Original Article

Role of Soluble Transferrin Receptor – An Iron Marker in Hemodialysis Patients

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Abstract

Background: Iron status assessment is crucial in end-stage renal disease hemodialysis (ESRD-HD) patients because iron deficiency may cause unresponsiveness to erythropoiesis-stimulating agent. Soluble transferrin receptor (sTfR) is a potential iron marker that is not influenced by inflammation, and the results among studies are still conflicting. This study evaluated the role of sTfR in determining iron deficiency in ESRD-HD patients. **Methods:** This cross-sectional study was conducted at the Hemodialysis Unit in Cipto Mangunkusumo Hospital, Indonesia, from August to September 2018 and included 127 ESRD-HD patients. The sTfR level, sTfR index (sTfR/log ferritin), iron status, ferritin level, and complete blood count were assessed. Transferrin saturation (TSAT) was used as a reference. The role of sTfR was analyzed using the Chi-square test and receiver operating characteristic curve analysis. **Results:** The median sTfR was 3.0 (range, 1.0–8.5) mg/l, and the median TSAT was 23% (4.0%–100%). The sTfR level in ESRD-HD patients with absolute iron deficiency was 3.9 (1.9–8.5) mg/l, in those with functional iron deficiency was 3.5 (1.9–5.4) mg/l, and in those with no iron deficiency was 2.6 (1.0–6.4) mg/l. The previous sTfR cut-off value of 2.5 mg/l had a sensitivity of 83.3%, specificity of 48.2%, positive predictive value (PPV) of 44.3%, and negative predictive value (NPV) of 85.4%, whereas the new sTfR cut-off value of 2.71 mg/l had a sensitivity of 83.3%, specificity of 56.5%, PPV of 48.6%, and NPV of 87.3%. TSAT and index TSAT were not influenced by inflammation. **Conclusion:** The cut-off sTfR value of 2.71 mg/l is better than 2.5 mg/l to determine the iron status in ESRD-HD patients.

Keywords: *CKD, hemodialysis, inflammation, iron, transferrin receptor*

Introduction

Anemia is a common complication in chronic kidney disease (CKD) patients.[1] The prevalence of anemia in Manado, Indonesia, was as high as 80%–90% in 2016 and increased as CKD worsened.[2] Additionally, it was shown to be associated with an increased risk of cardiovascular complications and a reduced quality of life.[1] The common cause of anemia in CKD is depletion of erythropoietin production by the kidneys, which is often exaggerated by iron deficiency.[2,3] Iron deficiency in patients with CKD and on hemodialysis (HD) is usually caused by occult gastrointestinal bleeding, blood trapping in an HD dialyzer or line, bleeding at HD access, and an inflammatory state that can decrease iron absorption and availability.[2] Iron deficiency results in a suboptimal response to an erythropoiesis-stimulating agent (ESA). ESA therapy in an iron deficiency state should involve iron supplementation simultaneously. Thus, it is very important to assess the iron status in end-stage renal disease HD (ESRD-HD) patients.[1]

Administration of high-dose ESA in ESRD-HD patients is often incompatible with the increase in hemoglobin level. This can be confusing for the clinical doctor. Moreover, serum ferritin and transferrin saturation (TSAT) cannot provide correct information about the patient's iron status. The accuracy of the ferritin and TSAT tests depends on their sensitivity and specificity. Several studies with response to iron therapy and bone marrow biopsy with iron staining have reported that TSAT of more than 20% shows good sensitivity in determining absolute iron deficiency, compared to serum ferritin which is less than 200 ng/ml.[1,4] This is the reason for the use of TSAT as a reference standard in this study. Besides, TSAT is routinely

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performed in the laboratory, noninvasive, easily available, and can be approved by the patient.

The gold standard approach to identify iron deficiency is iron-stained bone marrow aspiration, which is invasive and impractical in daily practice, and thus, it has been largely substituted with ferritin and TSAT. Soluble transferrin receptor (sTfR) is one of the parameters to identify iron deficiency accurately, even in an inflammatory state.[1,4,5] Serum sTfR indicates the transferrin receptor amount expressed mainly by erythroid cells that can be affected by intracellular iron. In iron deficiency, increased expression of the transferrin receptor will cause an increase in serum sTfR. The sTfR index can be calculated using serum sTfR and ferritin. This index represents total body iron supply and iron availability for erythropoiesis. It has been suggested to be a better parameter than sTfR alone.[5,6] ESRD-HD patients often experience functional iron deficiency, where iron in the body cannot be optimally used; thus, ESA therapy will be ineffective. This study was aimed to evaluate the diagnostic performance of sTfR for iron deficiency in ESRD-HD patients.

