Original Research Article

Serum transferrin receptor-ferritin index as a marker of iron deficiency anemia in active inflammatory bowel disease patients in Indian population

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ABSTRACT

Background: Anemia is the most common complication in IBD (inflammatory bowel disease). The aim of the study was to assess the sTfR-F (soluble transferrin receptor-ferritin) index as an early marker of IDA (iron deficiency anemia) in IBD.

Methods: Retrospective cross sectional study has 480 cases of IBD (group I) with controls 220 (group II), serum, tHsCRP, serum, iron, TIBC (total iron binding capacity), sTfR, ferritin, fecal calprotectin, vitamin B12, and folic acid were assessed.

Results: In study I, group I was compared with group II showed (66.5%) patients had active disease and in that 65.0% of UC, 32.1% of CD and 2.9% others colitis anemia. In study II, subgroup I 56.4% had IDA subgroup II 7.3% had ferritin between 30-100 mg/ml subgroup III 23.3% had ferritin>100 mg/ml (ACD, anemia of chronic disease) subgroup IV 5.6% had vitamin B12 and folic acid deficiency excluding sTTR-F analysis. In study III, subdivided to identify IDA with sTfR-F index as group A 60.8% had sTfR-F index>2, group B 32.6% had sTfR-F index=1-2 and group C 3 (6.2%) had sTfR-F index<1. Initially diagnosed IDA was 56.4%, in addition with group A, IDA has increased by 66.5%. In study IV, in IDA, sensitivity of sTfR-F index was100%, sTfR 89% and SF 85%. Specificity of sTfR and sTfR-F index were 80.60% and SF has low specificity 73.90%. In study V, a statistical significance was seen more in female than male and in children than in adults with sTfR-F index in IDA.

Conclusions: sTfR-F index as an early diagnostic marker, in differentiating IDA, ACD and combi in IBD patients.

Keywords: Ulcerative colitis, Crohn’s disease, Anemia

INTRODUCTION

IBDs including ulcerative colitis (UC) and Crohn’s disease are chronic inflammatory disorders that involve an autoimmune inflammatory response directed against the gut mucosa by an unknown cause.1 Anaemia in IBD is according to several previous studies commonly due to either IDA, ACD or combined anaemia (IDA and ACD simultaneously).23 IDA may result from gastrointestinal bleeding, malnutrition or malabsorption of iron.45 ACD is a consequence of upregulation of hepcidin expression, decrease erythropoetin production and inhibition of erythropoiesis caused by inflammatory mechanisms underlying IBD pathogenesis.4 The recognition of the cause of anemia in IBD patients allows implementation of efficient therapeutic option. However, there is no single, reliable marker of iron homeostasis since all traditional hematological and biochemical iron status parameters are influenced by inflammatory process underlying IBD.5
Iron deficiency can occur with or without anaemia has been reported to affect 13-90% of IBD patients, depending on some characteristics of the studied population (e.g. gender, age at diagnosis, disease activity, hospitalization and previous surgeries). Besides that, inflammatory mechanisms also lead to decreased iron uptake from the intestinal epithelium, thus providing a very complex two-way interactive pathophysiologic pathway between IDA mechanisms and inflammation.

In the European Crohn’s and colitis organisation (ECCO) guidelines from 2015 iron deficiency was defined as serum ferritin<30 µg/l in patients without signs of active disease and in the case of inflammation, iron deficiency cannot be ruled out with ferritin<100 µg/l. IDA is defined as iron deficiency in combination with haemoglobin below 120 g/l for nonpregnant women and below 130 g/l for men. In most cases, IBD-associated anaemia is due to the combination of chronic IDA and ACD, differentiation between IDA and ACD can be made by assessment of stainable iron in bone marrow. However, bone marrow examination is an invasive procedure resulting into inconvenience and discomfort to the patient. Red cell indices and iron parameters such as TIBC show considerable overlap. In general, IDA is associated with a serum ferritin value below 20 ng/ml whereas a serum level above 100 µg/l excludes iron deficiency in majority of cases. Serum ferritin being an acute phase reactant increases nonspecifically in inflammatory conditions despite the presence of iron deficient stores and values between 30 and 100 µg/l fall in the diagnostic gray zone.

sTfR is a truncated form of the transferrin receptor present on erythroblasts in bone marrow and many other cells. Measurement of sTfR is a new marker of iron metabolism that reflects body iron stores and total erythropoiesis. sTfR is not influenced by chronic or acute inflammation; therefore, it could be a more reliable index in diagnosing IDA in patients with IBD. Especially the sTfR-F index is gaining an increasing value in the evaluation of anemia for diagnosing IDA in the setting of chronic inflammation or discriminating iron deficiency in the absence of anaemia. Recently it has been proven to be unaffected by acute phase response like ferritin. Thus it can be introduced as a promising marker to reflect the iron deficiency in the presence of acute and chronic infection or inflammation.

