Soluble Transferrin Receptor and Soluble Transferrin Receptor-Ferritin Index for Evaluation of The Iron Deficiency in Cirrhotic Patients

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ABSTRACT

• ***Objective:*** *Iron delivery to erythroblast is mediated by the interaction of plasma transferrin with the cell surface transferrin receptors. Measurement of serum soluble transferrin receptor (sTfR) has been introduced as a promising new index for iron depletion.*

Our aim is to evaluate the value of sTfR concentration and soluble transferrin receptor-ferritin index (TfFI) in the diagnosis of iron deficiency anemia (IDA) in cirrhotic patients.

• ***Material and Method:*** *Thirty-five Grade A (Child Pugh) cirrhotic patients and 29 healthy subjects were enrolled into this study. Complete blood cell count and biochemical analysis including sTfR were performed in all patients and control subjects. Transferrin saturation (TS) was calculated by using the formula 100x serum iron concentration/total iron binding capacity, and TfFI was estimated with sTfR concentration/log ferritin. IDA was diagnosed when TS was below 15% together with a concordant peripheral blood smear.*

• ***Results:*** *IDA group had higher sTfR and TfFI levels when compared to cirrhotic patients with anemia of chronic disease (ACD) (sTfR; 7.77±4.00 vs 3.70±0.98 mg/l, p<0.001, z=3.495, TfFI; 5.98±3.14 vs 1.59±0.48 g/l, p<0.001, z=3.550) and healthy subjects (sTfR; 7.77±4.00 vs 2.99±0.78 mg/l, p<0.001, z=4.503, TfFI; 5.98±3.14 vs 1.87±0.78 g/l, p<0.001, z=3.699). Patients in IDA group had lower serum ferritin levels when compared to ACD group (93±64 vs 290±122, p=0.002, z=-3.051). Sensitivity, specificity and diagnostic values of sTfR and TfFI were 90% vs 90%, 87% vs 94% and 88 vs 92 respectively.*

• ***Conclusion:*** *Serum sTfR and TfFI may be used together to distinguish the iron deficiency state in cirrhotic patients.*

• ***Key Words:*** *Transferrin receptor, ferritin, iron-deficiency anemia, liver cirrhosis.* ***Nobel Med 2009; 5(1): 19-23***

ÖZET

SİROTİK HASTALARDA DEMİR EKSİKLİĞİNİN DEĞERLENDİRİLMESİNDE SOLUBL TRANSFERRİN RESEPTÖRÜ VE SOLUBL TRANSFERRİN RESEPTÖR-FERRİTİN İNDEKSİNİN YERİ

• **Amaç:** Demirin eritroblastlara ulaştırılabilmesi plazma transferrinin hücre yüzeyindeki transferrin reseptörlerine bağlanmasıyla gerçekleşir. Son zamanlarda serum solubl transferrin reseptörü düzeyi (sTfR), demir eksikliği tanısı koymada gelecek vaat eden bir inceleme olarak karşımıza çıkmaktadır. Bu çalışmada sirotik hastalarda demir eksikliği anemisi (DEA) tanısında sTfR ve solubl transferrin reseptör-ferritin indeksi (TfFI) düzeylerinin önemi araştırılacaktır.

• **Materyal ve Metod:** Hepsi Child A (Child-Pugh sınıflandırması) evresinde olan 35 sirotik hasta ile 29 sağlıklı birey çalışmaya alındı. Hasta ve kontrollere tam kan sayımı ile sTfR düzeylerini de içeren biyokimyasal analiz yapıldı. Transferrin saturasyonunun (TS) hesaplanmasında 100x serum demir düzeyi/total demir bağlama kapasitesi formülü kullanıldı. sTfR düzeyi/log ferritin ile TfFI hesaplandı. TS <%15 bulunan hastalarda beraberinde periferik yaymada uyumlu bulgular varlığında DEA tanısı konuldu.

• **Bulgular:** DEA grubunda sTfR ve TfFI düzeyleri kronik hastalık anemisi (KHA) olan sirotik hastalara (sTfR; 7,77±4,00 vs 3,70±0,98 mg/l, p<0,001, z=3,495, TfFI; 5,98±3,14 vs 1,59±0,48 g/l, p<0,001, z=3,550) ve sağlıklı kontrollere (sTfR; 7,77±4,00 vs 2,99±0,78 mg/l, p<0,001, z=4,503, TfFI; 5,98±3,14 vs 1,87±0,78 g/l, p<0,001, z=3,699) kıyasla daha yüksek bulundu. DEA grubunda serum ferritin düzeyleri KHA grubuna kıyasla daha düşük bulundu (93±64 vs 290±122, p=0,002, z=-3,051).

