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The Relation Between Fecal Calprotectin and the Rate of Clinical Activity of Ulcerative Colitis

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Abstract

Background: Inflammatory bowel disease is a disorder with unknown origin in which environment, genetics, and immunity play a part. Although colonoscopy and biopsy are expensive and invasive, are used to monitoring the mucosal inflammation. Fecal calprotectin is a non-invasive test which has attracted a lot of attention. We aimed to determine the relation of fecal calprotectin and clinical activity of ulcerative colitis.

Methods: This cross-sectional study took place with the confirmation o f ethics committee o f Mashhad University o f Medical Sciences in 2014 - 2015. Patients with diagnosis of ulcerative colitis were included; demographic information was recorded and clinical activity of disease was evaluated. Fecal calprotectin was measured by the quantitative ELISA method and results of laboratory studies and clinical examinations were analyzed by SPSS software version 16. Level of significance was considered less than 0.05.

Results: Seventy patients were studied (male = 56%, average age = 38 $\pm$ 15). 25 patients (36.2%) were newly diagnosed. The average period of disease was 4.1 ± 5 years. 25 individuals (35.7%) had mild, 19 individuals (27.1%) had moderate, and 26 individuals (37.1%) had severe disease. Averages of fecal calprotectin in mild, moderate, and severe disease were 132 $\pm$ 111, 119 $\pm$ 44 and 141 $\pm$ 78 μg/ g, respectively. Averages of fecal calprotectin in mild and moderate disease (P = 0.874), in mild and severe (P = 0.925) and in moderate and severe disease (P = 0.662) were not significantly different.

Conclusions: Although fecal calprotectin in severe ulcerative colitis is higher, it has no relation with disease clinical activity.

Keywords: Colitis, Ulcerative, Inflammatory Bowel Disease, Calprotectin

1. Background

Ulcerative colitis, the most common inflammatory bowel disease (IBD), is a disorder with unknown origin in which environmental factors; genetics and immunity play a part (1, 2). The regulated function of cytokines has faded in inflammatory bowel disease. In fact, these cytokines are secreted from CD4 cells that are activated in the mucosal membrane of the intestine, but their unbalanced activity in a person who is genetically susceptible, will lead to the onset of this disease (2). Ulcerative colitis (UC) is an intestinal chronic disease and though the exact reason is unknown, but considering the epidemiology of this disease in different geographical parts of the world, environmental factors and lifestyle play their part in those who are genetically susceptible (1, 2). For unknown reasons this disorder is more common in Scandinavian and developed countries (3). This disease affects men and women equally. The most prevalent age of onset is between 15 - 30. Various studies seem to be necessary to determine the behavior of this disease in different geographical areas (4-6).

The follow up of patients with ulcerative colitis is mainly based on clinical criteria, to some extent laboratory criteria and colonoscopy. One of the most well-known scales to evaluate the severity of disease is the “truelove” scale which is a combination of clinical and laboratory data (7). Various studies have shown that clinical findings do not always match with disease activity in colon mucosa. Therefore, there have been always efforts to find a suitable substitute which has a good relation with mucosal inflammation. In recent years, calprotectin which is a main protein in macrophages and granulocytes has been suggested

as an index to evaluate the disease activity in intestinal mucosa (8). Calprotectin can be found in different cells, tissues, and body fluids. This protein mainly exists in monocytes and neutrophils and also can be expressed with lesser extents in epidermal and endothelial cells. In neutrophils, this proteins approximately consists about half of the total cytosolic proteins (30% - 60%) (9). Following the activation of neutrophils or the attachment of monocytes to endothelial membrane, calprotectin is released and its amount increases in serum or body fluids. The increased amount of calprotectin can be detected in inflamed tissues during an inflammatory disease such as rheumatoid arthritis, cystic fibrosis, multiple sclerosis or HIV infection and it can also be observed in the stool of patients suffering from inflammatory diseases or colorectal cancers (10).

Calprotectin has attracted a lot of attention in recent decade as a marker for diagnosis, response to treatment and monitoring in inflammatory bowel diseases (Crohn's disease, ulcerative colitis) and also malignancies of the digestive system (11). Fecal calprotectin (FC) is increased in these disorders and this increase differs based on the type of the disease and its severity. Fecal calprotectin is routinely used as an evaluative test in Norway and England.

