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Original Article

Evaluation of Fecal Calprotectin before and after Anti-TNF Therapy in Patients with Inflammatory Bowel Diseases

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ABSTRACT

Background:
Ulcerative colitis (UC) and Crohn's disease are chronic relapsing disorders in which their clinical activity and treatment response are evaluated by various indices. The response rate is unpredictable, and for its evaluation, we use invasive procedures like colonoscopy and blood samples to address this issue. The current study was designed to evaluate the accuracy of fecal calprotectin before and after treatment with anti-tumor necrosis factor (TNF) in patients with inflammatory bowel disease (IBD).

Materials and Methods:
46 patients with IBD who were a candidate for anti-TNF therapies were evaluated and the correlation between fecal calprotectin and disease activity before and after 5 months of treatment was studied.

Results:
Fecal calprotectin and disease activity after treatment with anti-TNF correlate, but before treatment, there was no correlation in the patients with UC.

Conclusion:
Fecal calprotectin not on its own but alongside other tests could be used for follow-up treatment.

Keywords: Inflammatory Bowel Diseases , Anti-TNF, Fecal calprotectin.

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INTRODUCTION

Inflammatory bowel diseases (IBD) including, ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory relapsing disorders that affect the gastrointestinal (GI) tract. Although the pathogenesis of IBD is still elusive, it is usually considered to be triggered by inadequate mucosal immune responses to commensal microbiota in genetically susceptible people (1). Clinical activity indices, such as Harvey–Bradshaw index (HBI) in CD and the Mayo Score in UC, demonstrate patients' subjective well-being state rather than mucosal healing (MH)(2). While MH is the ultimate target of therapy, its evaluation requires

the invasive procedure of colonoscopy(3). In clinical practice, inflammatory markers such as C-reactive protein (CRP), lactoferrin, and calprotectin have been introduced to evaluate response to the treatment (4,5).

Calprotectin is a calcium-binding protein, for practical purposes, could be considered as a neutrophil-specific agent(6). However, small levels of this protein are present in other phagocyte cells. Calprotectin is measured by an enzyme-linked immunosorbent assay (ELISA) on a sample of stools below one g(7). Many studies estimate the standard range of calprotectin to be 10 to 50 or 60 µg/mg when quantitative tests are applied(8). Nevertheless, rates above 200 µg/mg have a higher positive pathological predictive value, and values of 500 to 600 µg/mg almost assured pathology outcomes(9).

Calprotectin is most commonly used in diagnostic examinations of patients with suspected IBD. High concentrations of fecal calprotectin may be useful for discriminating IBD from specific GI disorders such as irritable bowel syndrome (IBS) (10).

Objective

Because of the low cost, non-invasiveness, and accessible measurement of this protein, It is important to evaluate the role of fecal calprotectin concentration in the diagnosis, recurrence rate, and prediction of treatment response in patients with IBD. In the present study, we aimed to identify the validity (diagnostic accuracy) of the fecal calprotectin level as a biomarker of disease activity in patients with IBD before anti-tumor necrosis factor (TNF) therapy. Moreover, we aimed to determine the application of this protein as a marker of treatment response.

MATERIALS AND METHODS

In this prospective cohort study, 46 patients with IBD (26 with UC, and 20 with CD) were enrolled. The inclusion criteria were as follows 1: IBD diagnosed based on the clinical, colonoscopy, and histopathological standards by gastroenterologists, and liver disease specialists. 2: non-responder to the first-line treatment and naive candidate of anti-TNF therapy. The exclusion criteria were severe cardiovascular disease, active infectious diseases, tuberculosis, and age less than 18 or above 70 years. Additionally, patients who were unwilling to complete a written consent form to use their registered information in future studies were also excluded. HBI(Harvey Bradshaw Index) as a numeric index of CD activity is composed of five parameters: general well-being, abdominal pain, number of liquid stools per day, abdominal mass, and complications. This index is calculated by a limited physical examination for abdominal mass and patient recall from the previous day. Mayo score consists of stool frequency, rectal bleeding, mucosal appearance at endoscopy, and physician rating of disease activity used to assess the severity of UC. In our study, HBI for CD and Mayo score for UC patients were calculated before and 5 months after treatment with anti-TNF. HBI cut-off for the clinical response and the clinical remission was defined as below five, while this cut-off for Mayo score is considered three. Stool calprotectin is easily measured by commercially available ELISA test at the same intervals and compared with the response to treatment, and the amounts more than 250 µg/mg is defined as high levels.

