Comparative study the levels of plasma transforming growth factor-β1, serum crp, fecal lactoferrin, and fecal calprotectin as biomarkers for disease activity in Egyptian patients with ulcerative colitis

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Aim This study aimed to analyze the utility of transforming growth factor-β1 (TGF-β1), C-reactive protein (CRP), fecal lactoferrin (LF), fecal calprotectin, and the Mayo score for severity of ulcerative colitis (UC) in monitoring disease activity in Egyptian patients with UC.

Patients and methods This study was carried out on 130 patients with UC and scored according to the Mayo score for severity of UC. Patients and controls were exposed to fecal and blood samples to assess TGF-β1, CRP, fecal LF, and fecal calprotectin.

Results The values of TGF-β1, CRP, fecal LF, and fecal calprotectin in UC patients (n=130) compared with controls (n=30) were as follows: TGF-β1: 489.32±315.68 versus 5.93 ±1.81 pg/ml, CRP: 15.97±9.13 versus 3.17±0.95 mg/l, fecal LF: 497.06±448.95 versus 7.01±4.00 μg/g, fecal calprotectin: 809.70±554.36 versus 36.33±15.51 μg/g (for all $P<0.001$). The parameters of Mayo Score that determine the severity of ulcerative colitis correlated significantly with TGF-β1 (Spearman's rank correlation coefficient r=0.925), CRP (r=0.957), LF (r=0.932), and calprotectin (r=0.953). TGF-β1, CRP, fecal LF, and calprotectin levels were significantly lower in UC patients with inactive disease (TGF-β1: 46.4±37.1 pg/ml; CRP: 4.8±1.3; LF: 28.6±28.3 μg/g; calprotectin: 71.7±24.2 μg/g; $P<0.001$ for both LF and calprotectin, but $P>0.05$ for both TGF-β1, and CRP) compared with patients with mild (TGF-β1: 343.4±110.7 pg/ml; CRP: 9.8±2.1; LF: 177.8±66.8 μg/g; calprotectin: 459.0±206.7 μg/g; $P<0.001$), moderate (TGF-β1: 640.6±141.0 pg/ml; CRP: 18.6±3.5; LF: 561.0±181.9 μg/g; calprotectin: 1080.8±224.1 μg/g; $P<0.001$), and high active disease (TGF-β1: 814.5±132.9 pg/ml; CRP: 27.1±3.0; LF: 1048.3±296.8 μg/g; and calprotectin: 1421.7±95.5 μg/g; $P<0.001$). The overall accuracy for the detection of histopathologic active disease was 87.7% for TGF-β1, 89.2% for the Mayo score for severity of UC, 84.6% for CRP, 90% for fecal LF, and 91.5 for fecal calprotectin.

Conclusion Fecal LF, fecal calprotectin and TGF-β1, and CRP correlated significantly with the Mayo score for UC and histopathology. Furthermore, calprotectin and LF are appropriate markers that can distinguish endoscopic and histopathologic inactive from active disease. Also, TGF-β1 and CRP were used as suitable markers to differentiate mild from moderate and the moderate from high active disease. Thus, these four biomarkers may be used for surveillance of UC activity.

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Introduction

Ulcerative colitis (UC) is a form of chronic inflammatory relapsing bowel disease. The recognition of inflammatory activity is critical to enable clinical decision-making and for customization of therapy [1]. A standard based on clinical manifestations and/or endoscopy is proposed to define remission in UC. Diverse disease activity indices are attainable for UC and various symptom-based activity scores, composite scores, and patient assessment scoring systems are available for use and have been published [1–4]. Two widely used scores are the Mayo UC Disease Activity Index by Sutherland [5] and the Ulcerative Colitis Endoscopic Index of Severity [6]. Both include clinical and endoscopic parameters. The Mayo index has the advantage that both the clinical and the endoscopic parts can be used separately. Furthermore, it is easy to calculate and to an increasing extent can be used in clinical practice [7]. Colonoscopy and biopsy are the most commonly used measures in the evaluation of intestinal mucosal inflammation of patients with UC, but these techniques risky and costy to the patient [8]. Multiple standard markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, and platelet count are used to aid diagnosis and evaluation of the disease. However, these markers have low specificity for bowel inflammation [9].

Transforming growth factor-β1 (TGF-β1) is a polypeptide composed of 112 amino acids, encoded by a gene located on the long arm of chromosome 19 and produced by lymphoid and nonlymphoid cells.

It exists in five different isomers. The effects of TGF-β1 on cell growth and differentiation, suppression of immune response, wound healing (inducing fibrosis and scar formation), have been confirmed and it is also involved in angiogenesis, the development of tumors, and inflammatory processes [10]. It plays an important role as a stimulant of fibrosis and myofibroblast activation and in a biological process called epithelial-to-mesenchymal transition (EMT) in colonic diseases [11]. EMT is a well-documented biological process that is important in normal tissues and organ development and in the pathogenesis of diseases (such as chronic inflammation-related fibrosis, colorectal carcinogenesis, cancer invasion, and in mucosal healing). The inhibition of EMT appears to reduce chronic inflammation-induced wall fibrosis in the colon [12]. In inflammatory bowel disease (IBD), TGF-β1 is produced and secreted from colonic epithelia and also from the cells in the lamina propria; it controls cell growth and plays a role in healing and fibrosis [13].

