Original Article

Serum Human Trefoil Factor 3 is a Biomarker for Mucosal Healing in Ulcerative Colitis Patients with Minimal Disease Activity

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Abstract

Background: The goals of treating ulcerative colitis (UC) have shifted from clinical remission to mucosal healing. Non-invasive biomarkers are required to assess mucosal healing as endoscopic assessment is inconvenient for patients. Enhanced expression of trefoil factor 3 (TFF3, a mucin-associated peptide) is observed after injury of the gastrointestinal tract. The present study was designed to evaluate TFF3 as a biomarker of mucosal healing in patients with UC.

Methods: This cross-sectional study included consecutive patients with UC (18–65 years old, disease duration >3 months, either left-sided colitis or pancolitis) who had a Simple Clinical Colitis Activity Index (SCCAI) <6. Colonoscopy was done to assess the presence or absence of mucosal healing (defined using the Baron score) in all patients. Serum level of TFF3 was assessed in all patients and 20 healthy controls.

Results: Seventy-four patients were included [mean age 37.2 ± 10.9 years, 47 males, median disease duration 4.8 years (IQR 3–8.3), median SCCAI = 0] in the study. Forty-three patients had mucosal healing (Baron score 0 or 1) and 31 did not (Baron score 2 or 3). Median TFF3 level in patients without mucosal healing was significantly higher than that in patients with mucosal healing [1.5 (IQR 1.2–1.9) vs 1.1 (IQR 0.8–1.3) ng/ml, p = 0.01] and healthy controls [0.85 (IQR 0.7–1.2) ng/ml, p < 0.001]. A serum TFF3 level of <1.27 ng/ml (as determined by the receiver operating characteristic curve; area under the curve 0.73) had sensitivity, specificity, positive predictive value and negative predictive value of 70, 68, 75 and 62%, respectively, for identifying patients with mucosal healing.

Conclusion: Serum TFF3 can potentially be used as a biomarker to assess mucosal healing in UC patients.

Keywords: Trefoil factor 3; ulcerative colitis; mucosal healing

1. Introduction

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory disease of the colonic mucosa characterized by a relapsing and remitting course. The main objectives of treatment of ulcerative colitis have shifted from the previous goals of just achieving and maintaining clinical remission to the current goal of attaining mucosal healing.1,2 The important relationship between mucosal healing and long-term prognosis has come to be realized over time and has been documented in clinical studies.3,4 Mucosal healing as a therapeutic endpoint has become a common endpoint in clinical trials in addition to the more traditional subjective clinical activity indices.

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The current gold standard for mucosal healing is endoscopic assessment. Endoscopic assessment of mucosal healing is inconvenient for the patient and not readily accepted as a method of repeat assessment owing to the invasiveness of the technique. Hence, a simple, rapid, sensitive, specific, inexpensive, non-invasive marker to detect and monitor intestinal inflammation in inflammatory bowel disease (IBD) is needed.

The current understanding of the aetiology of UC includes altered epithelial barrier function and a dysregulated immune response in genetically susceptible individuals as a consequence of a multifaceted interaction among environmental factors, the commensal flora and the colonic immune system or an impaired mucosal barrier. A large body of evidence now supports the involvement of trefoil peptides in mucosal surface protection and repair after injury. Trefoil factors (TFFs) constitute a family of three mucin-associated peptides (TFF1, TFF2 and TFF3) that are widely expressed in a tissue-specific manner in the gastrointestinal tract. TFF3 is predominantly secreted by goblet cells of the small and large intestine and protects the gastrointestinal mucosa from a variety of insults. Enhanced expression of trefoil proteins is observed after injury in both the proximal and distal gastrointestinal tract, the site of damage in conditions such as peptic ulcer or inflammatory bowel disease. Although in vitro and animal studies have documented the crucial role of TFFs in the epithelial restitution of the gut, only one clinical study has investigated the clinical potential of TFFs in IBD. Grønbaek et al. showed that, in 48 UC patients, serum TFF3 levels were increased in active disease and correlated with disease activity indices. However, no study has evaluated the role of trefoil peptides as a biomarker for mucosal healing. In patients with significant disease activity, mucosal healing is not expected, but confusion arises in patients who are in clinical remission or have minimal disease activity. Hence this study aimed to evaluate serum TFF3 as a marker of mucosal healing in UC patients in clinical remission or with minimal disease activity.