Calprotectin is usually mentioned with synonym names such as calprotectin, S100A8/A100A9, MRP8/MRP14, calgranulin A/B and L1 protein. Considering the fact that calprotectin is basically expressed in myeloid cells, sometimes it is called as MRP8/MRP14 myeloid dependent protein. And because this protein is a part of S_100 protein family, it can be called as S100A8/S100A9. Calprotectin can have a local bacteriostatic and cytokine/like effect. A physiological role of this protein is still unknown and multiple studies related to this subject are now being carried out. The stool sample can be collected by the patient him/herself at home because in room temperature, fecal calprotectin can remain stable for 7 days (12). The purpose of this study is to determine the relation of fecal calprotectin and the clinical activity of ulcerative colitis.

2. Methods

This cross-sectional study took place in two university hospitals of Mashhad (Qaaem and Imam Reza hospitals) on a target population of 70 people suffering from ulcerative colitis. Patients were chosen by simple sampling. Exclusion criteria included small intestine involvement (e.g. celiac disease), colon cancer, taking nonsteroidal anti-inflammatory drugs (NSAID) and infectious colitis.

This study was approved in ethics committee of Mashhad University of Medical Sciences and before starting the procedure, an informed consent was obtained from all patients. Demographic information of patients including age, sex, results of study and clinical criteria were carefully recorded on checklists which were provided for this reason.

For measuring calprotectin, a stool sample was also taken and sent for quantitative Elisa test (using East biopharm kit).

Blood indexes were evaluated by sysmex kx_21 auto analyzer machine on blood samples sent in container containing EDTA (ethylene diamine tetra acetic).

Severity of ulcerative colitis was classified based on the frequency of defecation, presence or absence of fever, tachycardia and erythrocyte sedimentation rate (ESR) (Truelove and Witts classification) (Table 1).

Table 1. Clinical Classification of Ulcerative Colitis Based on Truelove Criteria[a]

Classification	
Mild	< 4 stools/day, without or with only small amounts of blood
	No fever
	No tachycardia
	Mild anemia
	ESR < 30 mm/hour
Moderate	Intermediate between mild and severe
Severe	> 6 stools/day, with blood
	Fever > 37.5°C
	Heart beat > 90 beats/min
	Anemia with hemoglobin level < 75% of normal
	ESR > 30 mm/hour

Abbreviation: ESR, Erythrocyte sedimentation rate.
[a]Adapted from Truelove SC, Witts LJ and Cortisone in ulcerative colitis: final report on therapeutic trial. Br Med J 1955; 2:1041.

2.1. Statistical Analysis

Data was encoded and imported in SPSS software version 16. Comparison between quantitative data was done using t-student test and analysis of variance (ANOVA) and data's correlation coefficient was done using Spearman and Pearson tests. Analytical significance was considered less than 0.05.

3. Results

Demographic information of patients is described in Table 2, The results of laboratory tests are summarized in Table 3.

Table 2. Demographic Information of Patients

Demographic Information	Number
Age (on average)	38 ± 13 (years)
Sex	
Male	39 (56%)
Female	31 (44%)
Marital status	
Single	21 (30%)
Married	49 (70%)
Mean duration of disease	4.1 ± 5 (years)

Table 3. Results of Laboratory Tests on Targeted Population

	Standard Deviation ± Mean	Minimum	Maximum
White cell count (mL)	7917 ± 2289	3600	15300
Platelet count (mL)	298089 ± 68843	184000	348000
ESR (mm/hour)	21 ± 19	1	91
CPR			
Positive	55.7%		
Negative	24.3%		
Calprotectin (μg/g)	132 ± 84	1.3	572

Based on clinical criteria, 25 individuals (35.7%) suffered from mild disease, 19 individuals (27.1%) had moderate disease, and 26 ones had a severe type. The average of FC in patients with mild, moderate, and severe disease was respectively 132 ± 111 μg/g , 119 ± 44 μg /g and 141 ± 78 μg /g and this difference was not statistically significant (P = 0.687) (Table 4).

Table 4. The Mean of FC Bass on "Truelove "Clinical Criteria

	Standard Deviation ± Mean	ANOVA Test P Value
Mild	132 ± 11	0.687
Moderate	119 ± 44	
Severe	141 ± 78	

The averages of FC in mild and moderate disease (P = 0.874) and in mild and severe (P = 0.925) were not significantly different. The averages of FC in moderate and severe disease were not also significantly different (P = 0.662).