CRP is a protein composed of five monomers and is one of the most prevalent acute-phase reactant proteins. Under normal conditions, CRP is released from the liver cells in small amounts (1 mg/l). However, following inflammation, the production of CRP increased rapidly in hepatocytes under the effect of interleukin-6, the tumor necrosis factor-α, and interleukin-1b [1,7]. Mahmoud *et al.* [14] reported a significant association between CRP with clinical, endoscopic, and histological activity of the IBDs. CRP testing is widely available and relatively inexpensive; yet, an elevated serum CRP concentration is not exclusive to intestinal inflammation, given that enhanced production of the protein occurs in most systemic inflammatory diseases [15]. A high CRP level is mainly determined by the underlying cause, such as the activity of the disease, and its half-life is stable for ∼18 h. Despite these characteristics, there is heterogeneity in the CRP response between Crohn's disease whereas it presents a strong response and UC whereas the response is mild to absent [16].

Fecal markers may be more specific for evaluating the activity of intestinal diseases. Specifically, lactoferrin (LF) and calprotectin have been measured in stools and are directly proportionate to neutrophil migration in the gastrointestinal tract [17].

LF is a widely used fecal biomarker for intestinal inflammation. It is an iron-biding glycoprotein with a molecular weight of 80 kDa present in specific granules, especially in mature neutrophils [18–20]. Although it is an excellent measurable marker of neutrophil inflammation, various exocrine cells can also secrete small amounts of this protein that are often present in lower quantities in many fluids such as normal human milk, tears, synovial fluid, and serum [21]. Its existence in breast milk has raised considerations about the validity of low values of LF quantified in the stools of breastfed children. LF is not changeable in fecal samples at room temperature for up to 5 days, warranting samples to be sent to the laboratory [22]. During intestinal inflammation, neutrophils attack the mucosa and considerably increase LF concentrations, which can be measured easily in feces or lavage fluid of the gut [23,24]. Studies assessing LF in the diagnosis of IBD have indicated that it showed the same performance as fecal calprotectin and was better correlated than CRP with endoscopic mucosal inflammation [15,25,26].

Calprotectin is a calcium-binding and zinc-binding protein (heterodimer of S100A8/A9) that inhibits metalloproteinase, has antifungal activity, and promotes programmed cell death in cell culture [27–29]. It is the main protein present in monocytes and macrophages and constitutes about 60% of neutrophil cytosolic proteins [27,30]. This protein is stable in stool samples at room temperature for up 7 days and withstands heat and gut enzymatic degradation [31–33]. Although it is more changeable in early infancy, various studies have shown that high fecal calprotectin concentrations are directly related to the measurement of the neutrophil infiltrate in the gut mucosa as an indicator of infectious and inflammatory disorders [34,35]. It can be used to distinguish organic from nonorganic intestinal disease, and can be useful in the diagnosis of colorectal cancers and inflammatory conditions; it can also be useful in the estimation of relapse in IBD [27,36]. When the value of these markers is low, the diagnosis of active inflammation in the colon is not possible [37]. The aim of this study was to investigate the utility of TGF-β1, CRP, fecal LF, fecal calprotectin, and the Mayo score for assessment of the severity of UC in monitoring disease activity in Egyptian patients with UC.

Patients and methods

One hundred and thirty patients known to have active UC who presented to the outpatient clinics and were inpatients of the Gastroenterology Unit of Internal Medicine Department of El Hussien University hospital (documented clinically, endoscopically, and histologically) were enrolled in the study. Our patients were classified according to the Montreal classification of the extent of UC into 48 patients with proctitis, 39 patients with left-sided colitis, and 43 patients with

Table 1 Mayo score for the severity of ulcerative colitis

Score	Criteria
Stool frequency	
0	Normal
1	1–2 stools/day more than normal
2	3–4 stools/day more than normal
3	>5 stools/day more than normal
Rectal bleeding	
0	None
1	Streaks of blood with stool less than half the time
2	Obvious blood with stool most of the time
3	Blood alone passed
Mucosal appearance	
0	Normal
1	Mild disease (erythema, decreased vascular pattern, mild friability)
2	Moderate disease (marked erythema, absent vascular pattern, friability, erosions)
3	Severe disease (spontaneous bleeding, ulceration)
Physician global assessment	
0	Normal
1	Mild disease
2	Moderate disease
3	Severe disease

extensive colitis as shown in Table 1. There were 77 (59.2%) women and 53 (40.8%) men. Their mean ages were 40.18 (±11.51) years. Thirty (12 men and 18 women) healthy controls, who were members of staff of the hospital, with a mean age of 41.13 (±13.29) years, were included; they had no confirmed abnormality in the upper or the lower digestive tract. The Ethical Research and Review Committee of the Hospital approved the study protocol and informed consent was obtained from the participants.

TGF-β1 was measured in venous blood obtained after overnight fasting. Results were expressed as the mean of the results of the sample. Five milliliter of blood was obtained (without using a tourniquet) on EDTA. The blood samples were immediately placed on ice. The plasma was spun for 30 min in 1000*g* and later for 10 min at 10 000*g* to remove platelets (which contain a large amount of TGF) to obtain the platelet-poor plasma. The activation of TGF-β1 was performed by acidification of platelet-poor plasma with 2.5 N acetic acid/10 urea and then, pH was adjusted up to 7.2–7.6 using 2.7 N NaOH/1 mol/l HEPES. The analysis was carried out using enzyme-linked immunosorbent assay (ELISA) following the human TGF-β1 Immunoassay Protocol (Quantikine; R&D Systems, California, USA) method. According to the manufacturer, the TGF-β1 cut-off for positivity was 7.0 pg/ml.