Statistical Analysis

The categorical variables were reported as numbers and percentages, while the continuous variables were reported as median and interquartile levels (IQR). The statistical analysis was performed using SPSS software for Windows, version 25. A P value <0.05 was considered statistically significant. The Spearman's rank correlation coefficient rho (q) and paired t test were calculated to assess correlations between the two variables (FC, Mayo score, and HBI). The receiver operating characteristic (ROC) curves were drawn for the FC levels, and the area under the curve (AUC) was calculated for the diagnostic accuracy of FC.

RESULTS

A total of 46 patients with IBD (20 with CD and 26 with UC) were enrolled in this survey. The mean ages of the participants were 38.5 (30-55 years) in CD patients and 39.2 (20-69 years) in UC patients. 25 (54.3%) patients with IBD were male, and 21 (45.7%) were female. The number of men in the CD group was more than the UC patients (male/female ratio CD: 11/9, UC: 14/12). In the CD group, the main disease localization was pancolitis with small intestinal involvement (65%). While in 30% (6 cases) of CD patients, the disease had been documented as

isolated terminal ileum involvement. Of 26 patients with UC, most of them had pancolitis (80.7%), and five patients (20.2%) presented with left side colon involvement (Table 1).

Table 2 demonstrates the indices of disease activity for both groups before and after anti-TNF therapy. The mean HBI in CD was 14.8 before treatment and decreased to 5.6 after five months of receiving anti-TNF. This reduction, according to the Wilcoxon test, was significant (p<0.001). The same significant result was extracted from the UC group with a Mayo score of 8.65 before and 2.58 after treatment (p <0.001).

Assessment of the fecal calprotectin (FCP), CRP, and erythrocyte sedimentation rate (ESR) values in patients before and five months after anti-TNF therapy revealed that all these three parameters were higher in the first interval (before anti-TNF treatment) and had a reduction with treatment. In particular, there was a statistically significant difference in FCP levels before and after treatment (p < 0.001, Table 3).

The mean ESR in CD was 24.25 mm/h before treatment, which decreased to 15 mm/h after five months of receiving anti-TNF. This reduction was significant (p < 0.001). The same significant result was extracted from the UC group with ESR 37.31 mm/h before and 8.62 mm/h after treatment (p < 0.001).

The mean level of CRP in CD disease was 7.9 mg/L before treatment, which decreased to 5.3 mg/L after treatment. This reduction, according to the Wilcoxon test, was significant (p < 0.001). The same result was extracted from the UC group with CRP 8.62 mg/L before and 3.85 mg/L after treatment (p < 0.001)

Our result showed that the HBI score and FC-ELISA levels had a significant correlation in CD patients before anti-TNF therapy (r=0.488, p = 0.029), (Table 2). In contrast, among UC patients, this correlation between the Mayo score and FC-ELISA levels was not significant (r = 0.199, p = 0.33), (Table 4). To determine the correlation between HBI and Mayo scores with the FCP level after five months of being treated with anti-TNF, we used Spearman's rho test. Interestingly HBI and Mayo scores showed a very close correlation with the fecal calprotectin in patients with CD and UC (p < 0.001), (Table 5).

In 30/33 patients with response to treatment, fecal calprotectin less than 250 µg/mg were seen. It showed the sensitivity of this test as 91.7%. In 1/3 of the patients with no response treatment, FCL was higher than 250 µg/mg, which showed that the specificity was 40%, the positive predictive value was 84.6%, the negative predictive value was 57.1%, and accuracy rate was 80%.

DISCUSSION

IBD diagnosis, activity, and remission are considered to be a huge challenge for gastroenterologists as they require precise history and physical exams in addition to the lab data and colonoscopy. It is emerging that fecal calprotectin is being used as a surrogate marker for intestinal inflammation(11). However, the role of fecal calprotectin in disease activity and response to treatment remains sparse in recent studies. This research aimed to signify the accuracy of FCL in IBD patients as a marker of disease activity before and after treatment.

