Role of Quantitative Fecal Immunochemical Test in Assessment of Mucosal Healing in Patients with Ulcerative Colitis

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Abstract

Background: Ulcerative Colitis (UC) is a chronic inflammatory disorder of the colorectum that is characterized by a remission and relapse course. Mucosal healing has been considered the goal of treatment in UC because it reduces the risk of relapse and colectomy. Fecal biomarkers have emerged as an important easy, rapid and non-invasive tool for assessing and monitoring mucosal healing in patients with UC.

Aim of the Study: To determine if the q-FIT can evaluate the mucosal healing in UC patients.

Patients and Methods: The study was conducted on 60 UC patients who underwent colonoscopy in the Endoscopy Unit, Internal Medicine Department in Tanta University Hospital. FIT was examined in stool samples from all the patients. Mucosal status was assessed using the Mayo Endoscopic Subscore (MES).

Data were collected from all study patients including; clinical, demographic and laboratory data. Statistical analysis was carried out for all collected data and statistical significance was determined at a p-value <0.05.

Results: A total of 60 UC patients were evaluated with FIT results in conjunction with colonoscopies. The sensitivity and specificity of the FIT values (<100ng/ml) for predicting MH (MES 0 alone) were 0.93 and 0.80, respectively. The sensitivity and specificity when MH was defined as (MES 0 or 1) were 0.75 and 0.91, respectively. A significant correlations between FIT levels and the MES was observed (r=0.858, p-value=0.001 *).

Conclusion: Quantitative FIT can be a non-invasive and effectivebiomarker for evaluation of mucosal healing in UC.

Key Words: Ulcerative colitis – Mucosal healing – Quantitative faecal immunochemical test.

Introduction

ULCERATIVE Colitis (UC) is one of the 2 major types of Inflammatory Bowel Disease (IBD), along with Crohn's disease. It is a chronic inflammatory condition causing continuous mucosal inflammation

Correspondence to: Dr. Ahmad F. Abo El-Ezz Ahmed, E-Mail: ahmed_fikry64@yahoo.com. of the rectum and the colon. It is characterized by a relapsing and remitting course [1].

Patients with ulcerative colitis usually present with diarrhea, which may be associated with blood. Bowel movements are frequent and small in volume as a result of rectal inflammation. Associated symptoms include colicky abdominal pain, urgency, tenesmus, and incontinence [2]

The treatment strategy for UC consists of remission induction therapy in the active stage, followed by maintenance of remission after successful remission induction. UC patients are treated to achieve and sustain clinical remission, as indicated by the disappearance of clinical symptoms. Recently, however, not only clinical remission but also endoscopic Mucosal Healing (MH) is being pursued as the treatment goal for UC [3-5].

Endoscopically assessed mucosal healing is associated with improved outcomes for ulcerative colitis [6]. However, undergoing colonoscopy to determine mucosal healing is invasive and costly. Because clinical assessment in asymptomatic UC patients can underestimate mucosal disease activity, surrogate biomarkers of endoscopic disease assessment are appealing [7]. Therefore, non-invasive methods of evaluating and predicting mucosal status are eagerly desired.

Quantitative Fecal Immunochemical Tests (FITs) can measure hemoglobin concentrations in feces using an antibody for human hemoglobin. The amount of fecal blood most likely reflects mucosal status in UC. In particular, occult blood can be present in feces of UC patients in clinical remission but without mucosal healing. Such fecal occult blood can be detected by FIT, and therefore, a negative FIT result may reflect and predict mucosal healing non-invasively [8].

Patients and Methods

This study was carried out over 60 Egyptian ulcerative colitis patients who underwent colonoscopy in the Endoscopy Unit at the Internal Medicine Department in Tanta University Hospital in the period from February 2016 to March 2017.

Mucosal status was assessed using the Mayo endoscopic subscore classification. It consists of four subscores: Stool frequency, rectal bleeding, endoscopic findings; and physician's global assessment. Each one of these subscores ranges from 0 to 3, arriving at a final score between 0 and 12 (≤ 2 remission, 3-5 mild disease, 6-10 moderate disease, 11-12 severe disease).

Inclusion criteria:

All of the patients had an established diagnosis of UC according to endoscopic and histologic assessments.

Exclusion criteria:

- Patients who did not have FIT results within 1 month before colonoscopy.
- Patients with changes in abdominal symptoms or treatment after the FIT but before colonoscopy.
- If the colonoscopic examination was incomplete because of problems with the bowel preparation because the colonoscope could not be reached to the cecum.