Determination of fecal lactoferrin concentration

The stool samples were collected and placed in plastic containers, frozen, and stored at −72°C until analysis. Fecal concentration of LF was determined using an IBD-SCAN quantitative immunoenzymatic test (catalog no. 30351l; Techlab, California, USA). The test uses antibodies to human LF. The samples were diluted at 1 : 100, 1 : 400, 1 : 1000, and 1 : 4000 and further handled according to the manufacturer's instructions. Absorbance of the samples was determined using an ElizaMat 3000 reader (DRG MedTek, Warsaw, Poland). Fecal calprotectin was quantified using an 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 LF cut-off point to indicate positivity was 13 μg/g feces.

Determination of fecal calprotectin concentration

A single stool sample was collected from each patient in screw-capped plastic containers that avoids toilet, water artifact and simplifies laboratory sampling at initiation of study. The stool samples were frozen (−20°C) until determination of calprotectin. Fecal calprotectin was quantified using an ELISA test (Calprest; Eurospital). 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 was considered positive at 50 μg/g feces.

Blood samples for the measurement of a full blood count, CRP, and TGF-β1 were provided by the patients within 3 days before endoscopy.

Blood leukocytes (normal range: 4–11 g/l), hemoglobin (normal range for women 12–16 g/dl, for men 13–18 g/dl), a sedimentation rate (normal range for men and women ≤50 years ≤20 mm/h and 15 mm/h; normal range for persons older than 50 years ≤30 mm/h and ≤20 mm/h), as well as CRP (upper limit of normal<6 mg/l) were determined as routine laboratory values.

Inclusion criteria were disease duration more than 3 months, complete colonoscopy including at least six colonic biopsies from UC-affected colon and rectum, informed consent, age range from 18 to 65 years, fecal samples delivered from 3 to 1 day before colonoscopy and bowel preparation was not started until the fecal samples were collected. Exclusion criteria were incomplete colonoscopy, history of use of NSAIDs and/or antibiotics during the three months preceding enrollment, colorectal cancer, Crohn's disease, urinary incontinence (fear of contamination of fecal samples), inability to collect fecal samples, pregnancy, history of colorectal surgery, and alcohol abuse.

All patients were subjected to total colonoscopy to determine disease severity. Colonoscopic indications were included: (a) clinically active disease, (b) assessment of endoscopic activity after medical treatment; and (c) search for dysplasia in longstanding disease. The purpose of colonoscopic examination was to confirm the diagnosis, assess the extent of the disease, and obtain biopsy specimens using Pentax videoscope Ec-3840 L (Hoya Company, Tokyo, Japan). Biopsies were immediately fixed in 10% neutral-buffered formalin. Formalin-fixed paraffin-embedded samples were prepared for histopathology and stained by hematoxylin and eosin for histological grading. The degree of inflammation was graded on a four-point scale: normal (no significant inflammation), mild (elevated number of mucosal leukocytes but intact epithelium), moderate (aggregates of leukocytes with crypt abscesses and erosions but no ulceration of the epithelium), and severe (significant ulceration of the epithelium by mononuclear cell infiltrate). Histological grading was performed by a pathologist without knowledge of endoscopic or laboratory features.

Disease severity was determined using the Mayo Score for the Severity of Ulcerative Colitis, which includes a combination of four parameters (Table 1). The stool frequency ranges from 0 to 3 points, where '0' is normal, '1'=1–2 stools/day more than normal, '2'=3–4 stools/day more than normal, and '3'=5 or more stools/day more than normal. The rectal bleeding ranges from 0 to 3 points: '0'=none, '1'=streaks of blood with stool less than half the time, '2'=obvious blood with stool most of the time, and '3'=blood alone passed. The mucosal appearance ranges from 0 to 3 points: '0'=normal, '1'=mild disease (erythema, decreased vascular pattern, mild friability), '2'=moderate disease (marked erythema, absent vascular pattern, friability, erosions), and '3'=severe disease (spontaneous bleeding, ulceration).

Statistical analysis

Statistical analysis was carried out using the statistical package SPSS version 25 (Chicago, IL, USA). The data were expressed as mean±SD. They were compared using a Student *t*-test for comparison between two groups and the analysis of variance test when more than two groups were compared. The association between the Mayo Score for the Severity of Ulcerative Colitis, TGF-β1, CRP, fecal LF, and fecal calprotectin was assessed using an *f*-test. Also, Pearson's '*r*' correlation and the χ^2-test were used.

Results

One hundred and thirty patients with UC were included in the study. The mean age was 40.18 ±11.51 years and 59.2% were women. The mean duration of disease up to the current colonoscopy was 5.07±3.9 years (range: 1–16 years); none of the patients had a history of surgery. Disease location in UC patients was as follows: 48 (36.9%) patients had proctitis, 39 (30%) patients had left-sided colitis, and 43 (33.1%) patients had extensive colitis. All patients presented with variable grades of diarrhea (the mean number of motions per day was 7.07.9±3.97); in terms of rectal bleeding in our patients, no rectal bleeding was observed in 29 (22.3%) patients, streaks of blood with stool less than half the time were observed in 31 (23.8%) patients, obvious blood with stool was observed most of the time in 31 (23.8%) patients, and blood alone passed in 39 (30%) patients (Table 2).

The mean levels of TGF-β1, CRP, fecal LF, and calprotectin and were 492.24±311.40 (range: 6–900 pg/ml), 18.99±13.36 (2–48 mg/l), 498.15 ±447.8 (0.5–1500 μg/g), and 813.27±533.87 (range: 9–1500 μg/g), respectively (Table 3).

