searching for laboratory markers which are simple, sensitive, specific and non-invasive is fundamental to assess the extent of inflammation, activity of the disease, evolution and prognosis which can be used to assess response to treatment and the possibility of relapse. Our aim of the work was to investigate the diagnostic role of fecal calprotectin and serum MMP-9 in determining the activity of ulcerative colitis. **Patients and Methods**: 71 patients were included in the study and fecal calprotectin, serum MMP-9, ESR and CRP were measured in these patients to determine the disease activity of ulcerative colitis. **Results**: Fecal calprotectin concentration in the patients with active UC was significantly higher than that in inactive disease and in controls (387.21 ± 44.07 μg/g vs 103.62 ± 119.67 μg/g, 12.44 ± 3.65 μg/g, p = 0.000). Serum MMP-9 was found to be higher in patients with active UC than in patients with inactive disease (11.02 ± 5.29 vs 4.01 ± 1.72 ng/ml, p = 0.000). A significant difference was also found in the patients with active UC of mild, moderate and severe degrees. Also, strong positive correlation was found between fecal calprotectin and serum MMP-9 and the severity of the disease. The area under the curve of the receiver operating characteristics (AUCROC) was 0.949 and 0.941 for fecal calprotectin and serum MMP-9 respectively. **Conclusion**: Fecal calprotectin and serum MMP-9 can be used to differentiate between active and inactive forms of UC.

Keywords

Inflammatory Bowel Disease (IBD), Ulcerative Colitis (UC), Matrix Metalloproteinases (MMPs), Fecal Calprotectin (FC)

1. Introduction

Ulcerative colitis (UC) is a chronic, idiopathic, inflammatory large bowel disease (IBD) with recurrent attacks of exacerbation. Determination of the disease activity is an essential part of clinical management [1]. Nowadays, the most accurate way to evaluate the severity of UC and extent of inflammation is colonoscopy and biopsy [2]. This procedure allows direct visualization of the large bowel, and the opportunity to take intestinal tissue samples for histopathological studies. Unfortunately, this is invasive, limited by cost, and requires an experienced operator as well as an often impossible preparatory regimen [3] [4]. Also, inter-observer variation in endoscopic evaluation of disease activity could change clinical outcomes, and have a fundamental impact on treatment options [5] [6].

On the other hand, patient symptoms cannot effectively reflect the extent of disease and response to treatment, nor are they a reliable index of healing of intestinal mucosa. Therefore, for evaluating the disease activity and therapeutic efficacy, a combination of monitoring symptoms with clinical, laboratory and endoscopic findings is used in routine clinical practice [7].

The diagnosis of UC is based on clinical symptoms combined with radiological and endoscopic investigations. Employment of non-invasive biomarkers is needed. Non-invasive biomarkers have the potential to avoid invasive diagnostic tests and inhibit potential complications [8]. These biomarkers can be divided into serological and fecal categories, [9] including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), platelet count, white blood cell count, interleukin-6, tumor necrosis factor-a (TNF-a), interleukin 1-b, and anti-neutrophil cytoplasmic antibody (ANCA) [10] [11].

Potentially, fecal markers have the advantage of possessing higher specificity for gastrointestinal diseases like IBD because the feces is in direct contact with colon mucosa and consequently contains special markers of mucosal disease [12]. Fecal calprotectin is one of these markers, it is a major protein in the neutrophilic granulocytes and the macrophages [13], which accounts for 60% of the total protein in the cytosol fraction in these cells [14] [15]. This kind of protein can resist metabolic degradation caused by intestinal bacteria, and the protein is relatively stable in stools for up to one week at room temperature [16]. It can differentiate between patients with organic or non-organic intestinal disease, and can be useful in detecting colorectal cancer and inflammatory disorders, and can also be useful in predicting a relapse of inflammatory bowel disease [10].

