Credibility of the measurement of serum ferritin and transferrin receptor as indicators of iron deficiency anemia in hemodialysis patients

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Abstract. – Background and Objectives:
Anemia is a common complication in uremic patients. Erythropoietin therapy is prescribed in these cases; however, this treatment is not successful in iron deficient patients. Ferritin-based diagnosis of iron deficiency in these patients is a challenging task, as serum ferritin level may be high due to chronic inflammation and mask iron deficiency. In the current study we evaluated the credibility of another indicator of body iron supply, serum transferrin receptor, in hemodialysis patients in two University-based Hospitals in North of Iran.

Materials and Methods: In a cross-sectional study, 53 hemodialysis patients with a mean age of 56 ± 18.7 years and 30 persons with iron deficiency and normal renal function with a mean age of 20.1 ± 14.4 years were examined. All hemodialysis patients were on hemodialysis 2-3 times per week for 3-4 hours. All cases were examined for blood hemoglobin content, serum iron, CRP, serum ferritin and serum transferrin receptor levels. The reference ranges introduced by manufacturers were considered as standard ranges for analysis of the results. Using one sample T-test and Fisher’s exact test, data were analyzed. \( p < 0.05 \) was considered as significant.

Results: Hemodialysis patients had blood hemoglobin content below normal range (\( p < 0.05 \) for men, \( p < 0.001 \) for women) and CRP levels above normal range (\( p < 0.001 \)). In hemodialysis patients, serum ferritin level was significantly higher than control group (\( p < 0.001 \)), whilst serum transferrin receptor levels in the two groups were not significantly different (\( p = 0.69 \)), and both were above defined normal upper limit (\( p < 0.001 \) for iron deficient patients; \( p < 0.05 \) for hemodialysis patients).

Discussion: This study showed measurement of serum ferritin in the presence of chronic inflammation induced by renal failure cannot be a credible indicator of body iron supply, while under this certain condition serum transferrin receptor can more appropriately reflect the amount of body iron supply.

Key Words:
Ferritin, Transferrin receptor, Hemodialysis patient, Iron deficiency anemia.

Introduction

Anemia is a common finding in hemodialysis patients. There are numerous factors that relate renal dysfunction to anemia such as accumulation of uremic toxins (guanidosuccinic acid, phenols and polyamines which suppresses erythropoiesis, increased amounts of different inflammatory cytokines including interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-alpha) and interferon gamma that act as apoptosis inducers in erythroid precursors in bone marrow; raised quantities of free radicals and reactive oxygen species (ROS) – for instance nitric oxide (NO) and superoxide anions – synthesized by activated macrophages, affecting erythroid precursors; blood loss related to hemodialysis procedure and gastrointestinal hemorrhage; and dilutional anemia due to disturbance of kidney filtration and excessive retention of fluids.

Reduced production of erythropoietin – synthesized by endothelial cells of the capillaries surrounding kidney tubules – during the course of renal complication, is another contributing factor to anemia. Erythropoietin therapy – nowadays administered as recombinant human erythropoietin – is one of the main approaches to cure anemic patients. One of the most important reasons of unresponsiveness to this treatment is shortage of body iron supply. Therefore, a precise assessment of iron storage in hemodialysis patients is of vital value to identify patients with actual iron deficiency and adjust treatment accordingly.

The gold standard for identification of iron deficiency-induced anemia is iron stain on liver or bone-marrow biopsy samples. This is a precise approach, yet an invasive one, and in case of existence of any replacement it is better avoided. One alternative approach is evaluation of two red blood cell indices – mean corpuscular volume (MCV) and mean corpuscular hemoglobin con-
centration (MCHC) – and detection of microcytic and hypochromic red blood cells as confirmation of existence of iron deficiency. Nevertheless, these two parameters are affected by the existing inflammation. Measurement of transferrin saturation is another way of estimation of iron supply. However, this factor has wide daily fluctuations due to amount of serum iron and transferrin and dependent on food regiment. The above facts undermine the reliability of transferrin saturation as a valid indicator of iron storage in hemodialysis patients. Serum ferritin (SF) is another known marker of iron deficiency. Although measurement of ferritin – a protein of major importance in the process of iron storage – can provide an indirect estimation of body iron supply, it appears that pathologic and inflammatory conditions affect this serum constituent, too.

In search of a reliable approach to estimate body iron supply – not affected by inflammatory procedures and pathologic conditions – serum soluble transferrin receptor (sTfR) was introduced.

This study evaluated SF and sTfR levels in hemodialysis patients, referred to Fatemeh-Zahra and Valiasr Centers, two University based Hospitals in North of Iran.