In our patients, the severity of disease was determined using the Mayo Score for the Severity of Ulcerative Colitis. This score was subdivided into four subgroups: 29 (22.3%) patients were in remission, 31 (23.8%) patients had mild UC, 31 (23.8%) patients had moderate UC, and 39 (30%) patients had severe UC. The controls were healthy individuals from among the clinical and laboratory staff who were willing to

Table 2 Clinical characteristics of ulcerative colitis patients and controls

Parameters	Groups		
	Controls	Ulcerative colitis patients	*P* value
Age [mean±SD (range)] (years)	41.13±13.29 (22–62)	40.18±11.51 (20–60)	0.536
Sex [*n* (%)]			0.362
Male	12 (40)	53 (40.8)	
Female	18 (60)	77 (59.2)	
Number of motion per day [mean±SD (range)]	2.10±0.80 (1–3)	7.07±3.97 (1–15)	0.001*
Temperature (mean±SD)	36.91±0.22	37.6±0.55	0.001*
Rectal bleeding [*n* (%)]			–
0=None	–	29 (22.3)	
1=Streaks of blood with stool less than half the time	–	31 (23.8)	
2=Obvious blood with stool most of the time	–	31 (23.8)	
3=Blood alone passed	–	39 (30)	
Mucosal appearance [*n* (%)]			–
0=Normal	–	29 (22.3)	
1=Mild	–	31 (23.8)	
2=Moderate	–	31 (23.8)	
3=Severe	–	39 (30)	
Patient global assessment [*n* (%)]			–
0=Normal	–	29 (22.3)	
1=Mild	–	31 (23.8)	
2=Moderate	–	31 (23.8)	
3=Severe	–	39 (30)	
Duration [mean±SD (range) (years)	–	5.07±3.90	–
Disease location [*n* (%)]	–	–	–
Proctitis		48 (36.9)	
Left-sided colitis		39 (30)	
Extensive colitis		43 (33.1)	

$P \leq 0.05$, significant. $P > 0.05$, NS. *significant at $P > 0.05$

Table 3 Laboratory characteristics of ulcerative colitis patients (*n*=130) and controls (*n*=30)

Parameters	Groups					
	Controls		Ulcerative colitis patients			
	Range	Mean±SD	Range	Mean±SD	*t*-Test	*P* value
ESR (mm/h)	5–30	11.53±7.49	4–105	43.43±29.32	11.37	0.001*
CRP (mg/l)	2–5	3.17±0.95	2–30	15.97±9.13	10.24	0.001*
Blood leukocytes (g/l)	4.5–11	6.82±1.95	4.5–20	11.07±3.31	3.28	0.003*
Hemoglobin (g/dl)	13–17	15.31±1.19	9.4–14.5	11.64±1.15	–7.18	0.001*
Platelet count ($\times 10^9$/l)	180–410	281.27±77.50	195–655	412.59±142.49	0.528	0.565
TGF-β1 (pg/ml(	1–7	5.93±1.81	6–900	489.32±315.68	5.53	0.001*
Fecal lactoferrin (μg/g)	0.26–13	7.01±4.00	0.5–1500	497.06±448.95	5.38	0.001*
Fecal calprotectin (μg/g)	10–55	36.33±15.51	10–1500	809.70±554.36	4.88	0.001*

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TGF, transforming growth factor. $P \leq 0.05$, significant. $P > 0.05$, NS. *significant at $P > 0.05$

provide blood and fecal samples; 60% were women, mean age 41.1±13.3 years (range: 22–62 years).

On the basis of our results, the ability to differentiate between the various subgroups of the Mayo score for the severity of UC showed that the mean plasma TGF-β1 in patients with remission was 46.4 (±37.1), the mean plasma TGF-β1 in patients with mild activity was 343.4 (±110.7), the mean plasma TGF-β1 in patients with moderate activity was 640.6 (±141.0), and the mean plasma TGF-β1 in patients with severe activity was 814.5 (±132.9). Thus, the mean plasma TGF-β1 can be used to differentiate inactive indices from mild activity indices, mild activity from moderate activity indices, and moderate activity indices from high activity indices (for all, $P<0.001$).

The CRP and blood leukocytes in this study cannot differentiate inactive activity indices from mild endoscopic activity indices ($P=0.060$ and 0.088, respectively), but can differentiate mild activity indices from moderate activity indices and moderate activity indices from the severe indices (for all, $P<0.001$).

Table 4 Relationship between the Mayo score subgroups with transforming growth factor-β1, fecal lactoferrin, fecal calprotectin, C-reactive protein, and blood leukocytes

The Mayo score for the severity of ulcerative colitis	*N*	Plasma TGF-β1		Fecal lactoferrin		Fecal calprotectin		CRP		Blood leukocytes	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Remission (0–2)	22	6–100	46.4±37.1	0.5–150	28.6±28.3	10–98	71.7±24.2	2–6	4.8±1.3	4.5.–12	7.1±2.1
			P<0.001		*P*<0.001		*P*<0.001		NS		NS
Mild (3–5)	25	10–450	343.4±110.7	15–250	177.8±66.8	130–800	459.0±206.7	6–12	9.8±2.1	5–11	9.3±1.6
			P<0.001		*P*<0.001		*P*<0.005		*P*<0.001		*P*<0.001
Moderate (6–10)	39	30–780	640.6±141.0	20–770	561.0±181.9	50–1250	1080.8±224.1	14–24	18.6±3.5	11–15	12.1±0.8
			P<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001
Severe (>10)	34	50–900	814.5±132.9	50–1500	1048.3±296.8	1000–1500	1421.7±95.5	12–30	27.1±3.0	12.5–20	14.6±1.9

CRP, C-reactive protein; TGF, transforming growth factor. $P \leq 0.05$, significant. $P > 0.05$, NS.