Method:

All participants in this study were subjected to: Thorough history taking, full clinical examination, laboratory investigations in the form of: Full Blood Count, Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), serum albumin, quantitative fecal immunochemical test in stool and colonoscopy.

Sampling and laboratory investigations:

Sampling and all laboratory investigations were done in Clinical Pathology Department, Tanta University Hospitals. Fresh fecal sample is collected using Epitope Diagnostics Fecal Sample Collection Device. This test is intended for detection of fecal occult blood by the quantitative determination of human hemoglobin levels in stool samples by ELISA. The assay utilizes the two-site sandwich technique with two selected antibodies that bind to different epitopes of human hemoglobin. Assay standards, controls, and patient samples are added directly to wells of a microtiter plate that is coated with hemoglobin antibodies. Subsequently, a Horseradish Peroxidase (HRP)-conjugated human hemoglobin specific antibody is added to each well. After an incubation period, a sandwich of antibody human hemoglobin HRP-conjugated antibody is formed. The unbound antibody and buffer matrix is removed in the subsequent washing step. For the detection of this immune complex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader [9-11].

Colonoscopy:

The patients received a polyethylene glycol based electrolyte solution (e.g, moviprep or fortrans) for bowel preparation before colonoscopy. Total colonoscopy was done using an EPK-i5000 colonoscope (Pentax, Tokyo, Japan). Assessment of the mucosal status is done using the Mayo endoscopic subscore classification. Mucosal healing was defined as Mayo score '0' or '0 or 1' throughout the colorectum.

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package Version 20.0 (12). Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparisons between more than two populations were analyzed F-test (ANOVA) to be used and Post Hoc test (LSD). For abnormally distributed data, Kruskal Wallis test was used to compare between different groups and pair wise comparison was assessed using Mann-Whitney test. Significance of the obtained results was judged at the p-value <0.05.

Results

In our study, age of the patients ranged from (17-68 years) with a mean age of 35.65 ± 10.66 . There were 26 males (43.3%) and 34 females (56.7%).

As regard the clinical activity of the disease, there were 16 (26.7%) patients in clinical remission and 44 (73.3%) patients in clinically active disease.

Among the 16 patients in clinical remission, there were 12 (75%) patients with MES 0, 3 (18.75%) patients with MES 1, 1 (6.25%) patient with MES 2 and no patients with MES 3. This means that the relation between clinical and endoscopic remission was statistically significant (*p*-value=0.001) and that patients in clinical remission with MES 0 showed significantly lower FIT results than MES 1 and MES 2 (*p*-value=0.017). (Table 1).

While comparing between the MES and platelets count, platelet counts were not related statistically to mucosal healing (by MES) (*p*-value=0.251).

On the other hand, the comparison between the MES and TLC showed that there was statistically significant lower total leucocytic counts in patients with mucosal healing (by MES) than without (*p*-value=0.001). (Table 2).

On the other hand, the comparison between ESR results and MES were not statistically significant (p-value=0. 119). While there was statistically significant relation between MES and CRP values (p-value= 0.001). (Tables 3,4).

The comparison between FIT results and colonoscopic findings by Mayo score showed the number of the cases with negative FIT (less than 100ng/ml as cut-off value) was greatest in cases with Mayo 0 and gradually decreased as the activity of the disease increased. This means that patients in remission by Mayo score showed statistically significantly lower FIT results (*p*-value=0.001). (Table 5).

Our study has shown statistically significant positive correlation between FIT results and Mayo endoscopic score (*p*-value=0.001*) Fig. (1).

The diagnostic performance of FIT results in our patients when a negative FIT result was of less than 100ng/ml as the cutoff value, showed that fecal immunochemical test results in relation to mucosal healing (Mayo endoscopic score 0) were 93% sensitivity and 80% specificity. Fig. (2).



Fig. (1): Positive correlation between FIT results and Mayo endoscopic score.



Fig. (2): Area under ROC curve denoting sensitivity of fecal immunochemical tests for mucosal healing (Mayo 0).

Table (1): Patients with clinical remission in comparison with MES.

Mayo Clinical remission 0: N 12	
NI 12	
1N 12	
% 75.0%	
1:	
N 3	
% 18.75%	
2:	
N 1	
% 6.25%	
31	
N 0	
°⁄₀ 0°⁄₀	
Total:	
N 16	
% 100.0%	
Chi-square:	
Chi-square: x^2 26.243	
\hat{p} -value $0.001*$	

p-value=0.001* (Significant).