2. Materials and Methods

2.1. Design

This cross-sectional study was conducted in the IBD clinic at the All India Institute of Medical Sciences, New Delhi, between January 2012 and January 2013.

2.1. Participants

Patients aged between 18 and 65 years who had had UC for >3 months, with either pancolitis or left-sided colitis and with disease that was either clinically mild or in clinical remission [as defined by a Simple Clinical Colitis Activity Index (SCCAI) score of <6], were included in this study. Patients were excluded if they had a history of colorectal cancer or extensive bowel resection (ileosigmoidostomy and ileorectostomy), were taking aspirin and/or NSAIDS regularly, had been on oral steroids or steroid enemas in the last 3 months, or had initiated azathioprine treatment within the last 3 months. All the included patients were assessed by colonoscopy to note the presence or absence of mucosal healing. Colonoscopy was done by a single person (SS) and the procedure was recorded. The recording was then assessed in a blinded fashion by a senior expert (VA). The opinion of the senior expert was taken as final.

Mucosal changes were defined according to Baron's scoring system. Patients having grade 0 or 1 changes in the whole of the colon were categorized as showing mucosal healing. Patients with active lesions (grade 2 or 3) for >15 cm were categorized as having active mucosal changes. Patients having active mucosal changes covering <15 cm were not included in the study. Serum levels of TFF3 were assessed in all these patients and in 20 healthy subjects, taken as a control group (Figure 1).

2.3. Candidate tests

Five millilitres of blood was taken from each patient and control subject on the day of colonoscopy. The samples were stored at −80°C. Serum TFF3 (Biovendor Research) levels were measured by ELISA, and each sample was analysed in duplicate. The laboratory personnel performing the tests were blinded to clinical activity scores and endoscopic findings.

2.4. Sample size

To determine the accuracy of TFF3 as surrogate non-invasive biomarker for mucosal healing, sample size was calculated considering the prevalence of mucosal healing in the study population of 50%, absolute precision = 0.10, α error = 0.05 by using the nomogram for sample size to estimate sensitivity and specificity, as described by Malhotra et al. Sample size for an anticipated sensitivity of 90% and specificity of 91% was found to be 69 and 63 respectively. We chose a sample size of 70 patients.

2.5. Ethics approval and patient consent

Study approval was obtained from the institutional ethics committee and informed consent was obtained from each patient according to institutional guidelines.

Figure 1. Flowchart representing study design.
2.6. Statistical analysis
Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) depending on whether the distribution was normal or non-normal. Categorical variables were expressed as percentages and were compared using the χ² test. Continuous variables were compared with Student's t-test or the Mann–Whitney U-test as appropriate. Receiver operating characteristic (ROC) curves were used to assess the best cut-off for identifying the presence of mucosal healing or intestinal inflammation. SPSS software version 17.0 (SPSS Inc., CA, USA) was used for analysis. All analyses were two-sided and a p value of <0.05 was taken as statistically significant.

3. Results

3.1. Patient characteristics
Seventy-four consecutive patients who fulfilled the inclusion and exclusion criteria were included in this study between January 2012 and January 2013 (Figure 1). These comprised 27 females and 47 males with a mean age of 37.2 ± 10.9 years (Table 1). Median disease duration was 4.8 (IQR 3–8.3) years. Forty-seven patients had left-sided disease and 27 had pancolitis. Most patients (70/74) had a SCCAI of <3 (median 0 (IQR 0–2)), i.e. they were in clinical remission at the time of enrolment. Only 4 patients had mild disease activity clinically (3 patients had a SCCAI of 4 and 1 patient had a SCCAI of 5). At the time of enrolment, 43 patients were on mesalazine alone, 5 were on sulfasalazine alone, 23 were on a combination of mesalazine and azathioprine and 2 were on a combination of sulfasalazine and azathioprine, while 1 patient was not on any ongoing treatment. Fifty-four patients had a history of steroid intake. Forty-three patients had mucosal healing on colonoscopy and 31 patients did not. Median SCCAI was higher in patients without mucosal healing (4 (IQR 3–8) years). Fourteen patients (33%) had pancolitis.