The aim of this study was to investigate the clinical usefulness of the sTfR-F index in the evaluation of anaemia in patients with IBD and especially for differentiation between IDA, ACD and combi anemia.

METHODS

This is a hospital based retrospective cross-sectional study of 480 patients of IBD was done in department of biochemistry, Asian institute of gastroenterology, hospital, Hyderabad in South India. The study period was from December 2016 to 2019.

Inclusion criteria

All cases of IBD cases attending outpatient/ward in the gastroenterology department were included in the study and divided. On inclusion, the eligibility criteria were assessed and medical history was recorded.

Patient’s relevant age, gender, the classification and extent of the patients IBD were established.

Exclusion criteria

Cases of macrocytic blood picture, haemolytic anemia, aplastic anemia, CKD, CLD, patients with history of acute blood loss, blood transfusion, if they had a previous or current history of malignancies (except cutaneous), prior gastrectomy, systemic infections in the last 3 months, alcohol abusive use (daily alcohol consumption above 40 g), drug addiction or replacement therapy with iron, folic acid or vitamin B12 in the last six months and pregnant women or nursing mothers were not included in the study.

The study was approved by the institutional ethical committee.

Anaemia was defined according to the WHO criterion as haemoglobin level less than 13 g/dl for males and haemoglobin less than 12 g/dl for females. Anaemic patients were classified into 3 groups adopting the following criteria. Anemia due to vitamin B12 deficiency or anaemia caused by folic acid deficiency was diagnosed when there were low serum levels of these vitamins in patients with anaemia.

**IDA:** If ferritin was<30, TSAT <16% and hsCRP <10, then it was IDA.

**ACD:** When serum ferritin≥100, TSAT≥16% and hsCRP≥10, then it was ACD.

**Combined IDA/ACD:** When serum ferritin <100, TSAT<16% and hsCRP≥10, then it was combined IDA/ACD.

Blood sample collection

During the inclusion in the study, 3 ml blood was collected in a vial containing dipotassium EDTA for complete blood count. Fresh peripheral smear was made. 5 ml of venous blood was collected in an iron free plastic tube. Serum was separated for hematological and clinical chemistry were obtained. sTfR-F index was calculated based on the ratio sTfR/log ferritin.
Transferrin saturation (%) was calculated from iron and transferrin.

Complete blood count was done using Beckman Coulter autoanalyzer.

**Peripheral smear**

Wright stained peripheral smear was examined for RBC morphology. Serum HS-C-reactive protein (HsCRP) was done using latex agglutination test using kit CRP latex AU5800.

**Iron studies**

Iron studies were done with serum iron (Iron-Ferrozine, Beckman Coulter) serum TIBC (Beckman Coulter AU5800), serum transferrin receptor (sTfR) (immunoturbidimetry E 501 Roche Ltd, stool for Fecal calprotectin by CLIA in diasonin, vitamin B12, folic acid and serum ferritin by ECLIA Roche ltd.

**Statistical analysis**

The data collected during the current study were recorded and analysed statistically to determine the significance of different parameters by using graph pad instant statistical software. Results are expressed as mean±SD. The values between groups are compared using Quick-cal test. P value of 0.05 was considered statistically significant.

### RESULTS

**In study 1: Comparison between healthy control and total IBD, UC, Crohn’s patient**

**Groups:** The study group consisted of 480 IBD patients, in this total 106 (22%) were children and 374 (78%) were adults as of inclusion criteria 280 (66.5%) were active cases and 200 (33.5%) were inactive cases. According this study 44 (41.5%) children with UC, 53 (50%) children with Crohn’s disease and 9 (8.5%) colitis in children. In 236 (63.1%) adults with UC, 118 (31.5%) Crohn’s and 20 (5.4%). In this active phase of 280 IBD there were 182 (65%) UC patients, 92 (32.1%) Crohn’s while 8 (2.9%) had colitis, while 21 (28%). There was a slight male predominance 290 (60.4%) against female 190 (39.6%) The mean age was for 11.6±4.3 years old in children versus 50.2±12.6 years adults. Based on the applied criteria, iron deficiency was diagnosed in 158 (56.4%) subjects with only other anemia profile. Among these 158 (56.4%) patients fulfilled WHO criteria for IDA anemia, while 46 (7.3%) patients had combi anemia and 66 (23.3%) patients had ACD anemia. The frequency of iron deficiency did not differ significantly between patients with UC and Crohn’s disease. We identified iron depletion in 33 out of 46 (71.7%) children with UC and 17 out of 29 (58.6%) children with Crohn’s disease.