Tab	le (2):	PLT	and	TLC	values	in	comparison	with MES	S.
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MES	Range	Mean \pm SD	<i>t</i> -test <i>p</i> -value	
$PLTX10^{3}/ml$:				
0	160-430	276.611±89.361	1.403	0.251
1	210-330	272.429 ± 44.436		
2	190-380	283.666 ± 74.353		
3	160-500	325.423 ± 102.617		
$TLC X10^{3}/ml$:				
0	4-10.8	6.984±2.144	5.878	0.001*
1	4.5-12.9	7.885±2.775		
2	3.9-10.9	7.533 ± 2.374		
3	4.2-16.9	10.355±3.334		

Table (3): Comparison between ESR and MES of the studied patients.

Mayo	Range	Mean \pm SD	<i>t</i> -test	<i>p</i> -value
ESR (mm/h):				
MES0	5-140	38.56 ± 36.42	2.038	0.119
MES 1	10-40	22.86 ± 11.13		
MES2	20-95	45.56±27.44		
MES3	10-145	53.08 ± 31.02		

Table (4): Comparison between CRP and MES of the studied patients.

Mayo	Cl	Tatal			
	Pos.	Neg.	Total		
0-1:					
Ν	8	17	25		
%	32% 68% 100.0%				
2-3:					
Ν	26	9	35		
%	74.3%	25.7%	100.0%		
Total:					
Ν	34	26	60		
%	56.7%	43.3%	100.0%		
Chi-square:					
x^2	10.	623			
<i>p</i> -value	0.0	01 *			

Table (5): Comparison between FIT results and colonoscopic findings.

Mayo	F			
subendoscopic score (0-3)	Positive	Negative	Total	
0:				
Ν	6	12	18	
%	33.3%	66.7%	100.0%	
1:				
Ν	5	2	7	
%	71.4%	28.6%	100.0%	
2:				
Ν	8	1	9	
%	88.9%	11.1%	100.0%	
3:				
Ν	26	0	26	
%	100.0%	0%	100.0%	
Total:				
Ν	45	15	60	
%	75.0%	25.0%	100.0%	
Chi-square:				
χ^2	26	.307		
p-value	0.001 *			

Discussion

Ulcerative Colitis (UC) is a chronic inflammatory disorder of the colorectum that is characterized by a clinical course of remission and relapse. Clinical remission is defined as a Mayo stool frequency of 0 or 1 and a Mayo rectal bleeding subscore of 0 [13]. The clinical status of the disease does not always correlate with the mucosal status, so continued monitoring of clinical manifestations as well as mucosal healing is required [14].

The non-invasive, low cost, and rapid FIT is applied to assess the mucosal status [15]. Q-FIT uses antibodies directed against human hemoglobin [16].

The present study was conducted in the Endoscopy Unit at the Department of Internal Medicine in Tanta University Hospital and was carried out on 60 UC patients with variable degrees of activity according to Mayo score.

In our study, there was a slight female predominance as females represented 56.7% and males represented 43.3% of the patients. Nakari et al., [15] who studied the evaluation of mucosal healing of UC by FIT, documented that 49% of the patients were females and 51% were males.

Regarding CRP, our study showed that the increase in the mayo endoscopic score is associated with elevation in CRP values and it was statistically significant (*p*-value=0.001). This was in agreement with Solem et al., [17] who discussed the correlation of CRP with clinical, endoscopic, histologic, and radiographic activity in IBD.

Against to our study, Auzoux et al., [18] who studied the assessment of mucosal healing in inflammatory bowel disease, indicated that the increase in CRP is not specific to inflammatory illness and can also be observed in cases of sepsis or systemic inflammation.

As regard ESR, there was statistically nonsignificant comparison with mucosal healing according to MES in our study (*p*-value=0.063). This was in agreement with Lok et al., [19] who discussed the correlation between ESR and mucosal inflammation in Chinese ulcerative colitis patients.

Another study by Yoon et al., [20] who discussed the correlations of CRP and ESR with endoscopic activity indices in patients with ulcerative colitis, showed that ESR level were modestly correlated with endoscopic activity indices, including Mayo score, in UC patients with low sensitivity and specificity for detecting endoscopic remission. This discrepancy between the ESR and CRP results in assessment of the mucosal status may be explained by the application of these markers in systemic inflammation and so, they are non-specific to inflammation of the mucosa. Shinzaki et al., [21] studied the Leucine-rich Alpha-2 Glycoprotein as a novel serum biomarker for detecting mucosal healing during disease course in UC patients with normal CRP, explained that the inflammation is regulated mainly by cytokine[s] other than IL-6 that stimulates the synthesis of CRP in the liver.

