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Trefoil Factor 3 in human serum as a predictor of disease activity in Egyptian ulcerative colitis patients and its role in colorectal cancer

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Abstract

Ulcerative colitis (UC), a chronic idiopathic inflammatory disease, is caused by abnormal immune response to intestinal microflora. Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality. The gold standard to establish diagnosis and assess disease activity remains endoscopy and histopathology. Non-invasive biomarkers are required for timely diagnosis of CRC and to assess disease activity as endoscopic assessment is not accepted by most patients. Enhanced trefoil factor 3 (TFF3) expression is seen following gastrointestinal tract injury. In the current study, the significance of serum TFF3 as a potential diagnostic biomarker of disease activity in naïve UC patients, and its diagnostic accuracy in CRC patients were investigated. We collected serum and fecal samples from 20 cases with active UC, 20 CRC patients, and 20 normal controls. TFF3 levels were higher in patients with active UC than in controls (p<0.001). TFF3 cut-off value of 7.9 ng/ml could predict disease activity with sensitivity and specificity of 90% and 100%, respectively. However, the combination of TFF3, C-reactive protein (CRP), and fecal calprotectin (FC) was able to predict disease activity better than each biomarker alone by raising the sensitivity and specificity to 100%. There was no correlation between TFF3, FC, and endoscopic activity in UC assessed by ulcerative colitis endoscopic index of severity (UCEIS). In the CRC patient group, the serum level of TFF3 was significantly higher when compared to controls (p=0.012). TFF3 and the degree of dysplasia were significantly correlated (r=0.496, p=0.026). At a cut-off value of 5.9 ng/ml, serum TFF3 had a diagnostic sensitivity and specificity for CRC of 82% and 90%, respectively. In conclusion, serum TFF3 may be used as a non-invasive biomarker to predict disease activity in UC both alone and in combination with CRP and FC and it could have a potential role in diagnosis of CRC.

Keywords: Inflammatory bowel disease, colorectal cancer, biomarkers, fecal calprotectin, trefoil factor 3.

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Introduction

Ulcerative colitis (UC) is an idiopathic, chronic inflammatory disease of the colonic mucosa. UC mainly affects the superficial layers of the colon which appears endoscopically as mucosal erythema, edema, granularity, and ulcers. Based on clinical and endoscopic evaluations, UC activity can be categorized as mild, moderate, severe, or fulminant. Inducing and maintaining clinical and endoscopic remission is the goal of treatment to avoid long-term consequences.[1,2]

The current etiopathogenesis of UC includes interaction between environmental variables, commensal flora, and colonic immune system which results in altered epithelial barrier function and aberrant immunological response in genetically susceptible individuals.[3]

CRC is one of the leading causes of cancer-related mortality globally.[4] Most patients receive an accurate diagnosis when it is too late due to lack of timely diagnostic tools. Several biomarkers have recently been investigated for the diagnosis of CRC. However, due to the delay in early detection of CRC, there are still limits in clinical practice. [5,6] The most frequently used tumor marker for the diagnosis of CRC is carcinoembryonic antigen (CEA), but recent research has revealed that CEA has poor diagnostic utility due to its low sensitivity and specificity.[7,8]

Endoscopic evaluation is the most accurate way to assess UC activity and screen for CRC. The location, extent and severity can be established with this procedure, but its use is prevented by several drawbacks, as it is invasive, time-consuming, and expensive.[9,10] So, the identification of novel, non-invasive and reliable serum biomarkers are needed to accurately detect inflammation, monitor disease activity in UC patients and to improve the diagnostic accuracy for CRC.

To date, C-reactive protein (CRP) and fecal calprotectin (FC) are the two inflammatory biomarkers that have been explored the most. Despite the observed correlation between endoscopic activity indices and CRP, there are still insufficient data to support its usage in UC.[11] There are many encouraging results for FC,[12,13] but more research is required to determine the appropriate cut-off levels before its widespread application in clinical practice. Therefore, novel biomarkers are still required to identify intestinal inflammation in UC patients.

