Calprotectin: A promising non-invasive tool for ulcerative colitis monitoring

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Abstract: Background: Ulcerative colitis (UC) has a chronic and regressive nature. Common diagnostic methods (colonoscopy and biopsy) have the disadvantage to be invasive, time-consuming and expensive. Therefore, a new sensitive and specific marker of disease activity of UC is urgently needed in clinical practice. The aim of this perusal is comparing to the value of fecal calprotectin level with colonoscopy in determining the severity of UC. **Methods:** One hundred and forty patients with the presumptive diagnosis of UC were chosen. After elimination of exclusion criteria regular clinical and paclinical evaluation was done. All patients underwent total colonoscopy for determining the severity of disease. A single stool sample was collected at the beginning of the study and the calprotectin concentration was assessed by a commercially available enzyme-linked immune sorbent assay (ELISA). C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also measured and were compared with calprotectin in determining disease activity of UC. **Results:** In all, 144 UC patients were prospectively included in the study. The mean age was 40.01 ±15.19 years and 45.8% were males. Mean level of fecal calprotectin were 270.45± 107.71 µg/g. Disease extent in UC patients was as follows: proctitis (14.6%), proctosigmoiditis (21.5%), left-sided colitis (18.8%), extensive colitis (14.6%) and 30.5% of patients had no involvement in colonoscopy. Calprotecin level had significant positive correlation with size of ulcers (P<0.001, r=0.661). We found a consequential relationship between high calproteectin level and UCEIS score (P<0.001, r=0.736). There was not a strong correlation between calprotectin level and clinical severity of UC (p=0.155). Also there was no significant correlation between calprotectin level and time since the diagnosis of UC (p=0.113, r=0.213). **Conclusions:** In conclusion, our data suggest that the fecal calprotectin represent a suitable marker in patients with UC, which makes the test a promising non-invasive tool for monitoring disease activity.

[Hassan Neishaboori, Tarang Taghvaei, Iradj Maleki, Farshad Nagshvar, Hafez Fakheri, Vahid Hosseini, Seyed Mohammad Valizadeh. **Calprotectin: A promising non-invasive tool for ulcerative colitis monitoring**. *Life Sci J* 2013;10(3):1356-1360] (ISSN:1097-8135). http://www.lifesciencesite.com. 203

Keywords: Calprotectin, Ulcerative colitis, Activity, Colonoscopy, Biomarker

1. Introduction

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory condition of the large bowel characterized by a relapsing course[1].It is important to accurately evaluate intestinal mucosa inflammation in the management of these patients, particularly for the assessment of therapeutic effectiveness[2].The assessment of ulcerative colitis (UC) activity is based on a combination of symptoms, clinical examination, and endoscopic findings[3].

Currently, the 'GoldStandard' for the evaluation of disease activity is endoscopy with serial biopsies[4], but these examinations are invasive and expensive for the patient[5, 6].

Fecal markers may be more specific for assessing intestinal disease activity. Specifically, calprotectin has been detected in stools in direct proportion to neutrophil migration through the gastrointestinal tract[7, 8, 9].

Fecal calprotectin, an important granulocyte cytosolic protein, is closely correlated with fecal excretion of ^{111}indium labelled leucocytes, deemed to be the gold standard for measuring intestinal inflammation[10, 11].Calprotectinis remarkably stable in stool samples, and its fecal concentration is unchanged by stool storage for up to 7 days at room temperature [12].

This study aims to investigate fecal calprotectin concentration as a marker of disease activity by comparing it with Ulcerative Colitis Endoscopic Index of Severity (UCEIS) and laboratory indices of disease activity in UC patients.

2. Material and Methods

Out of 177 adult outpatients and inpatients with a previously confirmed diagnosis of UC referred for colonoscopy to the Department of Gastroenterology of the Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari,

Iran between May 2012 and February 2013, 144 patients were included. They were diagnosed on the basis of standard clinical, endoscopic, and histologic criteria[13].All patients had been informed for participation, and the study protocol was approved by the Ethics Committee of the Medical Faculty of the Mazandaran University of Medical Sciences.