Materials and Methods

In a cross sectional study, 53 hemodialysis patients (27 females and 26 males) who consented to take part, were examined as study group. All cases had definite renal failure and were in stage 5 of chronic kidney disorder, undergoing hemodialysis procedure 2-3 times per week, each time for 3-4 hours. Including criteria were: hemodialysis for more than 6 months, no iron supplement uptake for 3 weeks prior to sample donation, erythropoietin therapy with a fixed dose for at least 3 months and no erythropoietin administration for at least 86 hrs before sample donation, absence of hemorrhage, acute hepatic disease, and infection, and no blood transfusion and ascorbic acid intravenous injection. 10 ml whole blood was taken from each patient. Considering following criteria, control group consisting of 30 persons with a gender distribution similar to study group were selected: SF level lower than 15 ng/ml (cut off point for identification of iron deficiency), no iron supplement uptake three weeks prior to sample donation, absence of renal failure, hemorrhage, acute hepatic complication, and infection, and no blood transfusion and intravenous ascorbic acid injection. Following components were measured: blood hemoglobin content with reference range of 12.3-15.3 mg/dl in women and 14-17.5 mg/dl in men by Abbot cell counter (Abbott Laboratories, Abbott Park, IL, USA); serum iron via turbidimetry method (Hitachi 717 system, Boehringer Mannheim, Mannheim, Germany) with reference range of 50-160 µg/ml; SF via chemiluminescence immunoassay (CLIA) with Liaison instrument (DiaSorin, Saluggia, Italy) and reference range of 10-140 ng/ml for women and 15-220 ng/ml in men; sTfR via enzyme-linked immunosorbent assay (ELISA) and reagents from Bio Vendor (Modrice, Czech Republic) with normal range of 1-2.9 µg/ml; and C-reactive protein (CRP) with normal range of 0-10 mg/l (Pars Azmoon, Tehran, Iran).

Statistical Analysis

Using SPSS Statistics software V17 (SPSS, Inc., Chicago, IL, USA) data were analyzed. In order to compare obtained results with reference values T-test for one group was performed, and the difference between patients and control group was examined via Student’s t-test and Fisher exact test. \( p < 0.05 \) was considered as significant in all cases.

Results

In this study 53 hemodialysis patients with a mean age of 56 ± 18.7 years and 30 non-uremic iron deficient anemia cases with a mean age of 20.1 ± 14.4 were examined. The mean hemodialysis period was 25 ± 15 months. Blood hemoglobin in hemodialysis patients was 9.9 ± 0.3 mg/dl. The mean value was 9.7 ± 0.3 mg/dl for women and 10.1 ± 0.3 mg/dl for men, both below reference values (\( p < 0.05 \) and \( p < 0.001 \) respectively). Serum iron level in hemodialysis patients was 59.0 ± 3.9 µg/ml. CRP in study group was 12.1 ± 0.7 mg/dl. SF level was 6.3 ± 1.2 ng/ml in iron deficient anemic patients and 309.5 ± 27.4 ng/ml (297.6 ± 34.8 in women and 321.9 ± 34.1 in men) in hemodialysis patients. These results in female and male hemodialysis patients were significantly higher than reference ranges (\( p < 0.05 \) and \( p < 0.001 \) respectively). The difference of SF levels in hemodialysis and anemic patients was significant (Student’s t-test; \( p < 0.001 \)). sTfR level was 4.1 ± 0.5 in anemic patients and 3.7 ± 0.2 µg/ml in hemodialysis patients, both higher than normal val-
ues ($p<0.001$ and $p<0.05$ respectively). Considering sTfR levels, two studied groups were not significantly different from each other (student’s $t$-test; $p=0.69$) (Figure 1). The patients were distributed based on SF and sTfR levels. Patient distribution in study and control groups were significantly different based on SF measurement (Fisher exact test, $p<0.001$), whilst it was not significant based on sTfR value ($p=0.41$) (Table I).

**Discussion**

In healthy and most pathologic conditions, SF is a good indicator of the amount of iron supply. However, erythropoiesis, malnutrition, malignancies, hemolysis and certain inflammatory conditions such as infections, hepatic dysfunction and renal failure may affect SF level$^{12}$. The current study showed measurement of SF is not an appropriate approach to estimate body iron supply in the presence of inflammation initiated by renal failure.

This result is different than the findings of Rocha et al$^{10}$, who introduced measurement of SF as a standard way of estimating deposited iron supply in bone marrow in hemodialysis patients. They considered SF above 500 ng/ml as cut off level to determine iron deficiency in hemodialysis patients ($p<0.001$). Due to increase in ferritin activity in acute phase of renal failure, it appears the SF cut off level for determination of iron deficiency is probably higher in uremic patients than non-uremic people. Nevertheless, this study cannot finally make a direct quantitative correlation between SF and body iron supply and did not introduce an approach to use this component in estimation of the amount of body iron in hemodialysis patients. Furthermore, they did not make a comparison between SF and sTfR. Our finding of SF surge during renal failure-induced inflammation disqualifies this marker as an appropriate indicator of body iron supply.