A substantial body of research supports the implication of trefoil peptides in protecting and healing damaged mucosal surfaces.[14] Three mucin-associated peptides (TFF1, TFF2 and TFF3) are included in the trefoil factors (TFFs) which are widely expressed in the gastrointestinal tract in a tissue-specific manner. [15] The small and large intestine's goblet cells produce the majority of TFF3. Following damage to the proximal and distal gastrointestinal tract, such as peptic ulcer disease or inflammatory bowel disease (IBD), increased production of trefoil proteins is seen.[16]

Additionally, new studies suggested that TFF3 may facilitate tumor cell invasion by acting both directly on cancerous cells and indirectly on the vasculature. It has been suggested that TFF3 contribute to angiogenesis, invasion, apoptosis, and cell proliferation. Serum TFF3 has been found to be higher in cancer patients, suggesting that it may be used as a biomarker for cancer screening. [17-19]

Therefore, we conducted this study to investigate the role of serum TFF3 as a diagnostic biomarker of disease activity in naïve patients with UC, and to determine its diagnostic accuracy in CRC patients. Moreover, the correlation between serum TFF3 values, inflammatory markers, endoscopic indices of activity and degree of dysplasia in CRC group of patients was analyzed.

Subjects and Methods

Study design

This case control study was conducted in the IBD clinic at Ain Shams University Hospital, Cairo, Egypt, from November 2019 to November 2021. We consecutively enrolled 20 adult patients "≥18 years" diagnosed by clinical criteria and colonoscopy with biopsy as UC. They were presented in activity suffering from any of the following: fever, bleeding per rectum, diarrhea, abdominal pain, or extraintestinal

manifestations. In addition, 20 patients diagnosed as CRC were recruited from the surgery clinic. A control group of 20 apparently healthy participants with matched age and sex were also recruited.

Exclusion criteria were pregnancy, indeterminate colitis, infectious colitis, urinary incontinence (due to the risk of fecal samples contamination), history of colorectal surgery, history of active non-steroidal anti-inflammatory drugs (NSAID) intake (2 tablets/ week), steroids enemas or oral steroids intake in the previous three months or start of azathioprine treatment in the previous three months, and primary immunodeficiency.

Ethical considerations

The study protocol was reviewed and approved by the Research Ethics Review Committee of the Faculty of Medicine, Ain Sham University (Reference Number: MD 14/2020). Each study participant provided an informed consent before enrolled in the study.

Data collection

The study population was subjected to a well-designed data sheet covering the following topics: detailed medical history, physical examination, and baseline laboratory investigations. All patients underwent colonoscopy and multiple biopsies were taken, to confirm diagnosis, assess the severity, extent of endoscopic findings, and visualize any dysplastic changes as polyps or masses. Assessment of disease activity was according to the ulcerative colitis endoscopic index of severity (UCEIS). [20] Serum and fecal samples were collected. The serum levels of TFF3, CRP, erythrocyte sedimentation rate (ESR), and FC were assessed in all patients and the control group.

The UCEIS was calculated as a simple sum of the following three descriptors :vascular pattern (scored 0–2); bleeding (scored 0–3); and erosions and ulcers (scored 0–3). As a result, the UCEIS score ranges from 0 to 8. We classified the UCEIS scores into four groups: remission (UCEIS 0–1); mild (UCEIS 2–4); moderate (UCEIS 5–6); and severe (UCEIS 7–8).

ESR and CRP

ESR was performed by the Westergren method. C-Reactive Protein test was done by a latex agglutination test. Human anti-CRP complexed with latex particles shows a visible agglutination reaction in 2 minutes when combined with a patient's serum that contains C reactive proteins.

Enzyme immunoassay

A commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) was used to measure the serum levels of TFF3 according to the manufacturer's instructions.

Fecal calprotectin

Stool samples were examined for calprotectin using the point-of-care (POC) desk-top Quantum Blue Reader® technique (Quantum Blue® Calprotectin, Bühlmann Laboratories AG, Switzerland), according to the manufacturer's instructions. It is a lateral flow technology based on ELISA techniques. The POC device employs internal standards with a sensitivity of 300 µg/g and a range of 30-300 µg/g. Following the manufacturer's instructions, we added a 1:10 dilution with extraction buffer, which enabled us to obtain FC levels as high as 3000 µg/g. FC values exceeding the highest and lowest limits of the measurement ranges were recorded as 3000 µg/g and 30 µg/g, respectively.