Table 5 Correlation of the Mayo score for the severity of ulcerative colitis with transforming growth factor-β1, fecal lactoferrin, fecal calprotectin, and C-reactive protein

Groups	Parameters	
	r	*P* value
TGF-β1 (pg/ml)	0.925	0.001
Fecal lactoferrin (μg/g)	0.932	0.001
Fecal calprotectin (μg/g)	0.953	0.001
CRP (mg/l)	0.957	0.001

CRP, C-reactive protein; TGF, transforming growth factor. $P \leq 0.05$, significant. $P > 0.05$, NS.

The mean fecal LF in our study can be used to differentiate between the various groups of the Mayo score for severity of UC; in patients who had achieved remission, it was 28.6 (±28.3). The mean fecal LF in patients with mild activity was 177.8 (±66.8), whereas the mean fecal LF value among patients with moderate activity was 561.0 (±181.9) and the mean fecal LF value in patients with severe disease was 1048.3 (±296.8). These results showed a good significant relationship between fecal LF values and the different subgroups of Mayo score for the severity of UC ($P<0.001$).

Fecal calprotectin in this study can be used to differentiate between the various subgroups of the Mayo score for severity of UC; the mean fecal calprotectin in patients with inactive disease was 71.7 (±24.2), the mean fecal calprotectin in patients with mild activity was 459.0 (±206.7), whereas the mean fecal calprotectin value among patients with moderate activity was 1080.8 (±224.1) and the mean fecal calprotectin value in patients with severe activity was 1421.7 (±95.5). These results showed a significant relationship between fecal Calprotectin values and the different subgroups of the Mayo score for severity of UC ($P<0.001$; Table 4).

The results of the Mayo score for severity of UC in our study correlated significantly with the levels of plasma TGF-β1 (Spearman's rank correlation coefficient $r=0.925$), serum CRP ($r=0.957$), fecal LF ($r=0.932$), and fecal calprotectin ($r=0.953$; $P<0.001$; Table 5).

From the histopathologic viewpoint, 19 (14.6%) patients were negative and 111 (85.4%) were positive. There were significant correlations between the results of histopathological examination with plasma TGF-β1 ($r=0.559$), CRP ($r=0.522$), fecal LF ($r=0.552$), fecal calprotectin ($r=0.573$), and Mayo score for severity of UC ($r=0.576$; $P<0.001$; Table 6).

Table 6 Correlation of histopathology with transforming growth factor-β1, C-reactive protein, fecal lactoferrin, fecal calprotectin, and the Mayo score for severity of ulcerative colitis

Histopathology	Parameters	
	Spearman's rank correlation coefficient (*r*)	Significance
TGF-β1 (pg/ml)	0.559	0.001
Fecal lactoferrin (μg/g)	0.552	0.001
Fecal calprotectin (μg/g)	0.573	0.001
CRP (mg/l)	0.522	0.001
Mayo score	0.576	0.001

CRP, C-reactive protein; TGF, transforming growth factor.

Table 7 Receiver operator characteristic curve of fecal calprotectin, plasma transforming growth factor-β1, the Mayo score, fecal lactoferrin, and C-reactive protein by positive histopathology

Test result variable(s)	Area	Standard error	Asymptomatic significance	Asymptomatic 95% confidence interval	
				Lower bound	Upper bound
Fecal calprotectin (μg/g)	0.968	0.014	0.000	0.941	0.995
TGF-β1 (pg/ml)	0.957	0.016	0.000	0.925	0.989
Mayo score	0.955	0.017	0.000	0.921	0.989
Fecal lactoferrin (μg/g)	0.951	0.018	0.000	0.915	0.986
CRP (mg/l)	0.925	0.023	0.000	0.889	0.970

CRP, C-reactive protein; TGF, transforming growth factor.

The compatibility between the results of histopathological examination and the classification constructed on the basis of the variable cut-offs was analyzed for each variable and was described as the percentage of samples that were correspondingly recognized (specificity, sensitivity, positive predictive value, negative predictive value, and overall accuracy). The receiver operator characteristic analysis showed that areas under the receiver operator characteristic curve of plasma TGF-β1, CRP, fecal LF, fecal calprotectin, and the Mayo score for severity of UC were 0.957, 0.925, 0.951, 0.968, and 0.955, respectively (Table 7).

The specificity was the highest for both fecal LF and fecal calprotectin and the lowest for the Mayo score. The specificity rates for plasma TGF-β1, CRP, fecal LF, fecal calprotectin, and the Mayo score for severity of UC were 71.4, 74.1, 100, 100, and 58.6%, respectively. The sensitivity rates for plasma TGF-β1, CRP, fecal LF, fecal calprotectin, and the Mayo score for severity of UC were 88.6, 90.3, 89.5, 91.2, and 98.02%, respectively. Thus, the sensitivity was the highest for both the Mayo score and fecal calprotectin, but was relatively low for plasma TGF-β1. The positive predictive value and negative predictive value rates were 98.2, 26.3%; 91.8, 42.1%; 100, 31.6%; 100, 31.3%; and 89.2, 89.5%, respectively. The overall accuracy rates for plasma TGF-β1, CRP, fecal LF, fecal calprotectin, and the Mayo score for severity of UC were 87.7, 84.6, 90, 91.5, and 89.2%, respectively. Thus, the overall accuracy was the highest for both fecal calprotectin and fecal LF, followed by the Mayo score for severity of UC, then plasma TGF-β1, and finally the CRP (Table 8 and Fig. 1).

