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Determining the association between stool calprotectin level and colorectal involvement in ulcerative colitis patients

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ABSTRACT

Background and Objectives: Calprotectin is a cytosolic protein in granulocytes. Its amount in the stool is proportional to the amount of neutrophil migration from the inflamed intestinal wall to the mucous and indicates the amount of active inflammation in the mucous. It is used to help diagnose the phase of disease and the extent of colon involvement.

Methods: For 60 patients with ulcerative colitis (UC), who underwent colonoscopy and biopsy, 5-10 grams of stool samples were sent to Zanjan Buali's laboratory to measure calprotectin level by enzyme-linked immunosorbent assay (ELISA). The extent of colon involvement was measured by a colonoscopy exam including rectal involvement (proctitis), rectum and sigmoid (recto sigmoiditis), descending colon involvement (left side colitis), and proximal to the colon's splenic flexure (pan colitis).

Results: All of the 60 patients who participated had UC. The average of calprotectin was 555.18±179.41 with no significant relationship between calprotectin levels and the gender, no significant relationship between calprotectin levels and gender, as well as between colorectal involvement and calprotectin levels.

Conclusion: The level of fecal calprotectin may indicate the severity of colorectal involvement, but cannot show the extent of it. This inability is also present in different ages and genders. Therefore, the results say this marker cannot be used as a diagnosis of the extent of colorectal involvement in patients with UC.

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Abbreviations

ELISA, Enzyme-linked immunosorbent assay; IBD, Inflammatory bowel disease; CD, Crohn's disease; UC, Ulcerative colitis; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; CDC, U.S. Centers for disease control and prevention; CBC diff, Complete blood count with differential; Alb, Albumin; AST, Aspartate aminotransferase; ALT, Alanine transaminase; Alp, Alkaline phosphatase; SE, Stool exam; NSAIDs, Non-steroidal anti-inflammatory drugs; SD, Standard deviation

Introduction

Inflammatory bowel disease (IBD) is a chronic disease that causes inflammation and mucosal ulcers of the gastrointestinal system, which is characterized by converting between the remission and flare up of the disease. IBD consists of two Crohn's disease (CD) and ulcerative colitis (UC). In CD, inflammation and ulcers may occur in any part of the gastrointestinal lumen from the mouth to the anus. While in the UC, damage occurs on the mucosal surface of the colon. The characteristic symptoms of these two diseases include abdominal pain, diarrhea, and bloody stools. The extent of mucous involvement at UC has a relationship with the prognosis treatment method and type of drugs taken (1). The latest U.S. Centers for disease control and prevention (CDC) statistics state that on average the prevalence of UC is 238 cases per 100000 people in the adult population. Also, on average, out of every 100000 new patients, there are 8 cases with UC (2). Ulcerative colitis is a mucosal disease that usually involves the rectum and progresses to the proximal of the colon to involve all or part of the colon. In 40 to 50 percent of patients, ulcerative colitis is limited to recto sigmoid, in 30 to 40 percent of cases are proximal of the sigmoid, but it does not include the entire colon, and 20 percent of patients there is pan colitis (3). The diffusion to the proximal is continuous and there is not healthy mucous among the affected areas. Although in some cases healthy areas may be seen among affected areas, a biopsy of this healthy mucous will usually be abnormal. Therefore, several biopsies need to be made during endoscopy from healthy areas. Mucous may seem normal after symptoms subside, but after a long time of illness, it becomes atrophic and deformable (4). The main symptoms of UC include diarrhea, blood in the stool, tenesmus, mucous excretion, and abdominal pain. Extra-intestinal manifestations include skin manifestations of (erythema nodosum, pyoderma gangrenosum, psoriasis, etc.), rheumatic lesions (peripheral arthritis and ankylosing spondylitis, etc.). UC is associated with complications such as mega colon toxic, extensive bleeding, intestinal perforation, narrowing and malignancy, anal fissure, perianal abscesses or hemorrhoids. The severity of symptoms is proportional to the extent of the disease. In proctitis, fresh blood is usually excreted in the stool, and the anus is painful to the touch.

In proctitis and proctosigmoiditis, the flow of substances inside the intestine decreases, which causes constipation. If the involved area expands to the proximal, it will be accompanied by diarrhea (5).

Disease in the Active phase can be associated with increased erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), platelet counts, and reduced in hemoglobin level. The level of stool calprotectin is quite consistent with histological inflammation, the prediction of recurrence. In extremely ill people, albumin levels drop rapidly. Leukocytosis is not a good indicator of IBD. Also, proctitis and recto sigmoiditis rarely cause an increase in CRP. Diagnosis of the disease with history taking, clinical signs and symptoms, negative stool testing for microbes with sigmoidoscopic views and biopsy samples. If the disease is not in an acute phase, colonoscopy can determine the extent and activity of the disease. Histological views change later than clinical manifestations (6).