Based on the results of our study, endoscopic remission was significantly corresponding to clinical remission as 75% of patients in clinical remission were MES 0 and 18.75% of them were MES 1. This was in agreement with Lewis et al., [22] who used the non-invasive components of Mayo score (the stool frequency and rectal bleeding) to assess the clinical response in UC patients.

Opposite to our study, Meucci et al., [23] who studied a group of mild to moderate UC patients, demonstrated that there was no correlation between the rate of clinical remission and the degree of endoscopic activity.

In our study, MES 0 patients in clinical remission presented significantly lower FIT results than MES 1-3. This was in line with the results of Nakari et al., [24] who studied the correlation between FIT results, mucosal healing and risk of relapse in UC patients in clinical remission.

In the same context, Ryu et al., [25] demonstrated that fecal hemoglobin can be used as a marker of clinical disease activity in patients with UC, with a negative FIT accurately reflecting disease remission with the advantage of easy availability, fast results and low financial cost.

However, Jauregui-Amezaga et al., [26] reported that 27% of UC patients in clinical remission with mucosal healing relapsed within one year. This is attributed to the natural course of UC. Considering such fact, non invasive biomarkers that assess and indicate the continuation of remission would be relevant.

Consequently, a study performed by Hiraoka et al., [27] concluded that throughout the followup of UC patients who were confirmed to have MH with negative FIT, clinical remission continued as long as FIT was negative, and that positive conversion of the FIT, particularly with high values, could be correlated with clinical relapse a few months later. In this study, the predictability of MH was compared by FIT results. FIT results and colonoscopic findings by Mayo score showed statistically significant positive correlation in assessment of mucosal healing as *p*-value=0.001. This was in agreement with Nakari et al., [15] and Ryu et al., [25] who reported statistically significant positive correlation between fecal hemoglobin concentrations and MES (*p*-value <0.01 and <0.0001 respectively).

Conclusion:

These findings suggest that quantitative fecal immunochemical test was reliable, non-invasive, easy, rapid and low cost tool to reflect the colonic mucosal status in patients with UC. Therefore, a negative FIT was strongly correlated with mucosal healing, and so quantitative FIT can be considered a novel marker for ulcerative colitis diagnosis and follow-up.

Recommendations:

Future studies including larger number of patients and longer duration are required to better clarify the exact role of FIT in assessment of mucosal healing in UC patients, monitoring of the disease activity and to inform clinical decisions to modify the treatment.

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Conflict of interest:

None declared.

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دور فحص البراز المناعى الكيميائى الكمى فى تقييم إلتئام الآغشية المخاطية فى مرضى إلتهاب القولون التقرحي

إشتمل هذه البحث على ستين مريضا يعانون من إلتهاب القولون التقرحى فى مستشفى طنطا الجامعى فى الفترة من فبراير ٢٠١٦ إلى مارس ٢٠١٧، وتم آخذ التاريخ المرضى للمرضى وإخضاعهم للفحص الإكلينيكى الشامل وفحوصات معملية وهى صورة دم كاملة، دلالات الإتهاب (سرعة الترسيب والبروتين الإرتكاسى-سى)، نسبة الآلبيومين، وفحص البراز المناعى الكيميائى الكمى الذى يستخدم آجساما مضادة لهيموجلوبين الدم البشرى مما يؤدى إلى قياس كم الدم الخفى فى البراز مما يعكس مدى إلتئام الأغشية المخاطية لإلتهاب القولون التقرحى بطريقة آمنة. بالإضافة إلى ذلك، تم عمل منظار القولون للحالات بعد الحصول على الموافقة اللازمة.

وقد خلصت هذه الدراسة إلى إنخفاض ذو قيمة إحصائية بمستويات فحص البراز المناعى الكمى فى مرضى إلتهاب القولون التقرحى فى حالة السكون المرضى ومقياس "مايو" (٠)، وإلى وجود علاقة طردية ذات دلالة إحصائية بين مستويات فحص البراز المناعى الكمى وإلتئام الآغشية المخاطية ونتائج المنظار القولونى بإستخدام مقياس "مايو". آيضا وجود علاقة طردية ذات دلالة إحصائية بين مستويات فحص البراز المناعى الكمى ونتائج مقياس "مايو".