Sirotik hastalarda demir eksikliği tanısı koymada sTfR ve TfFI'in sensitivite, spesifisite ve tanı değerleri sırasıyla %90 vs %90, %87 vs %94 and 88 vs 92 bulundu.

• **Sonuç:** Karaciğer sirozu olan hastalarda demir eksikliği varlığının gösterilmesinde serum sTfR ve TfFI düzeylerinin ölçümünden yararlanılabilir.

• **Anahtar Kelimeler:** Transferin reseptörü, ferritin, demir eksikliği anemisi, karaciğer sirozu. **Nobel Med 2009; 5(1): 19-23**

INTRODUCTION

The transferrin receptor (TfR) is a cellular transmembranous protein found especially on cells of reticuloendothelial system (RES), cells requiring large amounts of iron such as erythroblasts and placenta. Iron delivery to erythroblast is mediated by the interaction of plasma transferrin with the cell surface transferrin receptors.[1] A soluble form of TfR, which is derived from the proteolytic cleavage of the extracellular segment, has been demonstrated in serum.[2] In recent years, the soluble serum transferrin receptor (sTfR) has been introduced as a promising new index for iron depletion. sTfR was shown to assess erythropoietic activity[3] and functional iron deficiency[4] which was used to distinguish iron deficiency anemia (IDA) from anemia of chronic diseases (ACD).[5] Ferritin is another protein that participates in iron metabolism and reflects body iron stores. However, any inflammation can activate the ferritin gene through cytokine-mediated pathway causing hyperferritinemia.[6]

Previous studies have shown that conventional tests such as mean corpuscular volume and serum ferritin used in the diagnosis of IDA are effected by hepatic cell injury in cirrhosis.[7] Although bone marrow aspiration remains the most definitive investigational tool for verifying IDA, less invasive tests with high diagnostic accuracy in predicting iron deficiency are needed.

In this study, we aimed to evaluate the diagnostic values of sTfR concentration, soluble transferrin receptor-ferritin index (TfFI), ferritin level and other related parameters for the differentiation of ACD from IDA in patients with cirrhosis.

MATERIAL and METHOD

Thirty-five patients with cirrhosis (19 women and 16 men) and 29 healthy subjects (13 women and 16 men) were enrolled into this study. Cirrhotic patients were further divided into 3 subgroups: 10 with IDA, 15 with ACD and 10 were non-anemic cirrhotic patients (NACP). Patients with anemia due to a hematological malignancy, hemolytic anemia, vitamin B12 or folate deficiency and those received iron supplement were excluded. The diagnosis of cirrhosis was based on the characteristic clinical and →

laboratory features of liver failure such as jaundice, ascites, encephalopathy, low serum albumin, elevated serum transaminase levels, prolonged coagulation tests, ultrasonographic findings, upper gastrointestinal endoscopy indicating esophageal varices, and/or liver biopsy. Patients with hepatocellular carcinoma or any liver mass suggestive of malignancy, concomitant renal impairment, thalassemia and other hematological disorders and those received blood transfusion, haematinics (iron, folate, vitamin B12) or had a history of active gastrointestinal bleeding in the previous month were excluded.

Anemia was defined as a blood hemoglobin level (Hgb) <130 g/l in men and <120 g/l in women. IDA was diagnosed when transferrin saturation % (TS) was <15% together with positive blood smear.[8] Anemia of chronic diseases was diagnosed when there is mild to moderate anemia in association with infections and chronic inflammatory or neoplastic diseases that persisted for more than a month with no evidence of IDA or other causes of anemia.[9] Hgb, mean corpuscular volume (MCV), red blood cell count (RBC), RBC distribution width (RDW) were determined using Sysmex SE 9000 Automated Analyzer. Serum iron concentration (SIC), total iron binding capacity (TIBC) and serum ferritin were measured with E170 Autoanalyzer.