3.1.1. Patients with mucosal healing (Table 1)
Of the 43 patients with mucosal healing, 65% were males. Mean age of the group was 37.4 ± 11.9 years and median disease duration was 4 (IQR 3–8) years. Fourteen patients (33%) had pancolitis and 29 (67%) had left-sided colitis. Thirty-one patients (72%) had a SCCAI of 0 at enrolment and only 2 patients had a SCCAI >3; thus, most patients in this group were in clinical remission. Thirty-eight patients were on mesalazine, 5 were on sulfasalazine and 19 were on azathioprine. Thirty (69%) patients had a past history of steroid intake.

3.1.2. Patients without mucosal healing (Table 1)
A total of 31 (41.8%) of the patients who were enrolled did not have endoscopic remission or healing. This included 19 males and 12 females with a mean age of 36.9 ± 9.6 years. Median disease duration in this group was 5 (IQR 3–9) years. Median SCCAI score was 1 (IQR 0–2). Twenty-nine patients in this group also had a SCCAI <3, i.e. were in clinical remission at the time of enrolment, whereas only 2 patients had a higher SCCAI (4 and 5 respectively). Twenty-nine patients were on mesalazine at the time of inclusion and 6 of them were also on azathioprine. Four patients had a past history of azathioprine use; one of them was not on any treatment at inclusion. One patient was on sulfasalazine. Twenty-four (77.4%) patients had a history of steroid use.

3.2. TFF3 levels
The median TFF3 level in patients without mucosal healing was 1.5 (IQR 1.2–1.9) ng/ml, which was significantly higher than the level in patients with mucosal healing [1.1 (IQR 0.8–1.3) ng/ml, p = 0.01] and healthy volunteers [0.85 (IQR 0.7–1.2) ng/ml, p < 0.001] (Figure 2). An ROC curve was developed to establish the predictive value of serum TFF3 for identifying patients with mucosal healing. A serum TFF3 level of <1.27 ng/ml had sensitivity, specificity, positive predictive value and negative predictive value of 70, 68, 75 and 62%, respectively, for the identification of patients with mucosal healing (area under the ROC 0.7281, 95% CI 0.604–0.851) (Figure 3). There was no difference in the median TFF3 levels between patients with and without mucosal healing. Serum TFF3 levels in UC patients were significantly higher than in healthy controls [1.2 (IQR 0.9–1.5) vs 0.85 (IQR 0.7–1.2) ng/ml; p = 0.004].

3.4. Relationship of TFF3 levels with disease extent and gender
Mucosal healing was more frequent in patients with left-sided colitis than in those with pancolitis (61 and 51% respectively), but the difference was not statistically significant. Serum levels of serum TFF3 were higher in patients with pancolitis compared with those with left-sided colitis, but the difference was not statistically significant [1.3 (IQR 1–1.6) vs 1.1 (IQR 0.9–1.4) ng/ml; p = 0.9]. There was no difference in median TFF3 values between males and females [1.2 (IQR 0.9–1.6) vs 1.2 (IQR 0.9–1.4) ng/ml; p = 0.38].

Table 1. characteristics of patients with ulcerative colitis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total patients (n = 74)</th>
<th>Presence of mucosal healing (n = 43)</th>
<th>Absence of mucosal healing (n = 31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>37.2 ± 10.9</td>
<td>37.4 ± 11.9</td>
<td>36.9 ± 9.6</td>
<td>0.84</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>47:27</td>
<td>28:15</td>
<td>19:12</td>
<td>0.74</td>
</tr>
<tr>
<td>Disease duration, years (median, IQR)</td>
<td>4.8 (3–8.3)</td>
<td>4 (3–8)</td>
<td>5 (3–9)</td>
<td>0.29</td>
</tr>
<tr>
<td>Disease extent (pancolitis/ left colitis)</td>
<td>27/47</td>
<td>14/29</td>
<td>13/18</td>
<td>0.40</td>
</tr>
<tr>
<td>SCCAI score (median, IQR)</td>
<td>0 (0–2)</td>
<td>0 (0–1)</td>
<td>1 (0–2)</td>
<td>0.003</td>
</tr>
<tr>
<td>Treatment (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mesalazine</td>
<td>43</td>
<td>21</td>
<td>23</td>
<td>0.06</td>
</tr>
<tr>
<td>mesalazine + azathioprine</td>
<td>23</td>
<td>17</td>
<td>6</td>
<td></td>
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<tr>
<td>Sulfasalazine</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sulfasalazine + AZP</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
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<tr>
<td>Past history of steroid use</td>
<td>54</td>
<td>30</td>
<td>24</td>
<td>0.59</td>
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</tbody>
</table>
Discussion