The human matrix metalloproteinases (MMPs) are a family of 24 zinc dependent endopeptidases. They are divided by domain structure and substrate preference into collagenases, gelatinases, stromelysins, and membrane type MMPs (MT-MMPs) [17]. The involvement of MMPs in inflammatory processes has been documented both in animal models with experimentally induced IBD and in intestinal cell lines as well as in cultures of inflammatory altered tissues [18]. MMP-9 has been demonstrated to be the main metalloproteinase implicated in the development of IBD [18] [19]. Recent studies suggest that it is epitheli-

al-derived and not neutrophil-derived MMP-9 that is responsible for the penetration of inflammatory cells into inflamed tissue [18] [20]. The demonstration that the changes of MMPs on the organ level are reflected by their concentration or activity in easily accessible biological material would aid in the diagnosis and differentiation and monitoring of the course, as well as effectiveness of IBD treatment. We aimed through this work to investigate the diagnostic role of fecal calprotectin and serum MMP-9 in determining the activity of ulcerative colitis.

2. Patients & Methods

The study included 71 patients (55 males and 16 females) divided into three groups including 24 (33.8%) patients as control group, 23 (32.4%) patients with inactive ulcerative colitis and 24 (33.8%) patients with active disease with mean age 40 ± 5 years. Control group consisted of patients with no organic or metabolic disorders which were excluded on the basis of diagnostic procedures. The patients included into the control group were diagnosed with functional disorders such as functional dyspepsia, irritable bowel syndrome, and functional constipation. Patients were admitted in Tropical Medicine & Gastroenterology Department, Qena Faculty of Medicine, South Valley University due to disease flare or for control examination. Patients with the coexistence of other severe systemic diseases, history of malignancy, liver diseases, or pregnancies were not included in our study.

Disease activity was estimated using Mayo score. This index, which combines clinical and endoscopic assessments, is the sum of scores from 4 components: stool frequency, rectal bleeding, sigmoidoscopic findings, and physician's global assessment. This disease activity index ranges from 0 to 12 and higher scores correlate with more advanced disease. In general, a patient is considered to be in remission if the Mayo score is 2 or below and to have severe disease if the score is above 10. Clinical response is generally accepted when the score decreases 3 points from the patient's initial baseline score [21].

Blood samples were drawn by venous puncture in a fasting state. Sera were obtained from clotted (30min, room temperature) and centrifuged (15 min, 1500 ×g) blood. Serum samples were stored at −80˚C until analysis. MMP-9 concentrations were estimated by Human ELISA Kit (abcam, United Kingdom). This assay employs an antibody specific for Human MMP9 coated on a 96-well plate. Standards and samples are drained into the wells and MMP9 present in a sample is attached to the wells by the immobilized antibody. Then we washed the wells and we added biotinylated anti-Human MMP9 antibody. After removing unbound biotinylated antibody, HRP-conjugated streptavidin is added to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color appears in proportion to the amount of bound MMP9. The Stop Solution turns the color from blue to yellow, and the color intensity is measured at 450 nm.

The stool samples were dissolved, and 50 - 100 mg of the sample was suspended with 2500 - 5000 μL of the fecal extraction buffer, and was homogenized;

then, the suspernatant was diluted to 1:50, and the calprotectin was analyzed by ELISA kit (Eaglebioscinces, USA). Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with antibody to calprotectin. After a short incubation period, the plate is washed and horseradish peroxidase (HRP) conjugated human calprotectin specific monoclonal antibody is added to each well. After the second incubation period, a mixture of solid-phase antibody—human calprotectin—HRP conjugated monoclonal antibody" is formed. Then, we removed the buffer matrix and unbound monoclonal antibodies in the next step. For the detection of this immunocomplex, we incubated a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader at 450 nm. Serum high-sensitive CRP was measured by the latex particle-enhanced immunoturbidimetric kit (Omega Diagnostics, UK). Data on hemoglobin, white blood cell count (WBC) and ESR were obtained from patients' medical records.

The study was approved by ethical committee of Qena Faculty of Medicine-South Valley University. Written informed consent was obtained from all patients before treatment.