Serum transferrin, sTfR and C-reactive protein (CRP) concentrations were measured by Cobas Integra 400 Analyzer. Reference values of our laboratory were 50-175 g/100ml for SIC, 250-410 g/100ml for TIBC, <5 mg/liter for CRP and 30-400 ng/ml (men) and 13-150 ng/ml (women) for ferritin. TS was calculated using the formula TS%=100x(SIC/TIBC) and TfFI was estimated with sTfR concentration/log ferritin. Statistical analyses were performed by using unpaired student t-test, Mann Whitney's U-test and Chi-square test. Pearson test was used for correlation analyses. Receiver operation characteristic (ROC) curve analysis was used to assess the diagnostic values of variables in the determination of IDA. Informed consents of the patients were taken before the study, and this study was approved by the local institutional ethics committee.

Table 1: Laboratory tests reflecting the iron status of the patients and the healthy subjects in the control group.

	IDA (n=10)	ACD (n=15)	NACP (n=10)	Control (n=29)
Male/Female	5/5	7/8	4/6	16/13
Age (years)	56±17	55±11	57±18	48±7
Hgb (g/liter)	97.1±22.5	109.3±13.6	142.7±12.3	143.6±8.6
SIC (µg/100ml)	25±9	123±48	82±31	78±24
MCV (fl)	84±10	96±8	89±4	88±3
sTfR (mg/l)	7.77±4.00	3.70±0.98	4.48±1.78	2.99±0.78
Transferrin (g/l)	2.10±0.60	1.74±0.46	2.16±0.97	2.84±0.53
TS (%)	12±5	41±25	28±10	30±10
TfFI	5.98±3.14	1.59±0.48	2.14±0.64	1.87±0.78
RDW (%)	17.2±1.6	15.5±1.6	14.3±1.1	14.1±0.6
CRP (mg/l)	8.32±14.08	33.85±22.65	8.51±3.62	2.64±1.22
Ferritin (ng/ml)	93±64	290±122	366±190	88±42

IDA: Iron deficiency anemia, ACD: Anemia of chronic disease NACP: Non-anemic cirrhotic patients index STfR: Soluble transferrin receptor, SIC: Serum iron concentration, TS: Transferrin saturation, TfFI: Soluble transferrin receptor-ferritin, RDW: red cell distribution width

Table 2: Mann-Whitney U Test for comparing parametric variables between groups.

Variable	IDA/Control	IDA/ACD	IDA/NACP
Age	NS	NS	NS
Hgb	<0.001 t= - 6.377	NS	<0.001 t= -5.615
SIC	<0.001 t= -9.853	<0.001 t= -7.683	<0.001 t= -5.234
MCV	NS	0.002 t= -3.522	NS
Ferritin	NS	0.002 z= -3.051	NS
sTfR	<0.001 z= 4.503	<0.001 z= 3.495	0.009 z= 2.609
Transferrin	0.001 t= -3.679	NS	NS
TS (%)	<0.001 t= -5.118	<0.001 t= -7.065	0.001 t= -3.784
sTfFI	<0.001 z= 3.699	<0.001 z= 3.550	0.016 z= 2.419
CRP	NS	0.006 z= -2.740	NS

SIC: Serum iron concentration, IDA: iron deficiency anemia, NACP: Non-anemic cirrhotic patients NS: Non-significant, ACD: Anemia of chronic disease, sTfR: Soluble transferrin receptor, TS: Transferrin saturation, sTfFI: soluble transferrin receptor-ferritin index

Table 3: Chi-Square test for comparing diagnostic value of serum ferritin between IDA and ACD groups.

Groups	Ferritin		Total
	Low* (%)	Normal/High (%)	
IDA	1 (10)	9 (90)	10
ACD	0 (0)	15 (100)	15

*<30 ng/ml for men and <13 ng/ml for women, IDA: Iron deficiency anemia, ACD: Anemia of chronic disease, p=0.211, c^2=1.563.