Currently, the standard method for evaluating intestinal mucosal inflammation is endoscopy with a biopsy taking, and the active and remission phase is determined using endoscopic images. Although endoscopic evaluation is necessary to confirm the mucous condition, endoscopy is a costly and invasive, time-consuming and uncomfortable procedure for patients (7). In addition, it has been reported that preparation for endoscopy and endoscopy itself can exacerbate UC symptoms. Endoscopy is associated with complications such as visceral perforation, infection, bleeding, and side effects of medications like weaken the respiratory system and allergic reactions (8). Recently, some studies have focused on calprotectin of stool at UC and found Calprotectin's usefulness in diagnosing and evaluating disease activity and the extent of conflict, evaluation of the effect of the drug and control of relapse have been confirmed (9-11). Calprotectin is a protein of 36 Kilos Dalton attached to calcium and zinc that represents 60% of cytosolic protein found in granulocytes (12-14). This heterodimer consists of S100A8 (MRP8, calgranulin A) and S100A9 (MRP14, calgranulin B) and was first discovered in 1980 (15, 16). The amount of calprotectin in the stool is proportional to the amount of neutrophil migration from the inflamed intestinal wall to the mucous and indicates the amount of active inflammation in the mucous. Calprotectin remains stable at room temperature in stool up to 7 days (17).

The level of calprotectin in the stool is about six times higher than its level in the serum. This makes the stool calprotectin test more sensitive for intestinal diseases in addition to its higher specificity (18). Uncertainty of diagnostic test results, delayed recovering of clinical signs and colonoscopy findings, colonoscopy invasiveness and painful and its impossibility in the acute phase of the disease and the need to perform multiple biopsies are all difficulties of the diagnose and follow up. Calprotectin can be a factor with simply measured, noninvasive, and high precision testing to help diagnose the phase of disease and the extent of colon involvement. The purpose of this study was to investigate the role of stool calprotectin as a non-invasive marker of inflammation to predict the extent of involvement in UC patients so that it may be used to monitor the disease's response towards treatment.

Materials & Methods

In this study 60 patients (new case) of UC referred to the gastrointestinal treatment center, that by the gastroenterologist with the help of clinical and colonoscopy criteria and the findings of the pathology it was diagnosed UC for them, participated in study regardless of gender. Calprotectin levels and the extent of colorectal involvement were determined in all patients and the association between them was found. For all patients the ESR, CRP, Complete blood count with differential (CBC diff), Albumin (Alb), Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (Alp) tests and Stool exam (SE) (positive or negative for amoeba and other infections) were requested. Subsequently, sensitivity analysis was guided, in which all communication was determined based on the age and sex of the patients.

For All 60 patient's colonoscopy and biopsy have done with the goal of achieving the presence or absence of inflammation. Also, 5-10 grams of stool sample in the graduated container was sent to Buali's laboratory to check the level of stool calprotectin. The stool sample can be kept at room temperature for up to 7 days, and after centrifuging the stool or using German kits, the stool calprotectin level was measured by the ELISA method. The diagnostic values (cut off point) for the kit were as follows:

µg/gr <50 is a sign of no inflammation

µg/gr = 50-200 mild organic diseases such as intestinal inflammation caused by NSAIDS, mild diverticulitis and intestinal inflammation in remission phase

µg/gr > 200 indicates active organic disease, gastrointestinal inflammation and malignancy

The extent of colon involvement in this study was measured by a colonoscopy examination that include rectal involvement (proctitis), rectum and sigmoid (recto sigmoiditis), descending colon involvement (left side colitis) and proximal to the Colon's splenic flexure (pan colitis).

The collected information was analyzed using SPSS statistical software. Chi square was used to determine the relationship between qualitative variables. In order to determine the relationship between continuous variables, if the data distribution follows the normal distribution, Pearson correlation coefficient was used and if the normal distribution is not followed Spearman's correlation was used.

Results

In this study, 60 patients were examined. According to Table-1, the number of men participating in the study was 35 persons (58.6%) and women 25 persons (41.4%). The extent of colon involvement in the participants of the study was including recto sigmoiditis 21 (34.5%), left colitis 23 (38.3%) and pan colitis 16 (26.6%). The average age of the participants was 29/39 (38 median) and the average calprotectin in the participants was 555.18 (560). In total. No significant relationship was seen in all hypothesis in our research.

Table 2, showed the relation between Calprotectin levels with frequency of extent of involvement in the age categories and gender, there was no significant relationship between any of them, in age groups 19-24 (p=0.337), 35-52 (p=0.528), 53-70 (p=0.313) years, in gender men (p= 0.657) and women (p=0.271).

Table 1. Demographic information and calprotectin level.

Variables		Participant (Percent)	Mean ± SD	P-value
Age (19-70)	19-34	22(36.66%)	568.38 ± 192.63	**0.527**
	35-52	30(50%)	537.86 ± 182.04	
	53-70	8(13.33%)	583.37 ± 144.46	
Gender	Male	35 (58/6%)	539.61 ± 172.01	**0.439**
	Female	25 (41/4%)	577.25 ± 190.93	
The Extent of Colon Involvement	Recto Sigmoiditis	21 (34/5%)	491.4 ± 183.55	**0.415**
	Left Colitis	23 (38/3%)	550.13 ± 154.56	
	Pan Colitis	16 (26/6%)	555.18 ± 179.41	

No significant relationship between age and calprotectin was observed in the study patients (P= 0.527). Between the level of calprotectin and the gender of patients, no significant relationship was found (p=0.439) and there was no relationship between the extent of involvement and the level of calprotectin in all age groups 19-24 (p=0.337), 35-52 (p=0.528), 53-70 (p=0.313) years.

