

Serum Transferrin Receptor Assay in the Diagnosis of Iron Deficiency Anemia in Children

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Abstract: The present aimed to assess the utility of serum transferrin receptor (sTfR) and sTfR -ferritin index in diagnosing iron deficiency anemia (IDA) in children. Ninety children \leq 12 years of age with anemia (6-59 months; Hb < 11g/dl and 5-12 years Hb < 11.5g/dl) were recruited. Serum iron, total iron binding capacity, percent transferrin saturation (%TS) and serum ferritin were measured in all patients. Sixty-two children with IDA (SF < 15 μ g/l and/or %TS < 16) were included in the study. Thirty-five controls were also studied (non anemic children without ID). sTfR was measured (ELISA) in patients and controls and ROC curve made. sTfR was significantly ($p < 0.001$) higher in patients ($6.0 \pm 2.9 \mu$ g/ml) as compared to controls ($1.2 \pm 0.6 \mu$ g/ml). sTfR-F index showed a similar trend. The area under the ROC curve for sTfR was 0.985 indicating good diagnostic ability. The sensitivity and specificity of sTfR $\geq 2.25 \mu$ g/ml for diagnosis of IDA was 90.3% and 94.1% respectively. sTfR and sTfR-ferritin index are useful tests for the diagnosis of IDA.

Keywords: anemia, iron deficiency, transferrin receptor.

INTRODUCTION

Iron deficiency (ID) is the most common nutritional deficiency in the world. ID and iron deficiency anemia (IDA) affect more than 3.5 billion people in developing countries [1]. Pregnant women (PW) and children are particularly susceptible to deficiency of iron. A compilation of global data from several large studies, revealed that 50% of anemia in women and children is attributable to deficiency of iron [2]. With its high prevalence in India, it remains a severe public health problem for preschool children, PW and non pregnant women [3].

Iron deficient pre-school children suffer from delayed growth and neurologic development, behavioral disturbances, cognitive dysfunction and increased susceptibility to infection. Some of these effects are not reversed entirely even after iron therapy and correction of anemia [4]. Early diagnosis and prompt treatment of IDA may help ameliorate some of these ill effects.

The conventional laboratory tests of iron status which are widely used in clinical practice include serum iron (SI), total iron binding capacity (TIBC), percent transferrin saturation (%TS) and serum ferritin (SF). These tests are influenced by acute phase responses thus making their interpretation difficult [5]. Absence of stainable iron in the marrow is the gold standard for diagnosis of IDA but is invasive and painful. There is thus a need for a non invasive and sensitive test which can be done on peripheral blood for the detection of ID.

Transferrin receptor (TfR) is a transmembrane protein which mediates the uptake of iron by

erythroblasts. Virtually all cells have TfR on their surface but almost 80% of these receptors are in the erythroid marrow. Iron deprivation results in prompt induction of receptor synthesis [5]. A truncated form of this receptor is present in plasma (sTfR), its concentration being proportional to the total cell mass of TfR [5]. Assays for sTfR are now available commercially and have been evaluated in some studies for the diagnosis of iron depletion, most of which have included adult patients [5-7]. Combining sTfR and logarithmic transformation of ferritin in the form of TfR/log ferritin ratio (TfR-F index) provides a good indicator of ID [5]. This study aimed to evaluate the utility of sTfR and TfR-F index as a diagnostic test for IDA in children. Area under the receiver operating characteristic (ROC) curve was used to determine a cut-off point of sTfR that could be used as a predictor of IDA as different cut-off values have been suggested by various authors.

METHODS

After obtaining informed consent from parents/guardians, ninety children ≤ 12years of age with anemia were recruited from the Department of Pediatrics. Anemia was defined as Hb<11g/dl in children 6-59months and<11.5g/dl in children between 5-12years [8]. Iron parameters including SI [9], TIBC [10], %TS and SF (ELISA) were measured in all patients. IDA was defined as SF<15µg/l and/or %TS<16% [11]. Sixty-two children with IDA were finally included in the study. Thirty-five non anemic children with no evidence of IDA were included as controls. The study received clearance from the Institutional Ethics Committee for human research. sTfR was measured by ELISA in all patients and controls using commercially kits (Biovendor). High sensitivity CRP (hs-CRP) was estimated using commercially available ELISA kits and values<6mg/dl were considered as normal. Complete blood counts were performed on an automated hematology analyzer LH500and a stained peripheral blood film (Wright stain) was examined in all patients and controls.