Materials and Methods

This cross-sectional study was conducted at the Hemodialysis Unit of Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from August to September 2018 and was approved by The Ethics Committee for Health Research Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital (No: 0087/UN2.F1/ETIK/2018).

The inclusion criteria were ESRD-HD patients aged ≥18 years undergoing routine HD (two to three times a week), agreeing to participate in the study, and signing an informed consent. One of the confounding factors in this study was that we did not exclude iron and ESA treatment 3 months before the study. Anemia was considered when the hemoglobin level was <13 g/dl for men or <12 g/dl for women.[7] Hemoglobin target was 10–11.5 g/dl. ESA or iron therapy was confirmed when patients received ESA or intravenous iron therapy for the last 3 months. Absolute iron deficiency was defined as ferritin <200 µg/l and TSAT <20%, functional iron deficiency was defined as ferritin >200 µg/l and TSAT <20%, and no iron deficiency was defined as TSAT ≥20%.[7] TSAT was used as a reference standard. Inflammation state was determined by C-reactive protein (CRP) >5 mg/dl.

Venous blood (9 ml) was drawn before dialysis from the HD access of each participant using a sterile syringe. The blood was then introduced into a 3-ml tripotassium ethylenediaminetetraacetic acid (K3-EDTA) tube (B. Braun, Melsungen, Germany) and a 6-ml clot activator tube (B. Braun). The sTfR level, iron status, ferritin level, and complete blood count were assessed in each participant. sTfR was measured by sTfR (Cobas®; Roche, Mannheim, Germany), a particle-enhanced immunoturbidimetric assay, on Roche Cobas c311, and the sTfR index was calculated by dividing sTfR (mg/l) with log ferritin (µg/l). The coefficients of variation of sTfR were 2.33% for control material level I and 1.04% for control material level II. Meanwhile, the total error allowable values were 9.9% for control level I and 8.4% for level II. Serum iron and the total iron binding capacity were measured on Architect ci8200 (Abbott, Wiesbaden, Germany). Ferritin was measured on Cobas e411 (Roche), and the complete blood count was measured on Sysmex XN-3000™ (Sysmex, Kobe, Japan). Demographics (age and sex) and clinical data (HD duration and ESA/iron therapy) were collected from medical records.

Statistical analysis

The minimum sample size needed for this study was 123 patients using diagnostic formula, assuming a sensitivity of 80% and a prevalence of 50%. All parameters were evaluated using the Kolmogorov–Smirnov normality test. For non-normal distribution, the median and range were used instead of the mean and standard deviation. Post hoc analysis (Tukey test) was used to compare the therapy groups. Diagnostic tests were analyzed using the Chi-square test and receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of sTfR were calculated. Effect of inflammation was also assessed. Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM Corp, Armonk, NY, USA) were used for data analysis. A P value <0.05 was considered statistically significant.

Results

The study included 127 ESRD-HD patients who met the criteria. The patient characteristics are presented in Table 1. The median HD duration was 5 years. Majority of the patients have received ESA therapy, and a few of them received iron therapy. Although ESA therapy was administered to a high proportion of patients, anemia occurred in almost all the patients, with the mean hemoglobin level being 9.5 g/dl. About 33% of patients suffered iron deficiency anemia and two-thirds of these patients had absolute iron deficiency. The median sTfR level was higher than the cutoff (2.5 mg/l), which indicated iron deficiency; on the other hand, the median TSAT still within normal range (>20%) [Table 1]. The sTfR level was divided according to therapy and iron level [Table 2]. The sTfR level did not differ among the therapy groups (P = 0.961). However, based on iron level, sTfR was lower in normal iron group than in absolute iron deficiency group ($P \leq 0.001$). [Table 2]

When using an sTfR cut-off value of 2.5 mg/l, the sensitivity and specificity were 83.3% and 48.2%, with PPV being 44.3% and NPV being 85.4%. However, when

Table 1: Baseline patient characteristics

Characteristic	*n* (%) (*n*=127)
Male sex	63 (49.6)
Age (years), mean (SD)	50.2 (1.2)
Hemodialysis duration (years), median (range)	5.0 (0.5-30.0)
ESA therapy	107 (84.3)
Iron therapy	15 (11.8)
Anemia	125 (98.4)
Patients who reached hemoglobin target therapy	42 (33.1)
Iron deficiency	
Absolute	29 (22.8)
Functional	13 (10.2)
Hemoglobin level (g/dl), mean (SD)	9.5 (1.4)
Hematocrit, mean (SD)	28.4 (4.4)
Serum iron (µg/dl), median (range)	51.0 (11.0-204.0)
TIBC (µg/dl), mean (SD)	235.0 (48.9)
Transferrin saturation, median (range)	23.0 (4.0-100.0)
Ferritin (µg/l), median (range)	256.6 (3.4-3658.0)
sTfR, median (range)	3.0 (1.0-8.5)
sTfR index, median (range)	1.3 (0.3-10.3)