Statistical analysis

Data were analyzed using IBM SPSS (Statistical Package of Social Sciences) version 23, USA. Using the Shapiro-Wilk test, the normality of the distribution of numerical data was examined. Numerical data that weren't normally distributed were presented as median and interquartile ranges. The Wilcoxon rank sum test (for two-group comparison) or the Jonckheere-Terpstra trend test (for comparison of multiple tanked grouped) were used to compare intergroup differences. The Conover post hoc test was used for post hoc comparison with application of the Bonferroni correction whenever the Jonckheere-Terpstra test showed statistically significant difference among the groups. Fisher's exact test (for nominal data) or the chi-squared test for trend (for ordinal data)

were used to assess differences between categorical data which were presented as number and percentage or ratio. The diagnostic value of serum TFF3 was evaluated using receiver-operating characteristic (ROC) curve analysis. A p value of <0.05 was considered statistically significant.

Results

The current study enrolled 20 UC patients with a mean age of 40±12.675 years, 10 (50%) patients were males. According to UCEIS, 6 (30%) patients were in mild activity (score 2-4), 13 (65%) patients in moderate activity (score 5-6), and one patient (5%) was presented with severe activity (score 7-8). Three patients had extraintestinal manifestations in the form of pyoderma gangrenosum and axial arthropathy. The rest of patients' characteristics are summarized in Table 1. The study enrolled 20 CRC patients. Of these, 18 (90%) were males and 2 (10%) females. Their ages ranged between 45 and 60 years (mean ±SD; 52.2 ±5.72 years). Of the 20 CRC patients, 17 (85%) patients had cancer colon, 2 (10%) patients with severe dysplasia, and 1 (5%) patient with mild dysplasia. The control group included 20 subjects, 14 (70%) males and 6 (30%) females with a mean age of 30.050±6.573 years.

Table 1. Characteristics of the 20 UC patients enrolled in the study.

	UC patients (Group I)
Age, mean±SD	40±12.675
Gender, n (%)	
Male	10 (50)
Female	10 (50)
Smoking habit, n (%)	
Smoker	7 (35)
Non-smoker	13 (65)
Family history of IBD, n (%)	
Yes	1 (5)
No	19 (95)
Extra-intestinal manifestations, n (%)	
Yes	3 (15)
No	17 (85)
Disease location, n (%)	
Proctitis	11 (55)
Left sided colitis	6 (30)
Pancolitis	3 (15)
Disease severity	
Mild	6 (30)
Moderate	13 (65)
Severe	1 (5)

In UC patients, the mean FC and CRP levels were 355.5±75.619 µg/g and 25.7±16.268 mg/dl, respectively. The mean level of TFF3 was 10.611±2.122 ng/ml which was significantly higher than the mean level in the control group (p<0.001) (Table 2). In addition, compared to controls, patients with CRC had significantly higher serum levels of TFF3 (6.567±0.872 vs 5.735±1.118 ng/ml) (p=0.012).

Table 2. Mean levels of ESR, CRP, FC, and TFF3 in patients with UC and the control group.

		U.C	Control	*p-value
ESR (mm/ hour)	Range	12 - 80	2 - 19	<0.001
	Mean ±SD	35.250 ± 21.393	9.550 ± 4.639	
CRP (mg/dL)	Range	6 - 65	0 - 6	<0.001
	Mean ± SD	25.700 ± 16.268	0.750 ± 1.446	
TFF3 (ng/ml)	Range	6.5 - 13.5	3.95 - 7.9	<0.001
	Mean ± SD	10.611 ± 2.122	5.735 ± 1.118	
Fecal calprotectin (µg/g)	Range	200 - 500	25 - 240	<0.001
	Mean ± SD	355.500 ± 75.619	113.100 ± 66.660	

*$p \leq 0.05$ is significant.

Correlation of the TFF3 level with FC, and UCEIS

TFF3 was positively correlated with FC, however the correlation did not reach statistical significance (r=0.315, *p*=0.176). Furthermore, serum levels of TFF3 did not correlate with UCEIS (r=-0.12, *p*=0.615).

Correlation of the TFF3 level with the degree of dysplasia

As displayed in Figure1, TFF3 showed significant positive correlation with the degree of dysplasia (r=0.496, *p*=0.026).