Discussion

The clinical course of UC is quite variable and is manifested by episodes of relapse and remission [1,2]. The most common histologic feature observed in UC is infiltration of the inflamed mucosa by abundant neutrophils at early stages of inflammation [3]. The major products of inflammatory cytokines, chemokines, proteases, and reactive oxygen derivatives, as well as a full complement of factors are the neutrophils; these released factors aggravate tissue injury and mucosal inflammation. Active mucosal inflammation in UC is accompanied by an acute-phase reaction and translocation of leukocytes to the gut. Thus, various proteins can be determined in both serum and feces [14]. Several laboratory markers have been developed for the diagnosis and prediction of UC patients. These include leukocyte counts, ESR, and CRP [1].

TGF-β1 plays a major role in the pathophysiology of IBD, and may be used as a sensitive marker of UC activity and can also be used as a marker to differentiate inactive from active UC [38,39].

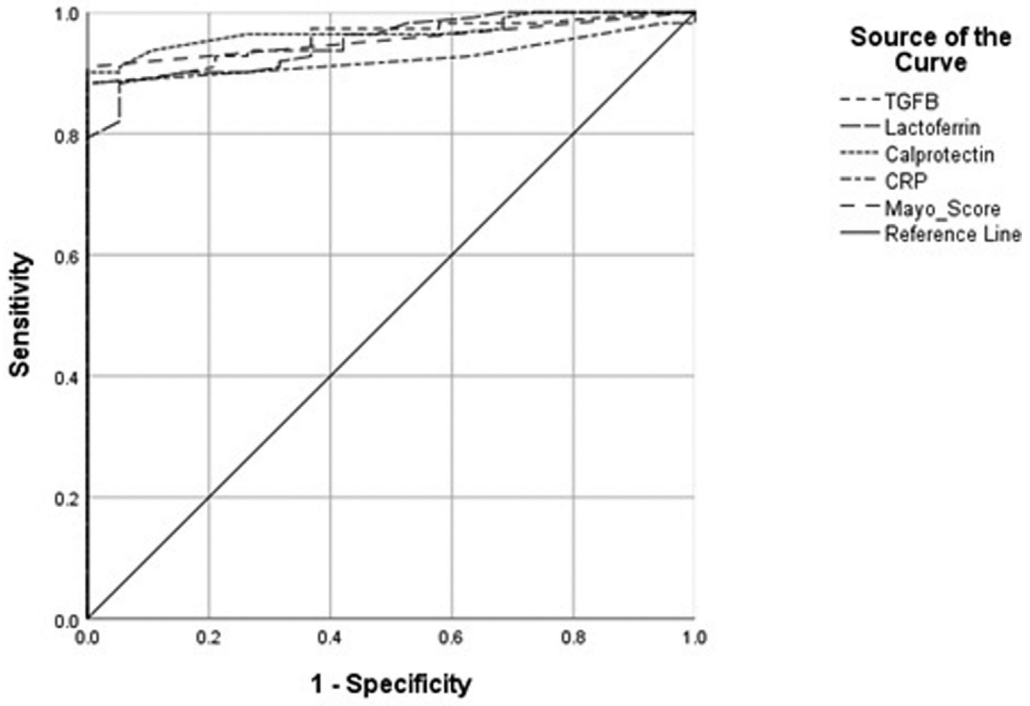
On the basis of our results, TGF-β1 might be considered a sensitive marker of UC activity and there was a

Table 8 Sensitivity and specificity

Marker	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Fecal calprotectin (μg/g)	≥50	91.2	100	100	31.3	91.5
Fecal lactoferrin (μg/g)	≥12	89.5	100	100	31.6	90
TGF-β1 (pg/ml)	≥9	88.6	71.4	98.2	26.3	87.7
Mayo score	≥2	98.02	58.6	89.2	89.5	89.2
CRP (mg/l)	≥6	90.3	47.1	91.8	42.1	84.6

Positive predictive value (PPV), negative predictive value (NPV), and the overall accuracy of fecal lactoferrin, fecal calprotectin, TGF-β1, clinical activity index, endoscopic activity index, CRP, and blood leukocytes in predicting histopathologically active disease. CRP, C-reactive protein; TGF, transforming growth factor.