**Table 1: Comparison between control and IBD patients.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>UC (%)</th>
<th>Crohn’s disease (%)</th>
<th>Colitis (%)</th>
<th>IBD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>200</td>
<td>280</td>
<td>171</td>
<td>29</td>
<td>480</td>
</tr>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (0-18)</td>
<td>50</td>
<td>44 (11.6±4.3) (41.5)</td>
<td>53 (12.2±2.8) (50)</td>
<td>9 (10.2±3.9) (8.5)</td>
<td>106 (22)</td>
</tr>
<tr>
<td>Adults (18-70)</td>
<td>150</td>
<td>236 (50.2±12.6) (63.1)</td>
<td>118 (47.3±7.9) (31.5)</td>
<td>20 (55.1±4.7) (5.4)</td>
<td>374 (78)</td>
</tr>
<tr>
<td>Male</td>
<td>110</td>
<td>197 (67.9)</td>
<td>79 (26.5)</td>
<td>16 (5.6)</td>
<td>290 (60.4)</td>
</tr>
<tr>
<td>Female</td>
<td>90</td>
<td>123 (64.7)</td>
<td>54 (28.4)</td>
<td>13 (6.8)</td>
<td>190 (39.6)</td>
</tr>
<tr>
<td>Disease severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>182</td>
<td>65 (65.0)</td>
<td>92 (32.1)</td>
<td>8 (2.9)</td>
<td>280 (66.5)</td>
</tr>
<tr>
<td>Inactive</td>
<td>100</td>
<td>50 (50)</td>
<td>79 (39.5)</td>
<td>21 (10.5)</td>
<td>200 (33.5)</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of hematological and iron profile between control versus UC and Crohn’s disease.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>UC</th>
<th>Crohn’s disease</th>
<th>Colitis</th>
<th>Control/ UC (p value)</th>
<th>Control/ Crohn's disease (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecalcalprotein</td>
<td>35.3±35.77</td>
<td>100.2±70.8</td>
<td>74.5±31.17</td>
<td>27.4±31.6</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron</td>
<td>70.5±43.1</td>
<td>101.5±26.16</td>
<td>34.5±33.2</td>
<td>68.0±77.8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TIBC</td>
<td>220±62.9</td>
<td>181.5±10.6</td>
<td>206.5±55.1</td>
<td>182±21.9</td>
<td>&lt;0.0001</td>
<td>0.1223</td>
</tr>
<tr>
<td>B12</td>
<td>786.0±89.0</td>
<td>422.0±22.83</td>
<td>221.5±31.8</td>
<td>214.0±14.1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Folic acid</td>
<td>4.7±2.19</td>
<td>6.3±0.28</td>
<td>8.8±3.04</td>
<td>6.2±4.42</td>
<td>&lt;0.0001</td>
<td>0.0143</td>
</tr>
<tr>
<td>Ferritin</td>
<td>167.5±0.70</td>
<td>92.5±106.7</td>
<td>36.0±332.5</td>
<td>91.0±108.8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Continued.
### Table 3: Comparison of the hematological profile between IDA, ACD and combi anemia.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IDA/158</th>
<th>ACD/66</th>
<th>Mixed/46</th>
<th>IDA/ACD (p value)</th>
<th>IDA/mix (p value)</th>
<th>ACD/mix (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STfR</td>
<td>5.7±1.8</td>
<td>2.2±2.1</td>
<td>4.0±2.3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron</td>
<td>42±2.82</td>
<td>61±4.24</td>
<td>28±18.38</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TIBC</td>
<td>262.5±24.78</td>
<td>382.5±194.4</td>
<td>286.5±16.26</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>0.0415</td>
</tr>
<tr>
<td>Ferritin</td>
<td>27±1.41</td>
<td>32±8.48</td>
<td>20±5.65</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Transferin saturation</td>
<td>11.5±0.707</td>
<td>14±1.14</td>
<td>7±7.07</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sTfR-f</td>
<td>5.2±1.838</td>
<td>1.4±0.424</td>
<td>0.8±0.41</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HS-CRP</td>
<td>5±1.69</td>
<td>10.8±0.424</td>
<td>13±3.53</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

In study II: Comparison of hematological and immunological parameters among different subgroups

Subgroup i: 158 (56.4%) patients had ferritin<30 ng/ml (IDA).