Mucosal healing is emerging as an important therapeutic goal with long-term impact in the therapy of ulcerative colitis.\(^\text{2,4}\) Endoscopic evaluation, though the gold standard for detection of mucosal healing, is limited by the facts that it is invasive, expensive and time-consuming, and thus less acceptable to both clinicians and patients. Moreover, repetitive endoscopic examinations cannot be an integral part of any management strategy for IBD. Various non-invasive biomarkers have been debated, but an ideal non-invasive marker for identifying mucosal healing is still to be found.\(^\text{22–27}\)

This was the first study to evaluate serum TFF3 in UC patients in clinical remission or with mild activity as a means of identifying mucosal healing. In this study 74 patients in clinical remission or with mild disease activity were enrolled and evaluated for endoscopic healing. In addition, sera was collected from 20 healthy volunteers as a control group. Fifty-eight percent of patients had mucosal healing, as determined by colonoscopy. Of 74 patients, 95\% were in clinical remission and 5\% had mild clinical disease activity. Serum TFF3 was significantly higher in patients than in healthy volunteers. Serum TFF3 levels were also significantly higher in patients who did not have mucosal healing than in those with mucosal healing. These results confirm the hypothesis that TFF3 levels are related to mucosal inflammation of the intestine and increase with the presence of mucosal damage.

Quantitative measurements of TFFs have been important tools for elucidating the biological functions of peptides, and we utilized this strategy to explore the role of these peptides as biomarkers for IBD. Clinical studies on the utility of trefoil peptides as a marker of activity in IBD patients are sparse. Vestergaard et al.\(^\text{28}\) did not find any significant fluctuations of TFF3 in three UC patients who underwent
The need for regular endoscopy in achieving the newly defined goal for patients with and without mucosal healing were significantly different and the values were more consistent within the subgroups; i.e., there was no significant overlap between the two subgroups.

The present study has several strengths. Clinical as well as endoscopic disease activity was correlated with serum trefoil peptide measurement; no large interindividual variations in peptide measurement were seen. Ninety-five percent of patients were in clinical remission. However, even in this group of similar patients serum TFF3 level had the ability to identify two subgroups: patients with and without ‘deep remission’ (both endoscopic and clinical remission). Therefore, within a subgroup of UC patients with mild disease activity or in clinical remission, serum TFF3 levels differ and can be used to identify those with endoscopic remission. Thus, serum TFF3 may be used as a surrogate marker of endoscopic remission and mucosal healing.

The main limitation of this study is that the surrogate marker was assessed at a single point of time. Further studies are needed to assess whether serial measurements of serum TFF3 would be more useful in assessing improvement in both clinical and endoscopic disease activity. Differences in serial measurements, if present, would be more useful than a single cut-off, which was used in the current study. Another limitation of the study is that faecal calprotectin could also have been measured and correlated with serum trefoil in these patients. Further validation is needed to establish the role of TFF3 as a surrogate marker of mucosal healing.

In conclusion, serum TFF3 level was able to identify patients with mucosal healing in a group of UC patients in clinical remission or with mild activity with reasonable sensitivity and specificity. It has potential for development as a serum biomarker that could decrease or with mild activity with reasonable sensitivity and specificity. It has potential for development as a serum biomarker that could decrease the need for regular endoscopy in achieving the newly defined goal of mucosal healing in patients with UC.

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References