Exclusion criteria were a history of resectional colorectal surgery, active NSAID intake, neoplastic colon polyps, active infectious diarrhea at the time of colonoscopy and colorectal malignancies. Thirty-three UC patients were excluded, 17because of withdrawal, 4because of NSAID intake, 4 because of colon polyps, 4 because of the diagnosis of indeterminate colitis diagnosis, 2 because of final diagnosis of Crohn's disease, 1 because of colon cancer and 1 because of pancreas cancer and Whippel surgery. A single stool sample was collected from each patient in screw capped plastic containers that avoids toilet water artifact and simplifies laboratory sampling at the beginning of the study. The stool samples were frozen (−20 °C) until calprotectin determination. Fecal calprotectin was quantitated using an enzyme linked immunoassay (ELISA) test (Calprest, Eurospital, Trieste, Italy).The results of the test samples were calculated by the standard curve and were expressed as micrograms per milliliter. According to the manufacturer, the calprotectin cut off point as positive was 50 μg/g feces. Blood leukocytes (normal range 2.6–7.8 g/L), hemoglobin (normal range for women 120–160 g/L, for men 140–180 g/L), a sedimentation rate (normal range for women and men younger than 50 years up to 20 mm/h and 15 mm/h, normal range for persons older than 50 years up to 30 mm/h and up to 20 mm/h), *C-reactive protein*(upper limit of normal <5 mg/L) as well as platelet count, serum albumin level, liver transaminases (AST/ALT (SGOT/SGPT),alkaline phosphatase, *total iron-binding capacity* (TIBC), ferritin, serum iron, uric acid and potassium were determined as routine laboratory values during 3 days before endoscopy. Stool exam was conducted to rule out infectious diarrhea. All patients underwent total colonoscopy for determining the severity of disease.

Disease activity was determined through using colitis activity index (CAI) byRachmilewitz[14], which includes a combination of laboratory parameters and clinical symptoms, namely weekly calculation of bowel frequency, blood in stool, well-being, abdominal pain, fever, extraintestinal symptoms, erythrocyte sedimentation rate, and hemoglobin level[14].

Severity of disease was determined by clinical symptoms and laboratory findings were divided to 3 subgroups. Mild disease considered with fewer than four stools daily, with or without blood in stools, no systemic signs of toxicity, a normal ESR and mild anemia with Hb≥75% normal level. Moderate disease considered with more than four stools daily, but with minimal signs of toxicity. Patients may display anemia (not requiring transfusions), moderate abdominal pain, and low-grade fever, 38 to 39 °C (100 to 102 °F).Severe disease, correlates with more than six bloody stools a day or observable massive and significant bloody bowel movement, and evidence of toxicity as demonstrated by fever, tachycardia, anemia with Hb≤75% normal level or an elevated ESR. Severity of disease was determined by colonoscopy assessed by UCEIS.

Statistical analyses were carried out using the statistical package Statistical Package for the Social Sciences (SPSS, Chicago) software version18. Results of numerical data are presented as mean ± standard deviation (SD).All the p values were two tailed; p values < 0.05 were considered statistically significant. The association between endoscopic disease activity and clinical activity, fecal calprotectin, CRP, and blood leukocytes was assessed by determination of the Spearman's rank correlation coefficient(r) for nonparametric correlations.

Table 1. Serological, biochemical findings in patients with Ulcerative colitis

Parameters	Result	Range
AST*, IU/L	18.13±5.63	(10-48)
ALT*, IU/L	17.99±7.35	(12-57)
Alkaline phosphatase, IU/L	225.84±66.78	(80-496)
White blood cell, No./mm3	7996. 30±2136.49	(3800-14900)
Hemoglobin, g/dL	12.19±1.86	(6.9-16.2)
ESR*	26.61±14.13	(3-80)
Platelet count (×1000), No./mm3	296.11±87.94	(165-834)
Creatinine, mg/dL	0.86±0.34	(0.4-4)
Albumin, g/dL	4.03±0.61	(2.5-5.9)
Potassium, mEq/L	4.26±0.39	(3.5-5.4)
Ferritin, ng/mL	68.76±43.07	(10-517)
Serum Iron, μg/dL	62.91±27.74	(10-198)
TIBC*, μg/dL	368.67±65.91	(32-520)

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ESR: Erythrocyte sedimentation rate; TIBC: Total Iron Binding Capacity. All Values are mean ± SD; otherwise noted.