ESA=erythropoiesis-stimulating agent, SD=standard deviation, sTfR=soluble transferrin receptor, TIBC=total iron binding capacity

Table 2: Determinant factors of the sTfR level

Determinant factor (*n*)	sTfR (mg/l)
Based on therapy	
ESA+iron therapy (*n*=14)	2.8 (1.5-6.4)
Only ESA therapy (*n*=93)	3.1 (1.0-7.3)
Only iron therapy (*n*=1)	2.9 (2.9-2.9)
No ESA or iron therapy (*n*=19)	3.3 (1.3-8.5)
Based on iron level	
Absolute iron deficiency (*n*=29)	3.9 (1.9-8.5)
Functional iron deficiency (*n*=13)	3.5 (1.9-5.4)
Normal iron (*n*=85)	2.6 (1.0-6.4)
All ESRD-HD patients	3.0 (1.0-8.5)

ESA=erythropoiesis-stimulating agent, ESRD-HD=end-stage renal disease hemodialysis, sTfR=soluble transferrin receptor

using a cut-off value of 2.71 mg/l, it showed the same sensitivity, but better specificity (56.5%), with area under the curve (AUC) being 74.9% [Figure 1]. The PPV and NPV of this new cut-off value was 48.6% and 87.3%, respectively. The associations between iron parameters and the iron deficiency status are presented in Table 3. The ferritin level, sTfR level, and sTfR index showed causative relationships with iron deficiency (all odds ratios >3; $P < 0.05$). The influence of inflammation on sTfR, sTfR index, and ferritin are presented in Table 4.

Discussion and Conclusions

Various studies on sTfR have been carried out on various populations with different cut-off values.[8,9] Gupta *et al*.[10] conducted a study in a population similar to this study (126

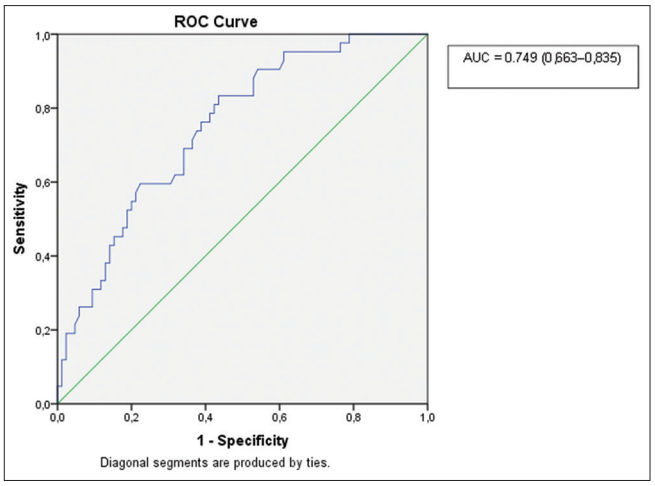

Figure 1: ROC curve analysis for STfR cutoff of 2.71. AUC = area under the curve, ROC = receiver operating characteristic, sTfR = soluble transferrin receptor

ESRD-HD patients) and found a sensitivity of 63.6% and specificity of 64.8% at a cut-off point of sTfR 3.0 mg/l with the gold standard of bone marrow iron staining. Our study found better sensitivity (83.3% vs. 63.6%) with a slightly lower specificity (56.5% vs. 64.8%). This study with sTfR cut-off values of 2.5 and 2.71 mg/l had a poorer diagnostic performance to detect iron deficiency when compared to the other study. Shin *et al*.[9] compared sTfR with bone marrow iron staining in not only ESRD-HD populations that performed bone marrow iron examination and obtained an AUC value of 94.4%, with a sensitivity of 85.4% and a specificity of 91.9% at the cut-off point of sTfR being 2.3 mg/l. The different results between this study and previous studies are likely due to differences in the gold standard, the study population, as well as the cut-off points. Additionally, this study had a PPV of 49%, indicating that 49% of individuals with a positive test result truly have the condition. Moreover, it had an NPV of 87%, indicating that 87% of individuals with a negative test result truly do not have the condition. As the sensitivity of sTfR for iron deficiency diagnosis was above 80%, sTfR could be used as a screening parameter to determine the iron status in ESRD-HD patients.

TSAT is influenced by inflammation states that are always present in ESRD-HD patients.[11] This study found that sTfR was better than TSAT in diagnosing iron deficiency, because when using TSAT with a cutoff of <20%, the rate of iron deficiency was 33.1%, whereas when using sTfR with a cutoff of 2.5 mg/l, it could identify iron deficiency two times more. In inflammation states, TSAT could be falsely increased leading to misdiagnosis in iron deficiency states.