Table 4: Sensitivity, specificity, positive predictive and diagnostic values of several parameters in the diagnosis of iron deficiency anemia in cirrhosis

	Sensitivity (%)	Specificity (%)	Positive Predictive value	Diagnostic value
Serum transferrin	80	53	0.50	64
Serum ferritin	13	95	0.67	60
MCV	70	73	0.64	72
sTfR	90	87	0.82	88
TfFI	90	94	0.90	92

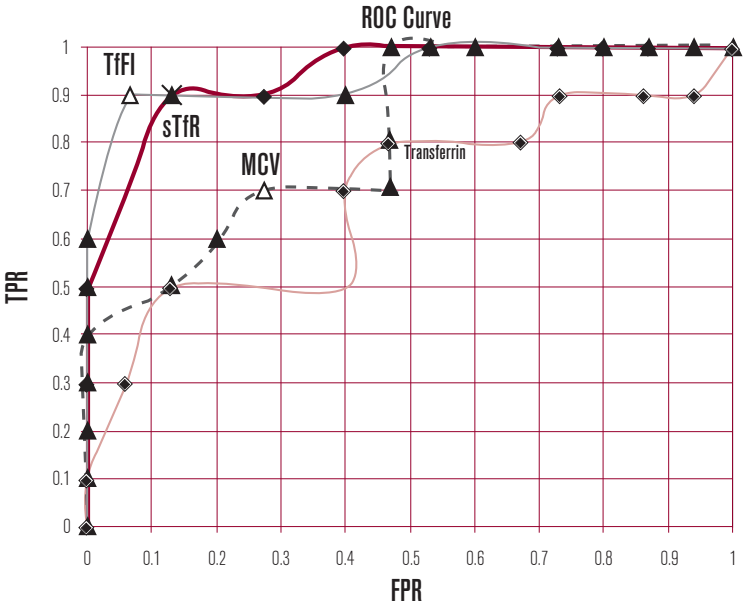

Figure. Receiver operation characteristic (ROC) curve analysis in the assessment of diagnostic values of soluble transferrin receptor-ferritin index (TfFI), transferrin, erythrocyte mean corpuscular volume (MCV) and soluble transferrin receptor (sTfR) for iron deficiency anemia in cirrhotic liver disease (FPR, false positive results; TPR, true positive results).

RESULTS

Median age of the cirrhotic patients was 60 (25-78) and was 48 (20-61) in control group. IDA group had lower blood Hgb and SIC when compared to both NACP (Hgb: 97.1±22.5 vs 142.7±12.3 g/l, p<0.001, t=-5.615, SIC: 25±9 vs 82±31 g/100ml, p<0.001, t=-5.234) and healthy subjects (Hgb: 97.1±22.5 vs 143.6±8.6 g/l, p<0.001, t=-6.377, SIC: 25±9 vs 78±24 g/100ml p<0.001, t=-9.853), however no difference was found between these groups when MCV and serum ferritin levels were taken into consideration (Table 1, Table 2). No statistical difference was found between blood Hgb and serum transferrin levels of IDA and ACD groups, while IDA group had lower MCV levels (p=0.002, t=-3.522). Serum transferrin concentrations of patients in IDA group were found decreased significantly when compared with healthy controls (IDA: 2.10±0.60 g/l, healthy group: 2.84±0.53 g/l, p=0.001, t=-3.679). IDA group had higher TfFI and serum sTfR levels when compared to ACD, NACP and controls (IDA vs ACD: sTfR; 7.77±4.00 vs 3.70±0.98 mg/l, p<0.001, z=3.495, TfFI; 5.98±3.14 vs 1.59±0.48, p<0.001, z=3.550, IDA vs NACP: sTfR; 7.77±4.00 vs 4.48±1.78 mg/l, p=0.009, z=2.604, TfFI: 5.98±3.14 vs 2.14±0.64, p=0.016, z=2.419 and IDA vs control group: sTfR; 7.77±4.00 vs 2.99±0.78 mg/l, p<0.001, z=4.503, TfFI: 5.98±3.14 vs 1.87±0.78, p<0.001, z=3.699) (Table 1, Table 2). No significant difference was found between the serum ferritin levels of IDA and ACD groups (p=0.211, c^2=1.563) (Table 3). ACD group had higher serum CRP levels when compared to IDA group (33.85±22.65 vs 8.32±14.08 mg/l, p=0.006, z=2.740).