3. Results

One hundred and forty four UC patients were sequentially and prospectively included in the study. The mean age was 40.01 ±15.19 years and 45.8% were men. The mean duration of disease to the current colonoscopy was 42.74±52.66 months (range, 1–360 months), none had history of surgery. Disease extent in UC patients was as follows: proctitis (14.6%), proctosigmoiditis (21.5%), left-sided colitis (18.8%), extensive colitis (14.6%) and 30.5% of

patients had no abnormal findings in colonoscopy. Mean level of fecal calprotectin were 270.45±107.71µg/g (ranging from 50–630 µg/g).Severity of disease determined by clinical symptoms and laboratory findings was divided to 3 subgroups. 31 patients were mild (21.5%), 27 patients were moderate (18.8%), 38 patients were severe (26.4%) and 48 patients were in remission (33.3%). Colon biopsy histology reports revealed that 120 patients (83.3%) had active UC and 24 patients were in remission pathologically (16.7%). Patient characteristics and laboratory findings are described in table1. Stool exam was normal in 65.3% of patients. Colonoscopy findings divided the patients into three subgroups based on UCEIS: vascular pattern, bleeding and erosion and ulcers. 30.6 % of patients had normal vascular pattern with arborisation of capillaries clearly defined, or with blurring of patchy loss of capillary margins, 15.3 % had patchy obliteration of vascular pattern and 54.1 % had complete obliteration of vascular pattern. 61 patients(42.4%) had no visible blood, 49 patients(34%) had mucosal bleeding with some spots or streaks of coagulated blood on the surface of the mucosa ahead of the scope, which can be washed away, 25 patients(17.4%) had mild luminal bleeding with some free liquid blood in the lumen and 9 patients(6.2%) had moderate to severe luminal bleeding with frank blood in the lumen ahead of endoscope or visible oozing from mucosa after washing intra-luminal blood, or visible oozing from a hemorrhagic mucosa.75 patients(52.1%) had normal mucosa, no visible erosions or ulcer, 39 patients(27.1%) had tiny (≤ 5mm) defects in the mucosa, of a white or yellow color with flat edge, 20 patients (13.9%) had larger (>5mm) defects in the mucosa, which are discrete fibrin-covered ulcers in comparison with erosions, but remain superficial and 10 patients(6.9%) had deeper excavated defects in the mucosa, with a slightly raised edge. Calprotecin level had significant correlation with the size of ulcers and increased when size increment (P<0.001, r=0.661). The UCEIS details are described in table2.

Table 2. Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score in patients with UC.

Score	Number	Percent (%)
0	44	30.6
1	2	1.4
2	13	9
3	32	22.2
4	14	9.7
5	17	11.8
6	11	7.6
7	6	4.2
8	5	3.5

We found a linear relationship between high calprotectin level and UCEIS score (P<0.001, r=0.736) (Figure1).Patients divided into 5 groups considering the clinical, histological and colonoscopy findings. 48 patients (33.3%) were in complete clinical remission. 24 patients (16.7%) were in histological remission.44 patients (30.6%) had normal colonoscopy. 120 patients (83.3%) had histological active disease.100 patients (69.4%) had active disease in colonoscopy. There was no significant relationship between calprotectin level and clinical severity of UC (p=0.155). Comparing calprotectin level and CRP, there was no significant relationship (p=0.165). Also there was no significant correlation between calprotectin level and duration of disease(p=0.113,r=0.213).