The median TSAT in this study was 23%, which is lower than the value of 32.7% in the study by Rocha *et al*.[12] This might be due to the longer duration of HD in this study. Although TSAT >20% reflects sufficient iron in the

Table 3: Distribution of participants according to iron parameters

Iron parameter	Iron deficiency		*P*	OR (95% CI)
	Positive	Negative		
Ferritin (µg/l)				
<200	29	27	<0.001	4.79 (2.16-10.64)
≥200	13	58		
sTfR (mg/l)				
≥2.71	35	37	<0.001	6.49 (2.59-16.24)
<2.71	7	48		
sTfR index				
≥1.4	32	25	<0.001	7.68 (3.28-17.96)
<1.4	10	60		

CI=confidence interval, OR=odds ratio, sTfR=soluble transferrin receptor

Table 4: Effect of inflammation state on sTfR, sTfR index, and ferritin

Parameter	CRP >5 mg/dl		CRP <5 mg/dl		*P*
	Median	Range	Median	Range	
sTfR	3.14	1.04-8.48	2.79	1.46-5.41	0.430
sTfR index	1.21	0.29-7.12	1.39	0.49-10.25	0.552
Ferritin	334	6.4-3658	176	3.4-1980	0.010

CRP=C-reactive protein, sTfR=soluble transferrin receptor

circulation according to the Kidney Disease Outcomes Quality Initiative (KDOQI), only 1.6% of the patients were not anemic. However, the median sTfR was 3.0 mg/l, which better reflected the anemic state (sTfR ≥2.71 mg/l) in this study. This study also showed that the sTfR level and sTfR index were higher in the absolute iron deficiency group than in the subjects with functional iron deficiency and no deficiency. Gupta *et al*.[10] also found that the sTfR level and sTfR index were significantly higher in CKD patients with iron depletion than in those without iron deficiency anemia. These findings indicate that the sTfR level and sTfR index can reflect iron deficiency in the CKD population.

In the group with only ESA therapy and in the group without ESA/iron therapy, the sTfR level and sTfR index were higher when compared with ESA + iron therapy group and only iron therapy group. These results indicate that ESA therapy is not effective in the condition of iron deficiency. Although ESA therapy was administered to most of our patients, the proportion of anemia was still high. Therefore, detection of iron deficiency anemia is very important in CKD patients, and the sTfR level and sTfR index can be used as iron markers. With regard to grouping according to iron deficiency, it was found that more severe iron deficiency was associated with a higher sTfR level and sTfR index, which is similar to the findings in the study by Gupta *et al*.[10] On evaluating the ferritin level, sTfR level, and sTfR index, the best parameter to predict iron deficiency was found to be the sTfR index, followed by the sTfR level and ferritin level. This result is consistent with the finding in a previous study that the sTfR index was better than sTfR alone.[6]

Among all participants, only a minority received iron therapy; however, the majority received ESA therapy. Compared with the study by Rocha *et al*.,[12] a larger proportion of patients received iron therapy (48% vs. 11.8%) and almost the same proportion of patients received ESA therapy (87% vs. 84.3%). In this study, the mean hemoglobin level was 9.5 g/dl, which is lower than the mean hemoglobin level of 11.3 g/dl reported by Rocha *et al*.[12] This might be attributed to more subjects receiving iron therapy and undergoing a shorter duration of HD in Rocha *et al*.'s study compared to this study. Anemia was noted in 98.4% of participants in this study. The third National Health and Nutrition Examination Survey stated that more than 70% of ESRD-HD patients had anemia.[13,14] The prevalence of anemia was high in this study, although most of the participants (84.3%) received ESA therapy. The reason might be iron deficiency, ESA resistance, or subclinical inflammation that causes an increase in the hepcidin level. Hepcidin can decrease iron absorption and iron release from the reticuloendothelial system.[13,14]

The present study has several limitations. The inflammatory status or hepcidin level was not assessed. This study also reported that no correlation was observed between sTfR and CRP (P = 0.43), like previous study.[11] Moreover, patients with occult bleeding were not excluded. This study also did not use iron-stained bone marrow aspiration as the gold standard; instead, TSAT was used as the gold standard for determining iron status. However, inflammation could influence the results of TSAT. This study also did not assess the response of iron therapy. More studies are needed to define the possible position of sTfR in the diagnostic flowchart of iron deficiency anemia in ESRD-HD patients, with bone marrow iron staining considered as the gold standard.

In conclusion, detection of iron deficiency is important in ESRD-HD patients. sTfR might be useful as a screening parameter to determine the iron status in HD patients with a cutoff of 2.71 mg/l. It is expected that appropriate iron therapy will make ESA therapy more effective, which will help overcome anemia, and this will improve the quality of life of ESRD-HD patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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