Serum sTfR and TfFI of the whole study population were found well correlated with their TS levels (p=0.044, r=-0.255; p=0.009, r=-0.327). A significant positive correlation was also found between sTfR and TfFI (p<0.001, r=0.686). Serum ferritin did not show any significant correlation with sTfR and TfFI. Cut-off point for sTfR was defined as 4.7 mg/l (sensitivity: 90%, specificity: 87%, positive predictive value: 0.82, negative predictive value: 0.93 and diagnostic value: 88) (Fig.). sTfR was found negatively correlated with serum transferrin (p=0.003 r=-0.365). Sensitivity, specificity, positive predictive value and diagnostic value of serum transferrin for IDA were; 80%, 53%, 0.50 and 64 (cut-off point: 1.8 g/l) which were 70%, 73%, 0.64 and 72 for MCV (cut-off point: 89.8 fL) and 90%, 94%, 0.90 and 92 for TfFI (Table 4). TfFI was shown to have best diagnostic value according to ROC curve analysis (MCV: Area under curve; 0.803±0.088, p=0.012, 95% CI=0.631-0.976; TfFI: Area under curve; 0.927±0.58, p<0.001, 95% CI=0.814-1.040; Transferrin: Area under curve; 0.700±0.114, p=NS, 95% CI=0.477-0.923; sTfR: Area under curve; 0.920±0.054, p<0.001, 95% CI=0.814-1.026 (Fig.).

DISCUSSION

Anemia is one of the major findings in cirrhotic patients that causes fatigue and deterioration in the functional capacity. Anemia in cirrhosis was related with iron deficiency due to the gastrointestinal blood loss from peptic ulcers and esophageal varices, increased plasma volume, hemolysis due to splenic sequestration, impaired iron utilization, folate deficiency and ACD.[10] It may be difficult to distinguish IDA from ACD in these patients. Decreased red blood cell life and inhibitory effects of interleukin-1 and tumor necrosis factor on erythroid precursors has been shown before in vitro studies.[11] Erythropoietin production in response to anemia is defective.[12]

Serum ferritin level can be increased in chronic liver diseases due to decreased clearance and/or increased release from damaged liver tissue. Therefore it can not reflect the iron stores of the body in those patients[7] as in our study where IDA and ACD groups did not differ when serum ferritin levels of the patients were taken into consideration. Expression of TfR by the reticuloendothelial system is directly proportional to cellular iron demand, and circulating level of sTfR reflects cellular TfRs. Thus sTfR can be used in the diagnosis of IDA.[13, 14]

In this study, sTfR levels of the patients in IDA group →

were found increased when compared with the others. However, elevated serum sTfR was also reported in ACD in other studies those suggested that sTfR is not superior to the serum ferritin in distinguishing IDA from ACD.[15, 16] As body iron stores decrease in IDA, both serum ferritin and sTfR levels undergo a characteristic sequence of changes.[17] During depletion phase of body iron stores, serum sTfR concentration remains stable while ferritin level shows a progressive decrease. Decreased hemoglobin synthesis after depletion of the functional iron compartment results in elevated serum sTfR. Then serum sTfR level reflects functional iron.[4] These two values can be combined into a ratio, which is reciprocally regulated sTfR/ferritin or sTfR/log ferritin index. This ratio has already been used in differential diagnosis of IDA.[4, 18, 19] In our study, both sTfR and TfFI were found higher in IDA group when compared to ACD, non-anemic cirrhotic and healthy control groups (Table 1 and 2).

Folic acid deficiency and/or deposition of lipoproteins on erythrocyte membranes results in normal or increased MCV in cirrhosis.[20] Although the difference between the mean MCV levels of the patients in IDA and ACD groups were statistically significant, sensitivity, specificity, positive predictive value and the diagnostic values were low when compared to sTfR and TfFI. Indeed, ROC curve analysis indicated that the sTfR and TfFI provided the highest diagnostic accuracy for discriminating IDA from ACD (Fig.).

Limitations to this study were the absence of bone marrow biopsy and influence of cirrhosis on the serum transferrin levels of the patients. Bone marrow biopsy could best reflect the bone marrow iron stores of our patients, however it would be highly invasive for the evaluation of iron deficiency. Therefore IDA was assessed according to serum TS and peripheral blood smear. Chronic liver diseases can alter transferrin synthesis that will resulted in low serum transferrin. In our study all of the patients had stage A cirrhosis according to Child Pugh classification and difference between serum transferrin levels of the ACD and IDA groups was not statistically significant.

In conclusion, serum sTfR and TfFI may be used together to distinguish the iron deficiency state in cirrhotic patients. Treatment of the iron deficiency state in those patients will improve the functional capacity and quality of life. However, such a treatment with false positive diagnosis will be useless or even can give harm to the hepatocytes.

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