Figure 1



ROC showing the relationship between fecal calprotectin, plasma TGF-β1, and the Mayo score for severity of ulcerative colitis, fecal lactoferrin, and CRP with positive histopathology. CRP, C-reactive protein; ROC, receiver operator characteristic; TGF, transforming growth factor.

significant increase in TGF-β1 concentrations in UC patients than the controls. It can also be used for the evaluation of inflammatory activity in UC and can differentiate mild from moderate, and moderate from high disease activity, but cannot differentiate mild active disease from inactive disease. Indeed, Kilic *et al.* [40] and Irena *et al.* [41] noted that in UC, the mean level of TGF-β1 was higher in patients with active disease than in patients with remission and can be used as a marker for differentiating these stages. Stadnicki *et al.* [42] investigated the expression of genes encoding for TGF-β1 and TβRI–III using RT-qPCR in patients with active and inactive UC and non-IBD controls. The localization and level of TGF-β1 protein in intestinal tissue was estimated by immunohistochemistry, and serum TGF-β1 concentrations were determined using ELISA. They found a significant increase in TGF-β1 gene expression and an increase in the expression of genes encoding receptor TβRI in patients with active UC compared with controls. The expression of genes encoding TβRII was found to be higher in patients with both active and inactive UC compared with the controls. Specific staining for TGF-β1 in fibroblasts was significantly greater in both active and inactive UC compared with controls. The serum concentration of TGF-β1 was significantly higher in patients with active UC compared with controls as well as in UC patients with left side/total colonic extension compared with those with disease limited to the rectum/rectosigmoid area. They concluded that increased TGF-β1 gene expression and its protein level, associated with an altered TGF-β1 receptor profile, indicate a fundamental role for TGF-β1 in intestinal inflammatory/repair processes in UC. Increased TGF-β1 serum concentrations correlated with progression of

disease [42]. Kanazawa *et al.* [43] studied the expression of TGF-β1 in paraffin-embedded samples from bowel tissue and the concentration in blood, basic fibroblast growth factor, endothelin-1, and vascular endothelial growth factor. They examined 11 patients with UC, 11 patients with Crohn's disease, and 10 healthy controls. The expression of TGF-β1 in the endothelial cells was not found in either the UC or the Crohn's disease group. They noted moderate or weak expression of TGF-β2 and TGF-β3 in the inflammatory cells in five cases of active UC and in four cases of active Crohn's disease. Some studies have been carried out in pediatric patients and it was found that TGF-β1 was significantly higher in patients with IBD in remission than those with active disease [44–46]. In another study, Wedrychowicz *et al.* [47] analyzed the influence of exclusive enteral nutrition on the serum concentration of TGF-β1 and vascular endothelial growth factor in 24 patients with Crohn's disease and 15 patients with UC; they found increased serum TGF-β1 in UC patients versus the Crohn's disease group and controls. In contrast to the previous studies, Liberek *et al.* [48] concluded that the four common polymorphisms of the TGF-β1 gene do not influence the susceptibility to or clinical parameters of IBD in the tested population of children.

CRP testing is widely available and relatively inexpensive; yet, an elevated serum CRP concentration is not exclusive to intestinal inflammation, given that enhanced production of the protein occurs in most systemic inflammatory diseases and its heterogeneity among individual patients on a genetic basis [14,15]. Also, it is estimated that ~15% of normal healthy individuals do not augment a CRP response [16]. These limitations have spurred the development of alternative tests, specifically stool biomarkers that have greater specificity for intestinal inflammation.

In this study, there was a significant increase in the CRP value in UC patients than the controls. It can also be used for the assessment of inflammatory activity in UC and can differentiate mild from moderate and moderate from high disease activity, but cannot distinguish inactive from mild active disease. On the basis of our results, the Mayo score for severity of UC and histopathologic findings were correlated significantly with CRP; thus, CRP may play an important role in the inflammatory process in UC. Our results are in agreement with those of Masoodi *et al.* [39] and Vilela *et al.* [49]. Other studies have suggested that UC has a weak CRP response [4,50]. Our explanation is that, in UC, the inflammation is confined to the mucosa, and also polymorphisms in the CRP gene are responsible for interindividual differences in CRP production in humans [14]. Thus, CRP does not seem to be an adequate biomarker for the assessment of disease activity in UC.

Stool biomarkers have become important tools to evaluate intestinal inflammation, whether because of infections, such as *Clostridium difficile* colitis or shigellosis, or as a result of IBD, UC, and Crohn's disease [3,18,19,51]. These fecal tests have the benefits of being noninvasive, rapid, simple, and relatively economic [23,52]. Various clinical studies have shown the utility of fecal biomarkers of inflammation in the diagnosis and prediction of disease activity; these include the stool assessment of sensitive biomarkers including neutrophil-granular proteins such as calprotectin and LF [14].

Polymorphonuclear neutrophils infiltrate the mucosa during intestinal inflammation, increasing the fecal LF concentration in proportion to neutrophil migration to the gastrointestinal tract [21]. Studies researching whether fecal LF can be utilized as a noninvasive marker to differentiate IBD from noninflammatory disorders, such as irritable bowel syndrome, have shown variable results [19,20,22,23].

Calprotectin is a calcium-binding and zinc-binding protein that suppresses metalloproteinase activity. Several studies have shown that high fecal calprotectin values are directly related to the quantification of the neutrophil infiltrate in the gastrointestinal mucosa as an index of inflammatory and infectious conditions [4,8].

Our results showed that fecal LF and fecal calprotectin concentrations were significantly elevated in patients than in controls. Also, fecal LF and fecal calprotectin were significantly differentiate inactive from mild, mild from moderate, and moderate from high active disease. Our findings were in agreement with those of Mahmoud *et al.* [14], Li *et al.* [28], and Lamb and Mansfield [29]; they reported that fecal LF and fecal calprotectin levels were significantly elevated in patients with active UC than in patients in remission and had a good relationship with disease activity index than the CRP and ESR values.