3. Statistical Analysis

Data was analyzed using SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Correlations between fecal CP, MMP-9, and clinical, endoscopic and histologic activity scores were determined by analysis of variance [ANOVA]. The p-value of equal to or less than 0.05 was considered statistically significant. The cut-off levels, sensitivity and specificity were calculated using the receiver operating characteristic [ROC] analysis. The cut-off level was calculated in the event that the value of the area under the ROC curve [AUC] was above 0.89, determined by the maximum value of Youden's index [sensitivity + specificity-1].

4. Results

The demographic data of the studied patients presented in Table 1 included a mean age of 40 ± 5 years with male predominance (77.5%). As regard to clinical activity of ulcerative colitis, 24 (33.8%) patients presented with active attack while inactive state of ulcerative colitis was detected in 23 (32.4%) patients. Based on Mayo score, mild disease was found in 37.5%, moderate disease in 33.3% while severe ulcerative colitis was found in 29.2% of patients. Mean fecal calprotectin level was 150.35 ± 173.58 μg/g, mean serum MMP-9 was 5.54 ± 5.18 ng/ml, ESR 18.65 ± 11.43 mm/h and CRP 22.44 ± 26.19 mg/L.

Table 2 shows association between fecal calprotectin, serum MMP-9, CRP And ESR with clinical activity of ulcerative colitis, fecal calprotectin level in active ulcerative colitis was 387.21 ± 44.07 μg/g which was higher than inactive (103.62 ± 119.67 μg/g) or control group (12.44 ± 3.65 μg/g) with statistically significant difference between active and inactive state ($p = 0.000$). Also, serum MMP-9 level in active ulcerative colitis was higher than inactive state of ulcerative

Table 1. Characteristics of study population.

		Mean ± SD
Age		40 ± 5
Sex	Male	55
	Female	16
Activity	Control	24 (33.8%)
	Inactive	23 (32.4%)
	Active	24 (33.8%)
Degree of activity	Mild	9 (37.5%)
	Moderate	8 (33.3%)
	Severe	7 (29.2%)
ESR		18.65 ± 11.43
CRP		22.44 ± 26.19
MMP9		5.54 ± 5.18
Calprotectin		150.35 ± 173.58
Hemoglobin (g/dl)		12.2 ± 1.8
Leucocytes (No./mm3)		10,055 ± 13,360

Table 2. Relation between laboratory investigations and clinical activity of ulcerative colitis.

		Mean ± SD	p-value
Calprotectin	Control	12.44 ± 3.65	0.000
	Inactive	103.62 ± 119.67	
	Active	387.21 ± 44.07	
MMP9	Control	1.53 ± 0.65	0.000
	Inactive	4.01 ± 1.72	
	Active	11.02 ± 5.29	
CRP	Control	2.64 ± 0.59	0.000
	Inactive	16.04 ± 15.57	
	Active	48.38 ± 26.52	
ESR	Control	14.67 ± 8.87	0.005
	Inactive	14.35 ± 9.19	
	Active	22.75 ± 10.38	

colitis (11.02 ± 5.29 vs 4.01 ± 1.72 ng/ml) (p = 0.000). CRP and ESR was significantly higher in active ulcerative colitis (p = 0.000 and p = 0.005 respectively) than in inactive or control group.

Fecal calprotectin was found to have a strong positive correlation with the degree of activity of ulcerative colitis (p = 0.000) (**Figure 1**). Serum MMP-9 varied significantly with the degree of activity of ulcerative colitis with elevated levels in more severe degrees of ulcerative colitis compared to moderate or mild degrees of severity and a significant positive correlation was found between serum MMP-9 and the degree of activity of ulcerative colitis (p = 0.000) (**Figure 2**).

On the other hand, CRP and ESR did not show significant correlation with the activity of ulcerative colitis.

The ROC curve is used to select the right cut-off point and to compare the diagnostic values of two or more diagnostic tests. The ROC analysis on the fecal calprotectin showed that a cut-off point of 240 μg/g had a 100% sensitivity and a 86.9% specificity for making a differentiation between active UC and inactive UC. As regard to serum MMP-9, a cutoff value of 4.5 ng/ml had 100% sensitivity and a 82.6% specificity to differentiate between active and inactive UC (**Figure 3**).