RESULTS

The age of patients ranged from 6m-12y with a Mean± SD of 3.4±3.3 years with majority (83.9%) of patients being between 6m-4y. The study comprised of 34(54.8%) males and 28(45.2%) females.

Complete blood counts

Table-1 shows the complete blood counts of patients and controls.

Anemia was mild, moderate and severe [12] in 46.8%, 43.5% and 9.7% patients respectively. Anemia

was not observed in any of the controls. MCV, MCH and MCHC were significantly (p<0.001) lower while platelet count and RDW were significantly (p<0.001) higher in patients as compared to controls. Examination of a stained PBF revealed microcytic, hypochromic blood picture in all patients.

Parameters of iron status

Biochemical parameters of iron status are shown in Table-2. Serum iron, %TS and SF were significantly (p<0.001) lower in patients as compared to controls and were reduced (SI<60 µg/dl, %TS<16, SF<15 µg/L) in all patients, being normal in the controls. An elevated (>400µg/dl) TIBC was observed in 45.2% patients.

sTfR level was significantly (p<0.001) higher in patients (6.0 ± 2.9 µg/ml) as compared to controls (1.2 ± 0.6 µg/ml) and was elevated(>1.7 µg/ml) in 59/62(95.1%) patients and 4(11.4%) controls. A ROC curve was constructed for sTfR which showed the AUC to be 0.985. sTfR ≥ 2.25 µg/ml had a sensitivity of 90.3% and a specificity of 94.1% for the diagnosis of IDA.(Figure1)sTfR-F index was significantly(p<0.001) higher in patients (84.5±87.6)as compared to controls(0.7±0.4).

sTfR showed a significant (p<0.001) positive correlation with TfR-F index and a negative correlation with Hb, SF, SI and %TS.

Hs-CRP was normal (<6mg/L) in all patients and controls.

Table-1: Complete blood counts of patients and controls

Parameter	Patients	Controls
Hb (g/dl)	7.7±1.7	12.7±0.9*
RBC(x10 ¹² /L)	4.64±0.88	4.70±4.7
MCV(fl)	59.1±7.9	82.9±10.2*
MCH(pg)	16.9±3.1	26.5±4.1*
MCHC(g/dl)	28.3±2.2	31.6±1.2*
TLC(x10 ⁹ /L)	8.8±2.2	7.9±1.6
Platelet count(x10 ⁹ /L)	387±159	274±72*
RDW(%)	18.4±2.4	12.4±1.7*

*p<0.001

Table-2: Biochemical parameters of iron status in patients and controls

Parameter	Patients	Controls
Serum iron (µg/dl)	28.8±9.8	90.9±19.7*
TIBC (µg/dl)	389.1±44.6	328.3±65.2*
%TS	7.5±2.6	28.1±7.8*
SF(µg/L)	2.7±2.3	39.1±9.8*
sTfR (µg/ml)	6.0±2.9	1.1±0.6*
sTfR-F index	84.5±87.6	0.7±0.4*