4. Discussion

We studied the relationship between calprotectin and the clinically severity of ulcerative colitis based on truelove criteria.

Although our results showed the maximum amount of fecal calprotectin in those with a severe type of disease but it did not show any significant difference between those with mild or moderate types.(P = 0.687) Our findings [in fact] confirm the results of the recent study that took place in China. Li.Z and colleagues studied three markers including fecal eosinophil cationic protein (FECP), fecal myeloperoxidase (FMPO), and fecal calprotectin (FC) on 59 patients and realized that despite the high amount of fecal calprotectin in those with severe disease, there is no statistically significant difference between groups with different severity of the disorder. In fact, a significant difference could be observed in other biomarkers (13). Sanborn WJ and colleagues conducted a study on 194 patients with ulcerative colitis and showed that setting "150" as a cut off for calprotectin does not have a proper accuracy for categorizing patients from the clinical severity and endoscopic point of view (14).

On the other hand, many recent studies have greatly emphasized on the role of calprotectin as a neutrophil derived biomarker in ascertaining clinical severity and endoscopic aspects of the disease. Some of these studies showed that the amount of calprotectin is significantly different among patients suffering from mild, moderate or severe colitis (15, 16). Also in a study performed on patients with intestinal inflammation, who were receiving infliximab, calprotectin was found valuable in predicting the relapse of the disease with the sensitivity of 91% and specificity of 82%. Another study which was done on patients with IBS and IBD, pointed an important role of fecal Calprotectin in differentially diagnosing and also measuring the severity of these disorders in patients with inflammatory bowel diseases (17).

Various reasons might be the cause of these differences in findings. It seems that the time during which sampling is done for conducting a test on secreted fecal calprotectin, is an important factor. In our study, the time of testing was not similar among the patients.

In 2015, Calafat studied the circadian changes of fecal calprotectin in patients with ulcerative colitis (UC). In his study measuring FC was done by quantitative rapid point-of-care test based on lateral flow assay immunochromography method. 56 samples from 8 patients were taken and studied. This study showed that the first sample taken in the morning has the highest amount of calprotectin , but it is not true for all patients thus he recommended that in those who suffer from ulcerative colitis, only one sample of

stool is not enough to be used for treatment follow up (10).

One of the other possible reasons that must be noticed is the type of the kit which is used and also the method of measurement. In our study we used quantitative Elisa method and East biopharm kit. In Calafat's study quantitative rapid point-of-care test based on lateral flow assay method was used. The method which was used in Austria's study was PhiCal test (Calpro AS, Oslo, Norway). In China, Jong's test was based on Nycotest Phical ELISA (Nycomed, Norway) kit (18). And in another study FC was measured by both Calpro Elisa and Buhlmann Elisa and it was concluded that Calpro Elisa is a more accurate method (19). Iglesias studies were done using Rapid test.

Another possible reason for this difference between the results of the studies might be due to the clinical criteria used for evaluating the severity of ulcerative colitis. In our study we used Truelove and Witts citeria which is a combination of clinical and laboratorial signs without considering the colonoscopic aspects while in other studies with different results, other criteria were used to describe the disease as light or silent (15, 16).

The other reason of the differences among the results might be due to the increscent or decrescent effect of disease expansion on the amount of calprotectin that is released in the stool which means that even with having a mild disease; the higher extension of colon's mucosa involvement might create a higher level of fecal calprotectin. Conversely, despite having a severe ulcerative colitis, the lesser expansion of the disease may lead to lesser levels of this marker.

It is suggested that future studies to be carried out in regard to unifying the patients based on disease extension in colonoscopy, and especially unifying the time of sampling in the early hours of a day in all patients. Also it is recommended that fecal calprotectin be measured several times a day and in this case, comments on the value of the fecal calprotectin in evaluating the severity of mucosal damage to those suffering from ulcerative colitis will be more accurate.

Conclusion: In this study, though the amount of fecal calprotectin was higher in severe disease compared to mild and moderate cases, statistically there was no significant difference between the level of fecal calprotectin and the clinical activity of ulcerative colitis.

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