5. Discussion

UC is a chronic, relapsing disease characterized by diffuse mucosal inflammation of the colon [22]. The precise etiology of UC is unknown; however, it is thought to be caused by an inappropriate inflammatory response to the gut contents in genetically predisposed individuals [23] [24]. The incidence and prevalence of ulcerative colitis have been increasing over time worldwide [25].

The diagnosis of UC is based on clinical symptoms combined with radiological and endoscopic investigations. Employment of non-invasive biomarkers is needed. Non-invasive biomarkers have the potential to avoid invasive diagnostic tests and inhibit potential complications [26].

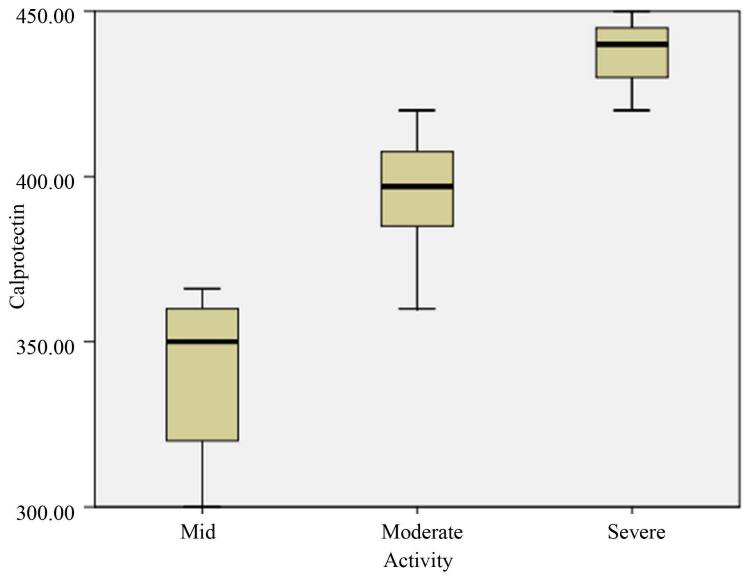

Figure 1. Correlation between fecal calprotectin (μg/g) and severity of ulcerative colitis. Fecal calprotectin was found to have a strong positive correlation with the degree of activity of ulcerative colitis.

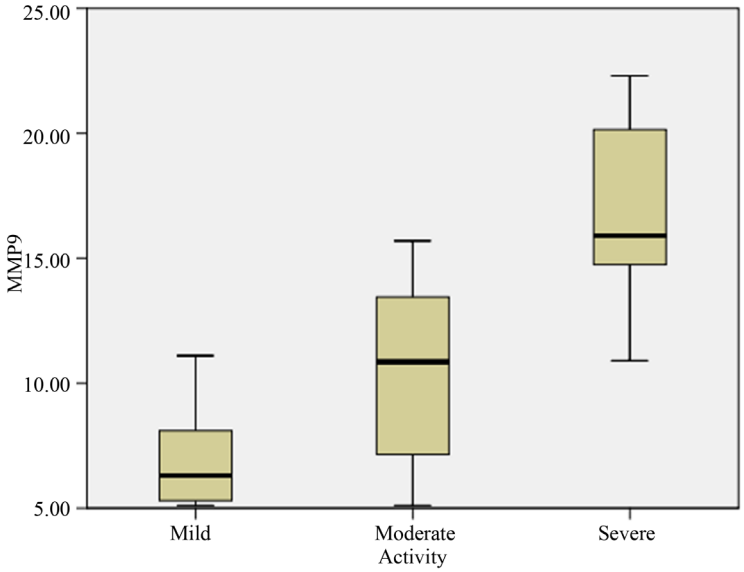

Figure 2. Correlation between serum MMP-9 (ng/ml) and severity of ulcerative colitis. A significant positive correlation was found between serum MMP-9 and the degree of activity of ulcerative colitis.