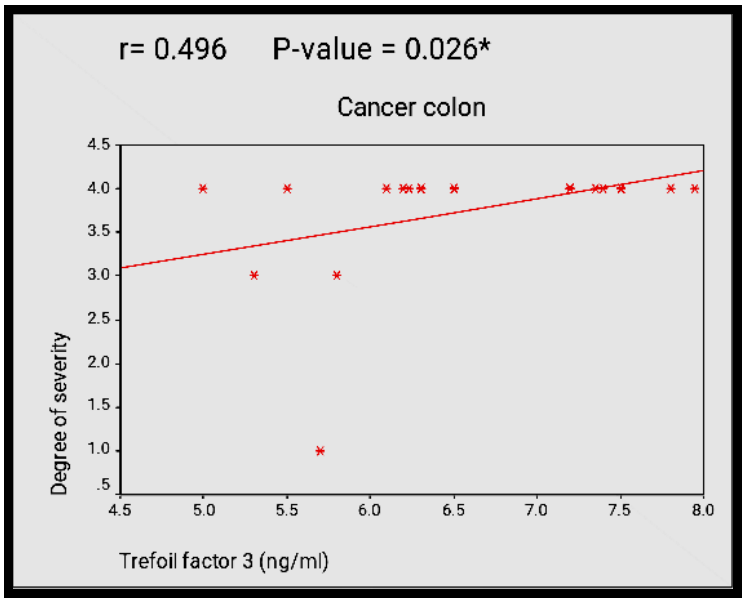

Figure 1. Scatter plot curve showing relation between TFF3 and the degree of dysplasia.

Diagnostic performance of different inflammatory markers in patients with active UC

To identify the best cut-off values for predicting active cases of UC, the ROC curve was plotted. A serum TFF3 level of 7.9 ng/ml had a sensitivity and specificity of 90 and 100%, respectively for identification of patients with active UC (Table 3, Figure 2). When combining the biomarkers together, the AUC for TFF3+CRP was 0.944, the AUC for TFF3+FC was 1, and the AUC for TFF3+CRP+FC was also 1 (Table 3, Figure 2).

Table 3. Diagnostic performance of different inflammatory markers in patients with active UC.

	Cutoff value	Sensitivity	Specificity	PPV	NPV
ESR (mm/ hour)	>16	80.0	95.0	94.1	82.6
CRP (mg/dL)	>2	100.0	95.0	95.2	100.0
TFF3 (ng/ml)	>7.9	90.0	100.0	100.0	90.9
FC (µg/g)	>240	95.0	100.0	100.0	95.2
TFF3+CRP		87.18	90	94.4	78.3
TFF3+FC		100	100	100	100
TFF3+CRP+FC		100	100	100	100

Positive predictive value =PPV; negative predictive value=NPV.

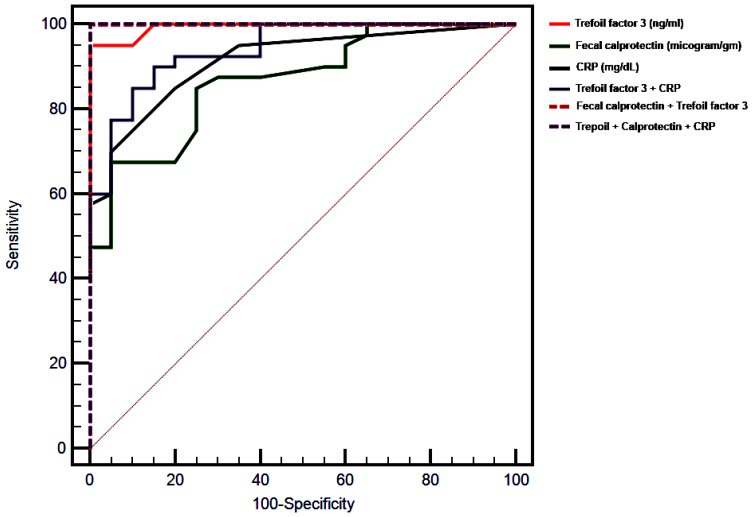

Figure 2. ROC curve analysis of different inflammatory biomarkers for predicting active UC.

Diagnostic accuracy of TFF3 for patients with CRC

The diagnostic efficacy of TFF3 for CRC patients was calculated using the ROC analysis. The sensitivity and specificity to distinguish between CRC patients and controls were 82 and 90%, respectively at an optimal cut-off value of 5.9 ng/ml. (Figure 3).