*p<0.001

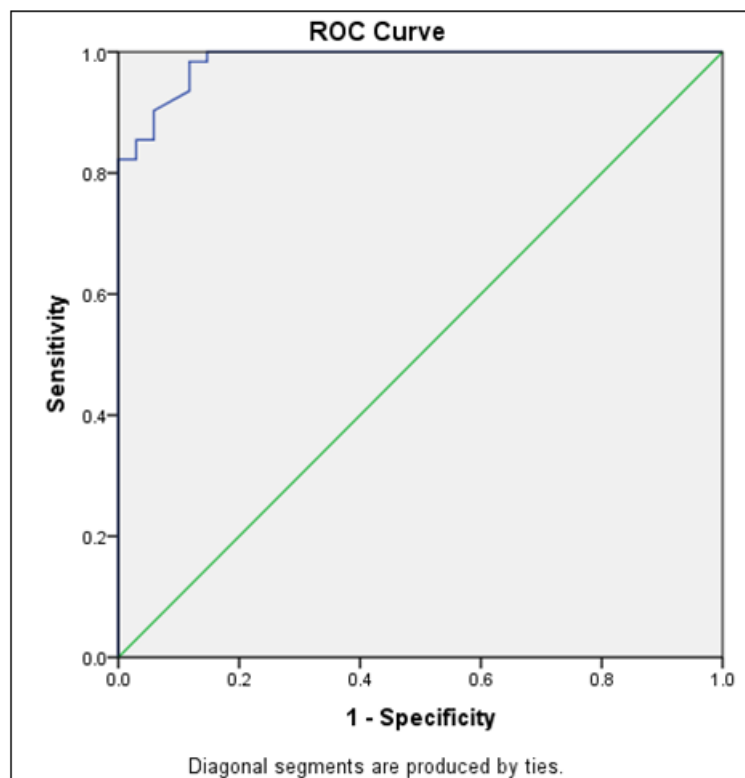


Fig-1: ROC curve for sTfR

DISCUSSION

IDA accounts for 50% of all cases of anemia and is particularly common in children [3]. Considering its magnitude, deficiency of iron significantly affects the quality of life of millions and also leads to changes, many of which are irreversible even after iron therapy [4]. Early diagnosis and prompt treatment of ID and IDA will help prevent these ill-effects.

The diagnosis of iron deficiency is traditionally based on a panel of tests including SI, TIBC, %TS and SF. SI and TIBC are subject to diurnal variation, fluctuation with dietary intake and alteration by co-existing inflammation [13]. SF, a measure of iron stores is an acute phase reactant and the levels rise with active infection/inflammation. The sensitivity of ferritin is hence low [14]. However, due to its high specificity it remains a commonly used test for the diagnosis of IDA. As staining of bone marrow for iron is invasive and causes inconvenience to the patient [5], there is need for a peripheral blood test which is sensitive for diagnosis of IDA. The utility of sTfR concentration as a diagnostic test for IDA remains an area of active research as the receptor levels are not affected by concurrent infection/inflammation.

A truncated version of the cellular transferrin receptor is present in serum (sTfR) [5]. As the receptor levels are affected by the rate of erythropoiesis, they are used to indicate depleted iron stores only when no other cause of abnormal erythropoiesis is demonstrated [12]. Combining sTfR with SF as TfR-F index increases the

sensitivity and specificity for diagnosing ID [15]. This study evaluated the usefulness of serum TfR and the ratio of sTfR/ferritin as a diagnostic test for IDA in children. We used area under the receiver operating characteristic (ROC) curve to determine a cut-off point of sTfR that could be used as a predictor of IDA.

A significantly ($p < 0.001$) higher level of sTfR was seen in children with IDA (6.0 ± 2.9 $\mu\text{g/ml}$) as compared to controls (1.1 ± 0.6 $\mu\text{g/ml}$). An elevated (> 1.7 $\mu\text{g/ml}$) sTfR was seen in 59/62 (95.1%) patients and 4 (11.4%) controls. As the supply of iron to tissues decreases, the expression of tissue receptor increases.⁵Elevation in sTfR is reported to be in proportion to deficit of iron in tissues and indicates the iron demand for erythropoiesis [14]. Punnonen *et al.*, studied 129 anemic patients and observed an elevated sTfR level in most patients with IDA. sTfR showed a significant ($p < 0.001$) positive correlation with TfR-F index and a significant ($p < 0.001$) negative correlation with hemoglobin.⁵Similar results were reported in a study on school children with prelatent ID, latent ID and overt IDA. The authors concluded that sTfR provides an alternative to the traditional biochemical panel for the diagnosis of ID and IDA [13]. sTfR and TfR-F index are reported to be stronger diagnostic indicators of ID than SF alone as was also seen in this study [16].

ROC curves prepared for sTfR as a diagnostic test for IDA revealed the AUC to be 0.985 indicating it to be a good indicator of ID. These observations are

similar to those of other authors who concluded that sTfR measurements effectively identify ID even in the presence of an accompanying inflammatory/infectious condition [5, 14, 17].

sTfR \geq 2.25 μ g/ml had a sensitivity of 90.3% and a specificity of 94.1% for the diagnosis of IDA. The cut off point for diagnosis of IDA is variably reported by various authors [5, 18, 19].

The significant negative correlation of sTfR and TfR-F index with Hb, SF, SI and %TS suggests that the use of these tests can improve the diagnosis of IDA.

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