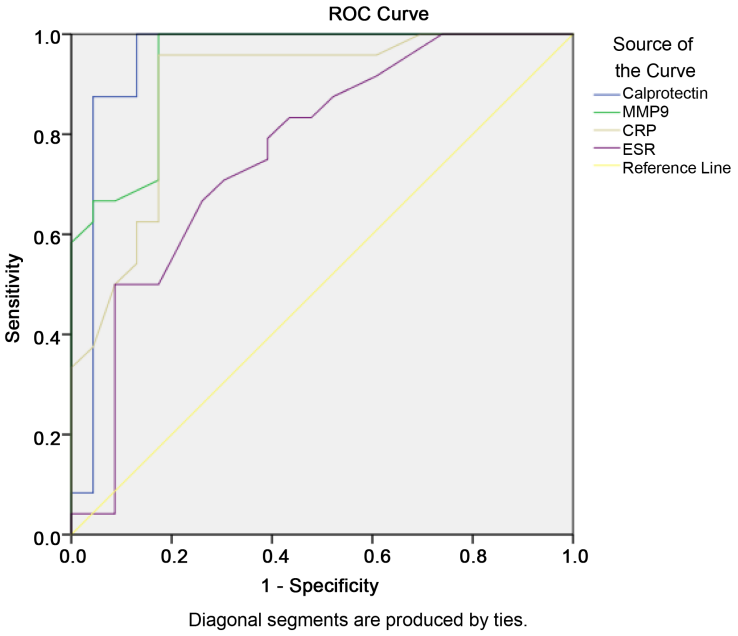

Figure 3. ROC curve analysis on the abilities of fecal calprotectin, serum MMP-9, CRP and ESR to differentiate between active and inactive UC. The ROC analysis on the fecal calprotectin showed that a cut-off point of 240 μg/g had a 100% sensitivity and a 86.9% specificity for making a differentiation between active UC and inactive UC. As regard to serum MMP-9, a cutoff value of 4.5 ng/ml had 100% sensitivity and a 82.6% specificity to differentiate between active and inactive UC.

Fecal calprotectin (FC) is a calcium-binding protein derived predominantly from granulocytes migrating via intestinal wall [27] [28] which constitutes 60% of cytosolic protein in human neutrophils [29] and about 5% of the total protein

[30]. Several clinical studies report the association between FC and the severity of intestinal inflammation [4] [31]. Accordingly, the level of FC is proportional to UC activity, and it can reliably distinguish active versus inactive disease [4] [32].

Expression of metalloproteinases is altered in diseases with inflammatory background [33] [34]. Many studies point to MMP-9 as a key enzyme engaged in the degradation of alimentary tract tissues in the course of IBD [18] [35]. MMP-9 is also involved in the shedding and activation of biologically active molecules, which further perpetuates pathological processes observed in IBD [35] and renders MMP-9 an interesting diagnostic and therapeutic target.

Our study results revealed that fecal calprotectin level was significantly higher in active than inactive or control group with strong positive correlation with the activity of ulcerative colitis determined by Mayo score. This was in accordance with Xiang *et al.* who reported that fecal calprotectin concentration in the patients with active UC was significantly higher than that in the inactive UC and in the controls [36].

Also, we found that serum MMP-9 concentration was significantly higher in patients with active ulcerative colitis than in patients with inactive state or in the control group. Serum MMP-9 levels showed strong positive correlation with activity of ulcerative colitis. Our results agreed with Lakatos *et al.* who noted that serum concentrations of MMP-9 were higher in UC patients compared to controls and correlated well with the disease activity [37]. Also, in agreement with our results, Malgorzata *et al.* reported that MMP-9 concentrations in active UC were significantly higher not only than those detected in controls but also with respect to concentrations measured in patients with inactive forms [38].

We found in the current study that fecal calprotectin with a cutoff value of 240 μg/g had 100% sensitivity and 86.9% specificity for differentiating active ulcerative colitis from inactive disease and that a serum MMP-9 level of 4.5 ng/ml had 100% sensitivity and 82.6% specificity to differentiate active state of ulcerative colitis from inactive disease. In conclusion, fecal calprotectin and serum MMP-9 can be useful as possible supportive markers allowing differentiation between active and inactive forms of UC.

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