Various studies have shown that patients with active IBD have elevated values of fecal LF and fecal calprotectin than patients in remission [7,9,31,53]. Hassan *et al.* [31] reported that clinical remission in UC does not necessarily suggest biochemical, endoscopic, or histological remission. Noninvasive fecal biomarkers such as fecal LF and calprotectin

are highly sensitive to mucosal inflammation and have the capability to increase our understanding of active inflammation in everyday patient care. Xiang *et al.* [8] concluded that fecal LF seemed to have good diagnostic value in differentiating IBD from irritable bowel syndrome, and was a more sensitive marker in patients with active IBD. Iman *et al.* [9] suggested that in IBD patients, fecal calprotectin was significantly elevated compared with controls, and may be used as a good marker in differentiating Egyptian patients with UC from healthy controls.

The fecal LF level has been shown to be a stable and accurate biomarker for the leukocyte degranulation observed in cases of intestinal inflammation. In particular, the fecal LF level has been proven to be a useful tool for diagnosing IBD [50] and differentiating between active and inactive disease [54,55]. Furthermore, the fecal LF level has been found to correlate well with the endoscopic severity of colonic IBD [23]. Sagawa *et al.* [55] reported that fecal LF level is a useful biomarker of the mucosal findings in UC. Although colonoscopy is the gold standard procedure for the diagnosis of IBD, the fecal LF level can be used as a sensitive biomarker in differentiating patients with active UC from patients in remission and from healthy controls. The neutrophils infiltrate the mucosa during intestinal inflammation and induce a marked increase in LF levels that can be measured easily in fecal matters or gut lavage fluid [23,56]. Studies analyzing LF for the diagnosis of IBD have shown that it shows efficacy similar to that of fecal calprotectin and correlated significantly better than CRP with mucosal inflammation as performed by endoscopy [24–26]. Menees *et al.* [57] reported that UC disease activity indices and ESR correlated with fecal LF in patients with IBD. In another study, LF and calprotectin can yield a simple, noninvasive assessment of intestinal inflammation and both correlated closely with each other, do not necessitate protein adjustment, and can differentiate patients with UC from healthy controls [58]. Alian *et al.* [7] reported that fecal calprotectin values were significantly increased in UC patients than controls and can differentiate inactive from mild, moderate, and high active disease.

Fecal LF and fecal calprotectin predict the severity of colorectal inflammation, with increased concentrations associated strongly with advanced histological grades of colorectal inflammation [7,8,17,19,53]. In our study, there was a significant correlation between fecal LF, fecal calprotectin concentrations, and the results of histopathological examinations. Our results are in agreement with those of Xiang *et al.* [8], Hassan *et al.* [31], and Mosli *et al.* [50], who reported that fecal LF and fecal calprotectin concentrations are related more closely to histologic than to macroscopic colonic inflammation and both biomarkers may represent an early inflammation that is not detectable macroscopically during colonoscopy.

Focusing on the evaluation of the relationship that might exist between the mucosal neutrophil infiltration represented by fecal LF, fecal calprotectin, TGF-β1, CRP, blood leukocytes, and the Mayo score for severity of UC, the present study showed that the Mayo score for the severity of UC correlated significantly with the fecal calprotectin, fecal LF, TGF-β1, and CRP, but not with the platelet count and sedimentation rate. Various studies have shown a good correlation between the values of fecal LF, fecal calprotectin, UC activity indices, CRP, and blood leukocytes in UC patients [7,54]. Also, Xiang *et al.* [8] and Mahmoud *et al.* [14] reported a significant correlation between fecal calprotectin, ESR, CRP, and the UC activity index in UC patients.

Histopathology is considered the gold standard criterion for the diagnosis of UC; the test probability (on the basis of sensitivity/specificity/positive predictive value/ negative predictive value and accuracy in percent) of fecal calprotectin, fecal LF, TGF-β1, the Mayo score for severity of UC, and CRP in predicting the positivity and negativity of UC showed that fecal calprotectin with a cut-off of at least 50 μg/g had the best overall accuracy (91.5%), followed by fecal LF, with a cut-off of at least 12 μg/g (90%), followed by the Mayo score for severity of UC, with a cut-off of at least 2 (89.2%), then the TGF-β1 with a cut-off of at least 9 pg/ml (87.6%), and finally CRP with a cut-off of at least 6 mg/l (84.6%) for the detection of active disease. These results are in agreement with most results reported by other researchers [7,41,43,50,59,60].

Conclusion

Fecal calprotectin and fecal LF are the two important biomarkers that can reliably differentiate inactive from active UC and have the potential to replace endoscopy in disease monitoring and are considered as an objective advances to grading the mucosal disease activity in patients with UC. These fecal biomarkers as screening tests may be useful in the selection of cases for endoscopic examination. The advantages of both fecal calprotectin and fecal LF are their ease of use, noninvasiveness, and relatively low cost. Despite being inferior to calprotectin and LF

measurements, the Mayo score for severity of UC showed a good correlation with these two fecal biomarkers. TGF-β1 can be used in the early diagnosis of UC exacerbation. It can be used for the evaluation of inflammatory activity in UC and correlated with the Mayo score for severity of UC and histopathology. Thus, TGF-β1 can be used as a marker for differentiating mild active from moderate and high active UC patients. Finally, CRP cannot differentiate inactive from mild active disease, but significantly differentiate mild, moderate, and severe disease as evidenced from the positive correlations with both the Mayo score for severity of UC and histopathology. Further studies are needed to determine the value of fecal calprotectin, fecal LF, TGF-β1, and CRP in other organic diseases, their guidance for choosing the best way of treatment, and their use as markers of remission and favorable management. Nevertheless, more studies with larger patient groups are necessary.

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Conflicts of interest
There are no conflicts of interest.

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