Table 2. The relation between Calprotectin levels with colorectal involvement in different age and gender.

Variables		**The Extent of Colon Involvement**	**Participant (Percent)**	**Mean ± SD**	**P-value**
Calprotectin		-	38(%)	555.18 ±179.41 (560)	-
Gender	Male	Recto Sigmoiditis	13(36.1%)	539.61 ± 172.01	0.657
		Left Colitis	14(38/9%)		
		Pan Colitis	9(25%)		
	Female	Recto Sigmoiditis	8(33/3%)	577.25 ± 190.93	0.271
		Left Colitis	8(33/3%)		
		Pan Colitis	8(33/3%)		
Age (Year)	19-34	Recto Sigmoiditis	4(18/2%)	568.38 ± 192.63	0.337
		Left Colitis	9(40/9%)		
		Pan Colitis	9(40/9%)		
	35-52	Recto Sigmoiditis	13(34/4%)	537.86 ± 182.04	0.528
		Left Colitis	11(36/6%)		
		Pan Colitis	6(20%)		
	53-70	Recto Sigmoiditis	4(50%)	583.37 ± 144.46	0.313
		Left Colitis	2(25%)		
		Pan Colitis	2(25%)		

Discussion

In this study, we examined the relationship between stool calprotectin levels and the extent of colorectal involvement. Our results showed that there was no significant association between stool calprotectin levels and the extent of colon involvement.

In a study, TIDE and colleagues also investigated the relationship between stool calprotectin levels and mucosal involvement and tissue changes in patients with ulcerative colitis.

Their results also had a significant relationship showed between calprotectin levels and mucous involvement in such a way that low levels of calprotectin were associated with mucosal healing (19).

It was similar to our in measuring the level of calprotectin in the stool of patients with ulcerative colitis, but unlike our study, they did not investigate the extent of colon involvement in these patients. In a study of Kawashima and colleagues in 2015, it was shown that the greater amount of mucosal damage and the severity of ulcerative colitis cause the higher level of stool calprotectin so to evaluate the mucosal inflammation this marker can be useful (20).

Calafat and colleagues confirmed the relationship between ulcerative severity and calprotectin levels, and reported the average calprotectin level 3887, while this amount was equal to 555/18 in the current study. This result is not unexpected because Calafat studied on patients with severe ulcerative colitis and found the relationship between the level of stool calprotectin and the severity of the disease, as mentioned in the above studies. Therefore, as the severity of colon involvement increases, the amount of calprotectin in stool increase (21).

In a study that Taghvaei and his colleagues conducted in the 2015. The results of his study showed that the UC lesions were as follows: Proctitis (11.6%), Proctosigmoiditis (18.5%), Left Colitis (15.8%), pancolitis (11.7%) and normal endoscopy (42.4%). In summary, they suggested that stool calprotectin be used as a reliable tool to assess disease activity in patients with ulcerative colitis. In addition, their findings showed a significant correlation between stool calprotectin levels and mucosal healing (26). In a study of Zijlstra and his colleagues in 2016 in the Netherlands, it was shown that the level of stool calprotectin is not a good factor for distinguishing IBD from juvenile polyp, and in both of these diseases High levels of stool calprotectin were reported. In this study, 742 patients with IBD reviewed and entered the study (27).

Differences in the results of different studies can refer to differences in the location of calprotectin evaluation and the different outcomes reviewed in different studies. While other studies looked at the severity of the disease, we looked at the extent of the involvement. Some studies also looked at the mucous levels of calprotectin. As we know, calprotectin is a cytosolic protein found in granulocytes, so the higher the calprotectin, the greater the migration of neutrophils from the inflamed intestinal wall to the mucous, and it indicates the active phase of inflammation. Therefore, its level can increase with inflammation and the greater the severity of the disease, the higher the level of calprotectin. As it was seen, the average calprotectin in this study was equal to 555 which is higher than the 200 cut off level for organic active diseases and malignancies. So, although calprotectin levels have not been relationship to the extent of the disease, they can indicate the severity of the disease and are associated with the presence of active disease.

On the other hand, the extent of the disease does not necessarily mean its severity. As a result, greater breadth has led to more tissue involvement and has led to an increase in calprotectin levels, but this has little effect on the increase in calprotectin levels, which has not been significant.

Conclusions

With a glimpse of the results, it can be concluded that this marker cannot show the extent of the involvement at all, this inability is also present in different ages and genders. Therefore, it is not recommended to use this marker as a diagnosis of the extent of involvement in patients.

Declaration

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Authors' contributions

HN, ZMS, MJ, FT designed the study concept, collected and interpreted the data and drafted the manuscript.

Ethics approval

This study was given ethical approval by Zanjan University of Medical Sciences (Registration Number: A-11-918-8).

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