Subgroup ii: 46 (7.3%) patients had ferritin between 30-100 ng/ml mixed IDA/ACD (combi anemia).

Subgroup iii: 66 patients (23.3%) had ferritin >100 ng/ml (ACD).

Subgroup iv: 10 (5.6%) patients had vitamin B12 and folic acid deficiency excluding sTfR-F analysis. We had excluded vitamin B12 and folic acid deficiency.

In study III: Comparison of the hematological profile between IDA, ACD and combi anemia

Anemia with controls: In subgroup again subdivided based on serum ferritin 30-100 ng/ml in the presence of inflammation, to identify IDA with sTfR-F index as group A: 28 of 46 patients (60.8%) had sTfR-F index>2;
group B: 15 patients (32.6%) had sTfR-F index=1 - 2 and group C: 3 patients (6.2%) had sTfR-F index<1.

Initially only subgroup I was diagnosed as IDA (56.4%), in addition with group A, IDA has increased by 66.5%. so overall IDA was diagnosed in 186 of 280 patients (66.5%), with the sTfR-ferritin it increased by 10.1%.

In study IV: The comparison of diagnostic efficiency of three markers for IDA, ACD detection in IBD patients, in terms of sensitivity, specificity

In IDA cases, sensitivity of sTfR-F index was 100%, sTIR alone was 89% and SF alone was 85%. Specificity of sTIR and sTfR-F index were 80.60% greater than SF which has low specificity 73.90%. In ACD sensitivity sTfR-F index and sTIR was 89.80% Serum ferritin 81.80%. So specificity sTIR was 100%, sTfR-F index 97.20%, SF 77.80%.

In study V: The comparison of STfR-F index between sex and age matched controls in IDA

A statistical significance (p<0.0001) was seen in female compared to male and in children when compared to adults with sTIR-F index in IDA.

![Figure 2: The comparison of diagnostic efficiency of three markers for ACD detection in IBD patients, in terms of sensitivity, specificity.](image)

DISCUSSION

In this study cases were well characterized through extensive medical record reviews. All biochemical analyses were performed in batch. This study we also compared both male and female IBD cases to matched controls. It was difficult to evaluate anemia in patients with inflammation as conventional laboratory tests for iron status were often unable to differentiate ACD from IDA. As a consequence, a wide range of reference limits for serum ferritin had been suggested to diagnose iron deficiency in patients with chronic inflammation. The gold standard for assessing iron status was staining the bone marrow iron with Perl’s stain. However, this procedure was invasive, expensive and painful. There were several possible causes of iron deficiency among IBD patients. It could be due to bleeding, exfoliation of cells or both in patients with bowel mucosa damage. It could also be explained by decreased iron uptake from the upper small bowel (Crohn’s disease) and low dietary intake caused by the avoidance of food due to gastrointestinal symptoms. STIR levels were expected to be highest in IDA as reported earlier by Dimitriou et al 2000, Malope et al 2001, Angeles et al 2006 and Hanif et al 2005 in different studies. Present study showed similar result. Serum ferritin levels reflected iron stores while sTIR levels reflected the degree of availability of iron for cells. Calculating the sTIR/log ferritin index (sTIR index) from these two measures provided an estimate of body iron over a wide range of normal and depleted iron stores. sTIR concentration was not usually affected by inflammation/infection but in conditions where iron deficiency coexisted with ACD sTIR rose secondary to underlying iron deficiency. Thus, sTIR levels were a better indicator of iron deficiency when it was associated with inflammation. sTIR was derived from the erythroid precursors in the bone marrow and from the reticulocytes, so it reflected the rate of erythropoiesis. The serum level of sTIR in iron deficiency also increased due to upregulated transferrin
receptor on the cell surface in response to increased cellular demand. Thus it was assumed that sTfR reflected the tissue iron supply reliably. Serum ferritin was the most specific marker of iron stores, but there occurred a relatively little change in its level after stores were fully depleted whereas sTfR levels rose with increase in iron deficiency. This indicated that serum ferritin was the most sensitive index of iron status whereas sTfR was more sensitive when there was functional iron deficiency. Moreover, sTfR levels also reflected the rate of erythropoiesis, so its specificity decreased as a sole marker of iron deficiency. Thus because of this reciprocal relationship between sTfR and serum ferritin, the serum TfR/ferritin ratio reflected the iron status over the entire range.