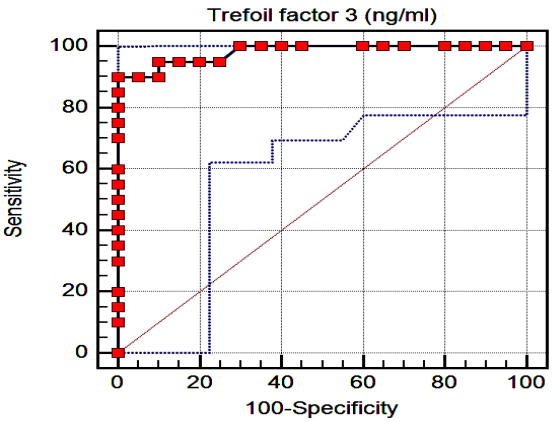

Figure 3. ROC curve analysis of TFF3 for predicting CRC patients.

Discussion

Currently, the most precise method for assessing disease activity in IBD patients and screening for CRC is endoscopy with intestinal biopsies.[10] However, repetitive endoscopic examinations are invasive, expensive, and hardly accepted by the patients. For many years, various non-invasive biomarkers have been studied to monitor disease activity and screen for CRC, but an ideal non-invasive biomarker is still needed. [21-24] As a factor that helps to preserve the integrity of the gut mucosa and promoting tumor cell proliferation and invasion, TFF3 is proposed as a potential candidate to fill this gap. Consequently, this study intended to investigate the role of serum TFF3 as a diagnostic biomarker of disease activity in naïve patients with UC, and to determine its diagnostic accuracy in CRC patients.

In the current study, we found that the mean levels of TFF3 were significantly greater in UC active cases compared to the control group (p<0.001). A study by Nakov et al., 2019 [25] also reported that the mean levels of TFF3 in patients with active UC were noticeably greater than those with quiescent UC, which were comparable to those in the control group. Moreover, Srivastava et al., 2015[26] showed that serum TFF3 was significantly higher in UC patients compared to study controls. Additionally, there was a significant difference between patients with and without mucosal healing in terms of serum TFF3 levels. These findings support the notion that intestine specific TFF3 levels are correlated with mucosal inflammation and increase in the presence of mucosal injury.

Although many studies have documented the role of TFF3 in gut epithelial restoration,[27,28] few studies have investigated the clinical potential of TFF3 in IBD. According to Vestergaard et al., 2002,[29] three UC patients who had prednisolone treatment and showed improvements in their clinical conditions did not have a significant difference in their mean level of TFF3. Another study by Grønbaek et al., 2006,[30] found a correlation between serum TFF3 levels and disease activity indices in patients with UC. They also observed a tendency for TFF3 levels to decline in response to clinical improvement following steroid therapy.

In our study, there was no significant correlation observed between FC, UCEIS and TFF3. In contrast, two previous reports[25,31] demonstrated a significant correlation of TFF3 levels with FC and endoscopic indices. This might be due to the small sample size in our study.

Many non-invasive biomarkers were investigated over the years, but still an ideal marker to detect disease activity is needed. FC is the most studied and the most sensitive one. In the present study, and in consistence with the published literature,[32] FC at a cutoff value > 240 µg/g had a sensitivity better than TFF3 (95% vs 90%, respectively), and similar specificity (100%) in predicting disease activity. However, the combination of TFF3, CRP, and FC was able to predict disease activity better than each biomarker alone by raising the sensitivity and specificity to 100% as previously reported[31] (Table 3).

In line to our observation in the CRC group, a study by Qiang et al., 2017[33] reported that patients with CRC had serum TFF3 levels that were significantly greater than those of patients with polyps and healthy controls. Moreover, they found that early CRC patients (TNM stage I) had significantly higher serum TFF3 levels than healthy controls (p < 0.001).

The ROC curve analysis further suggested that TFF3 can be a useful biomarker for diagnosis of CRC at an optimal cut-off value of 5.9 ng/ml. Also, the study by Qiang et al., 2017[33] reported 74.2% sensitivity, and 94.8% specificity when using serum TFF3 for diagnosis of CRC.

The findings of our study should be considered with cautious as it has some limitations. These include small sample size, a single-center study design, TFF3 was assessed at a single point of time, and that the CRC patients were isolated not on top of UC.

In conclusion, our study findings indicated that serum TFF3 was able to predict disease activity in UC patients with reasonable sensitivity and specificity both alone and when combined with CRP and FC. Assessment of serum TFF3 may have a role in diagnosis of CRC.

Author Contributions

All authors have participated in study design, collecting data, analysis and interpretation of data, practical part, and writing manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Review Committee of the Faculty of Medicine, Ain Sham University (Reference Number: MD 14/2020).

Informed consent

Each study participant provided an informed consent before enrolled in the study.

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