The result from this study demonstrated that HBI in Crohn's patients and Mayo score in UC patients were related to treatment response. Therefore, with successful anti-TNF therapy, a significant reduction in disease activity is noticed. Before anti-TNF therapy, a significant correlation was achieved between HBI and FCL in patients with CD compared with the Mayo score and FCL in UC. In other words, a higher score of FCL suggests a higher amount of HBI and not a Mayo score before anti-TNF treatment. Pavlidis and colleagues showed in 26 patients with CD that FCL before treatment with anti-TNF was not correlated with response to treatment, but patients with response to treatment had lower FCL in the induction period(12).

The result from this study demonstrated that HBI in patients with CD and Mayo score in patients with UC were related to treatment response. Therefore, with successful anti-TNF therapy, a significant reduction in disease activity was noticed. Before anti-TNF therapy, a significant correlation was found between HBI and FCL in patients with CD compared with the Mayo score and FCL in UC. Moreover, a higher score of FCL suggests a higher amount of HBI and not a Mayo score before anti-TNF treatment.

In our study FCL, less than 250 µg/mg was the response to treatment, the sensitivity of this value was 91.7%, the specificity was 40%, and it showed false positive of this test is high. Sipponen and

Table 1: Location of involvement

Variables		Left colitis	Ileum	Pancolitis	Pancolitist and illeum	Total
Crohn	Count	0	6	1	13	20
	%Percentage	0%	30%	5%	65%	100%
Ulcerative colitis	Count	5	0	21	0	26
	%Percentage	19.2%	0%	80.8%	0%	100%

Table 2: Comparison of the effect of before and after treatment on HBI and Mayo score

Variables		Mean (Std)	Median IQR	Statistic value	(P value)
HBI	Pre	14.8 (5.89)	14 (12)	-3.93	(0.001)
	Post	5.6 (3.32)	4 (4)		
Mayo	Pre	8.65 (1.648)	9.5 (3)	-4.488	< 0.001
	Post	2.58 (0.902)	2 (1)		

Table 3: Comparison of the effect of before and after treatment oncalprotectine

	Mean (Std)	Median IQR	Statistic value	(P value)
pre-treatment	704.11 (397.24)	600 (280)	-5.3	<0.001
post-treatment	188.96 (330.83)	90 (143)		

Table4: Relationship between HBI, Mayo score, and calprotectin before treatment

variables		Calprotectin
HBI	Pearson Correlation	0.488*
	Sig. (2-tailed)	0.029
	N	20
Mayo	Correlation Coefficient	-0.199
	Sig. (2-tailed)	0.330
	N	26

Table 5: Relationship between Mayo and HBI and calprotectin after treatment

variable			Calprotectin
Spearman'srho	HBI	Correlation Coefficient	0.727**
		Sig. (2-tailed)	0.000
		N	20
	Mayo	Correlation Coefficient	0.605**
		Sig. (2-tailed)	0.001
		N	26

others showed that FCL more than 200 µg/mg was correlated with disease activity index (CDAI) with a 70% sensitivity and a specificity of 90%. On the other hand, both HBI and Mayo scores were correlated with FCL after treatment(13). This illustrated that with the response to therapy and reduction of the HBI and Mayo score, FCL decreases.

Our data are in line with previous studies; for instance, GT and colleagues speculated that FCL in 29/32 patients with the clinically active disease was elevated. While only 7/30 patients had an excessively high level in remission(14). There is a strong association between HBI and FCL, consistent with the study by Shuhei and others in which FCL was correlated to disease activity in 42 Crohn's and 113 UC patients(15). Moreover, Lin and colleagues showed that FCL was correlated with the CD activity index in 36 CD and 52 UC patients(10). Results of previous studies and our study revealed that with the response to therapy and decline in the Mayo score and HBI, the observed value of FCL decreased(10,16,17). Nevertheless, this correlation was not investigated before, and after therapy, so this aspect makes our research novel.

CONCLUSION

FCL before anti-TNF therapy was only correlated with disease activity in patients with CD. FCL is not a precise and accurate alternative to disease activity in IBD. This issue causes a certainty limit. Therefore the practical importance of FCL becomes dominant alongside other activity factors. Thus, large-scale, multicentre, and well-designed studies are recommended to evaluate and improve our conclusion.

In conclusion, FCL was observed to be correlated with disease activity in patients with CD before and after anti-TNF treatment, while the correlation was only observed in UC disease after therapy. FCL may not be the specific indicator of disease activity in IBD patients, so further studies with more sample size should be designed.

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CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

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