In present study, patients of IDA had sTfR/log ferritin index of >2.0 while all pure ACD cases had sTfR/log ferritin index <1.0. The variability in blood loss during menstruation and the subsequent changes in iron status may be a confounder, obscuring the possible risk associations with IBD in women. Previous studies had reported the prevalence of iron deficiency in IBD, for example, one Canadian study on 280 IBD patients reported the prevalence of iron deficiency to 20% for Crohn’s disease and 27% for UC patients, compared to 20% iron deficiency in our material on IBD patients prior to diagnosis, 17% among future ulcerative colitis and 27% among future Crohn’s disease. This indicated that the changes seen in iron status among IBD patients were present years before diagnosis. In women with IBD, previous studies had shown that iron deficiency was more prevalent compared to men we had seen same female predominance. In a Spanish study on 104 patients, the prevalence of around 50% in women compared to 20% in men with IBD was seen. A Swedish study of 373 patients with IBD reported a prevalence of iron deficiency and IDA of 6.8 and 6.2%, respectively, the total frequency of iron deficiency with or without anaemia of 13% (8% in male and 16% in female IBD patients). This was consistent with our finding that iron deficiency was present in around 31.3% of women compared to approximately 8.3% of men with IBD diagnosis. Thus, iron deficiency was more prevalent in women compared to men. However, because our controls were matched, we can see that prior to diagnosis women did not have a higher proportion of iron deficiency compared to controls as was the case for men. Ferritin levels <30 µg/l were reported in 24% of 150 IBD patients in a French study and 25% in a Scandinavian study on 429 patients. We found a slightly higher frequency of ferritin <30 µg/l, 56.4% but our patients were healthy at inclusion. None of the studies mentioned above included any control subjects.

Further studies on the interactions of iron metabolism with gut microbiota and the immune system were needed. Our findings emphasized the importance of knowing the aetiology of iron deficiency before starting treatment. It was also important to exclude causes of iron deficiency other than menstrual blood loss among women, not to postpone an IBD diagnosis. In our study, iron deficiency was seen in 14.7% of controls. We also had a slightly higher median ferritin concentration, 92µg/l, compared to 56µg/l as also seen in one of Scandinavian patients with active disease.

In the present study, all 158 IDA patients had significantly raised sTfR levels. And with the sTfR-ferritin index the percentage of IDA has increased to 66.5% with total increase to 186 patients. Bone marrow iron stain in 8/9 cases showed absence of iron stores suggesting that the ACD cases with raised sTfR actually had coexisting iron deficiency. Thus sTfR levels were helpful but not completely reliable in diagnosing IDA coexisting with ACD. It was seen in the present study that 100% patients of IDA and ACD with coexisting iron deficiency had sTfR/log ferritin index of >2.0 while all pure ACD cases had sTfR/log ferritin index >2.0. Though serum sTfR concentrations were elevated in the IDA than the ACD and ACD with IBD. To distinguish IDA and (ACD with IDA on the basis of iron status was still difficult). However, the detection of iron deficiency in ACD with IDA patients was very useful for the initiation of replacement therapy. Apart from sTfR, some other derived variables like ratio of serum transferrin receptor to the log ferritin which is known as sTfR-F index had also shown to have some promising value in diagnosis of anemia of chronic disease having IBD. Therefore the present study was undertaken to differentiate between iron deficiency anemia, anemia of chronic disease and ACD with IDA anemia using sTfR and sTfR-F index.

Limitation of our study was the rather small number of patients with active disease not allowing for firm conclusions with respect to the role of disease activity on sTfR-F index. Another regarding sTfR measurement, the assay was not widely available, it remained expensive and was not standardized among different laboratories. One more was females in IBD group could influence the results due to the possible influence of menstruation on the parameters of iron deficiency.

**CONCLUSION**

Our results demonstrate that estimate both serum sTfR and sTfR-ferritin indices more efficient and independent tool in acute IBD cases to diagnose and differentiate between pure IDA, ACD and ACD with coexisting iron deficiency thus providing a non-invasive alternative to bone marrow iron as sTfR/log ferritin index has role in identifying development of iron deficiency in ACD whereas log sTfR/FS ratio can differentiate pure IDA from ACD with or without iron deficiency, sTfR level has a comparatively more ability than serum ferritin in diagnosing IDA and ACD. However, sTfR and serum ferritin alone cannot definitely exclude coexisting iron deficiency in ACD. One of the most important advantages of the sTfR-F index is that it is independent of the inflammatory status, taking into account that no
correlation was found between this index and HsCRP levels or disease activity. The sTR-F index seems to be very efficient in the detection and diagnosis of IDA, among patients with IBD. Its detection rate is higher than sTR alone and of course higher than the other existing markers; therefore, it could be proposed as an added value parameter, which can help in early diagnosis of IDA in patients with IBD.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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