Transferrin receptor is a cell membrane protein involved in cellular transportation of trans-

![Figure 1](image-url). Blood hemoglobin, serum iron, CRP, SF, sTfR in hemodialysis patients and control group (Student’s $t$-test, $^*p<0.05$, $^{**}p<0.001$).
ferritin. This protein is necessary for iron trafficking through cell membrane and its production is regulated by cellular iron accumulation\(^\text{13}\). Reduced amount of cellular iron stimulates sTfR synthesis to promote iron absorption into the cell. In the case of cellular iron surplus, transferrin receptor shows reduction\(^\text{14}\). Considering above facts, measurement of sTfR can be considered as an approach to approximate body iron storage. Transferrin receptor is expressed in almost all body cells, but more than 80% of that is detected on cell surface of erythroid precursors\(^\text{8}\).

Some investigations have been previously carried out on sTfR and its application in identification of iron deficiency in hemodialysis patients. Tarng et al\(^\text{15}\) reported a reduction of sTfR in anemic hemodialysis patients, compared with non-uremic anemia patients. They also reported of similar levels of SF in both groups. High level of SF in control group contradicts general conditions of anemia. In order to define anemia, reliable criteria have to be chosen and, therefore, the conclusions of the mentioned work may not be generalized. In our study, ferritin level below normal range in non-uremic patients was considered as the standard of delineation of iron deficiency anemia, and similarity of sTfR levels in non-uremic iron deficient patients and hemodialysis patients was considered as an indicator of existing iron deficiency in the latter group. In the study of Tarng et al, sTfR level was reported to be less than control group \((p<0.001)\) which is different than our findings. Considering details of erythropoietin therapy and iron utilization may explain some existing discrepancies between the two studies.

In another study, Beguin et al\(^\text{16}\) found a negative correlation between basal levels of ferritin and sTfR in hemodialysis patients and concluded the two mentioned factors to be appropriate indicators of iron deficiency. In this study inflammation as an important factor affecting the results is not examined. Therefore, it cannot decisively be stated whether their finding is applicable under inflammatory conditions. The other issue about their experiment is the studied cases were continuously receiving iron supplement up to the time of sample donation. This condition may affect evaluated factors. By monitoring iron uptake of patients, we controlled this intervening component. Furthermore, we examined existing inflammation quantitatively and showed SF surge during inflammation makes ferritin-based evaluation of iron deficiency unreliable.

In line with our findings, Beerenhout et al\(^\text{17}\) found a positive correlation between transferrin saturation and sTfR level and showed SF and sTfR are independent from each other, under chronic inflammatory conditions associated with renal functional failure. They also found a positive correlation between SF and CRP levels, despite the fact that sTfR was independent of this inflammation marker. Using a different approach than ours, the cited study finds sTfR more reliable than SF in evaluating iron deficiency in hemodialysis patients.

In another study, Keskin et al\(^\text{18}\) found out in spite of similarity of a variety of biochemical indices in iron deficient anemia patients and hemodialysis patients, sTfR in iron deficient anemic patients and iron deficient anemic patients suffering from chronic disease was higher than healthy people and anemic patients who had chronic diseases with normal iron storage. The study indirectly confirms our finding that transferrin receptor is an appropriate marker in differentiation between iron deficiency anemia and anemia of chronic diseases.

In a number of studies it is stated the logarithmic ratio of sTfR to SF is better indicator of iron deficiency than sTfR alone\(^\text{11}\). This is a debatable statement, as ferritin itself surges dramatically during inflammatory conditions induced by renal dysfunction and, therefore, its combination with another marker may not be an appropriate way to identify iron deficiency in hemodialysis patients. Nevertheless, up till now no study with a sizeable sample group and satisfying criteria has confirmed this hypothesis.

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>SF 0-5 [ng/ml]</th>
<th>SF &gt; 15 [ng/ml]</th>
<th>sTfR &gt; 2.9 [µg/ml]</th>
<th>sTfR 1-2.9 [µg/ml]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficient patients (30 cases)</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hemodialysis patients (53 cases)</td>
<td>2</td>
<td>51</td>
<td>51</td>
<td>2</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Table I. Distribution of hemodialysis and iron deficient anemia patients according to SF and sTfR levels.
Another important advantage of sTfR is its application in hemodialysis cases with inflammation unidentifiable in clinical examination. As this is a common situation in hemodialysis patients, in case of uncertainty about existence of inflammation, factors such as SF and transferrin saturation that are affected by inflammatory conditions, must be substituted by reliable factors to estimate iron supply.

Anemia exists during the course of chronic inflammation, autoimmunity, malignancy or infection. Two distinct conditions, iron deficiency anemia and chronic disease anemia may co-exist. Therefore, identification of iron deficiency while a chronic disease is present, is a challenging task for clinicians. STIR can potentially differentiate between these two complications: sTIR is a definitive marker of iron deficiency, regardless of presence or absence of inflammation, and high levels of SF can be interpreted as existence of inflammation. Therefore, a combination of the results of SF and sTIR can lead us to evaluation of both iron deficiency and inflammation.

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