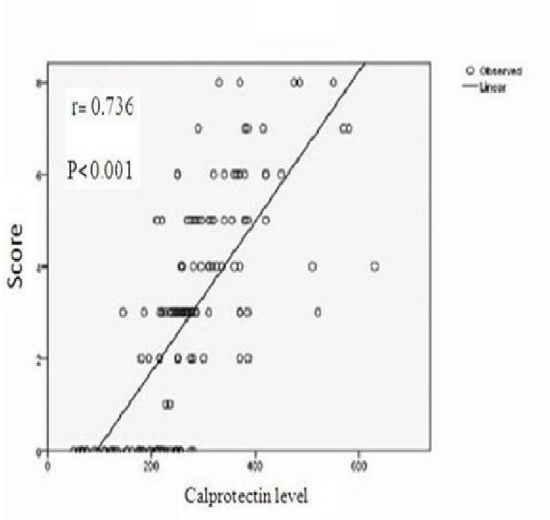

Figure 1. Relationship between high calprotectin level and UCEIS score.

4. Discussions

Ulcerative colitis is primarily characterized by mucosal inflammation of the colon with infiltration of neutrophils. Because of inflammation, relevant amounts of neutrophil-derived proteins are excreted into the gut lumen[15].The assessment of ulcerative colitis (UC) activity is based on a combination of symptoms, clinical examination, and endoscopic findings. Active inflammation in UC patients is associated with an acute phase reaction and migration of leukocytes to the gut. Thus, various proteins can be measured in serum and feces[16].

Taken together, our results indicated that calprotectin is suitable marker for monitoring disease activity in UC. The presence of calprotectin in feces is directly proportional to neutrophil migration toward the intestinaltract[17]. Furthermore, fecal calprotectin concentrations predicted the severity of colorectal inflammation, with increased concentrations strongly associated with advanced

histological grades of colorectalinflammation[18]. Platelet count, albumin, erythrocyte sedimentation rate and CRP are routinely used as inflammatory markers in blood when UC is suspected. However, these markers correlate poorly with histopathology[11, 19, 20].This was in concordance with our findings. It is well recognized that histology of colonic biopsy specimens is the most sensitive marker of colitis [20, 21, 22]and that macroscopic examination of the colon underestimates both the extent and the degree of inflammation compared with histology [21]. It is therefore interesting that fecal calprotectin concentration correlated more closely to histologic than to macroscopic colonic inflammation. This suggests that fecal calprotectin concentration may show that inflammation that is not detectable macroscopically during colonoscopy. As the new definition of treatment endpoints in IBD is changing, pathologic remission is considered in the newer references; hence calprotectin can be a noninvasive biomarker of this definition in UC patients. Several studies have already shown that fecal calprotectinis able to differentiate active from inactive UC[1, 20, 23-26].As fecal calprotectin closely correlates to endoscopically assessed mucosal damage[20, 27]and as patient acceptance of fecal sampling is high[28], this laboratory test could be useful to monitor the extent of mucosal damage in UC. Our results provide evidence that mucosal healing may noninvasively be assessed by measuring calprotectin as a surrogate marker. Accordingly, we did not find CRP and ESR to be useful in predicting relapse, in agreement with otherauthors[27,29,30]. These differences could be explained by the fact that calprotectin seems to be a direct marker of intestinal inflammatory activity, while serological biological markers (such as CRP and ESR) estimate inflammation indirectly[31].

In comparison to endoscopy this method is simple, noninvasive and inexpensive. However, fecal calprotectin can only reflect the excretion of neutrophils[2].

Calprotectin is stable in stools, directly associated with the inflammatory process, and easy to measure and has the potential to fulfill all criteria for an "ideal" test: simple, inexpensive, safe, noninvasive, convenient, acceptable for patients and staff, objective, reliable, and amenable to serial measurements to permit the assessment of therapeutic interventions. As such, fecal calprotectin measurement comes close to the requirements of the ideal test[32].

In summary, we demonstrated that fecal calprotectin was the only marker that could reliably discriminate inactive from active UC and has the potential to replace endoscopy in the diseasemonitoring[33].

In conclusion, our data suggest that the calprotectin in feces represent suitable markers in patients with UC, which makes the test a promising non-invasive, precise and simple tool for monitoring disease activity.

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