Value of Soluble Transferrin Receptors and sTfR/log Ferritin in the Diagnosis of Iron Deficiency Accompanied by Acute Infection

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Abstract There are many causes of anemia; the most common of these are acute and chronic infections, iron deficiency, or both. Identifying the cause is a very important step in management of anemia. So, we evaluated the usefulness of soluble transferrin receptor (sTfR) and of the sTfR/log ferritin in the diagnosis of iron deficiency anemia accompanied by acute infection. This study was conducted on 131 children aged 2–11 years old from those who attended the pediatric outpatient clinics in Menoufia university hospital. Hematological indices, iron balance and sTfR were evaluated and the sTfR/log F was calculated for each examined child. From the examined children four groups were distinguished: *Group I* (control): included 34 healthy children with normal iron status (66.7% males, age 4.2 ± 1.2). *Group II* (IDA): included 38 children diagnosed as iron deficiency anemia (47.4% males, age 4.9 ± 1.6). *Group III* (IDA + infection): included 26 children with infectious disease (upper respiratory tract

infection, otitis media, pneumonia, stomatitis, and urinary tract infection) and anemia meeting criteria of IDA (50% males, age 4.2 ± 0.7). *Group IV* (anemia + infection): included 33 children with infectious anemia without iron deficiency (56.2% males, age 5.06 ± 1.4). It was proved that sTfR and sTfR/log Ferritin were significantly higher in children with anemia due to iron deficiency, and in those with infection + iron deficiency, versus those with infectious anemia or in healthy children. The use of sTfR and sTfR/log ferritin improves the diagnosis of IDA in pediatric patients, especially in the presence of coexisting acute infection.

Keywords Anemia · sTfR · sTfR/log ferritin · Infection

Introduction

Anemia is one of the main deficiency diseases in the world and is characterized as a severe public health problem. The World Health Organization (WHO) estimates that anemia primarily affects 1.62 billion people worldwide and that the occurrence of iron deficiency is 2.5-fold higher [1].

Iron deficiency is the most common nutritional deficiency in the world and 15–20% of the entire population has iron deficiency anemia (IDA) [2]. Iron deficiency leads to many non-hematological disturbances which include growth and development, depressed immune function in infants; reduces physical work capacity; decreases the cognitive function in both infants and adolescents [3].

Evaluation of iron status in patients with anemia is based on complex hematological and biochemical assays, including serum iron concentration (Fe), unsaturated iron-binding capacity (UIBC), ferritin concentration (F), and calculating the total iron-binding capacity (TIBC) and

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transferrin saturation index (TSI) [4]. Also, Diagnosis of iron deficiency in infancy could be difficult because iron tests offer information limited to a single compartment and various infectious or inflammatory conditions, frequent in childhood, can alter them regardless of body iron status [5].

Unlike serum iron transferrin and ferritin values, serum transferrin receptor (sTfR) concentration, which has been used recently as a marker of iron status, is unaffected by acute phase reactions. Moreover, it is sensitive marker of body iron status [6].

Soluble transferrin receptor (sTfR) is a truncated extracellular form of the membrane transferrin receptor produced by proteolysis [7]. The parameter defined as TfR-F index is calculated in terms of sTfR/log ferritin and sTfR are good indicators of iron deficiency and could be useful in the differential diagnostics of anemia [8].

Aim of the Study

This study aimed to evaluate the usefulness of serum sTfR concentrations and sTfR/log Ferritin in diagnosis of iron deficiency accompanied by acute infection in children.

Patient and Method

This study was conducted on 131 children aged 2–11 years old from those who attended the pediatric outpatient clinics in Menoufia university hospital.

Written informed consent was obtained from the guardians of the children, and the study was approved by the ethics committees of the Menoufia Faculty of Medicine. The study conducted from 10/2014 till 10/2015.

A detailed history was collected for all study subjects. This was composed of (a) Personal history, including name, age, gender, residence, number of family members; (b) History of the present illness, evident pallor, infection, drug intake, bleeding disorder and/or mental status (abnormal behavior, school performance) and (c) Past history; including nutritional, developmental history, history of parasitic infestations (by asking about infestation by parasites or history of anti-helminthic treatment) and history of chronic diseases (such as, renal failure, cardiac disease and pulmonary diseases).

7 ml of venous blood were withdrawn from each child. 2 ml were transferred into a tube containing EDTA and used for determining the haematological indices [including haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and red cell distribution width (RDW)], whereas the remaining 5 ml were transferred into a plain tube, left to clot, centrifuged for 10 min at 4000 rpm and the serum separated and used for

determining C-reactive protein (CRP), ferritin, iron, total iron binding capacity (TIBC), transferrin and transferrin receptor (sTfR) using enzyme immunoassay. Total iron was determined in serum samples by colorimetric method using Spectrum diagnostics kit from Germany [9]. Total iron binding capacity (TIBC) was determined in serum samples by colorimetric method using Spectrum diagnostics kit from Germany [10]. Serum ferritin concentration was assessed using an enzyme-linked immunosorbent assay kit (Ramco Laboratories, INC., USA) according to the manufacturer's recommendation [11]. Human sTfR was assessed using an enzyme-linked immunosorbent assay kit (BioVendor ELISA kit, USA) according to the manufacturer's recommendation.

The inclusion criteria for healthy children were: normal perinatal period, growth, and development; normal physical exam; absence of hematologic or systemic diseases; neither infectious processes in the previous 2 months nor iron therapy in the previous 6 months.

Children with collagen, renal or liver diseases and children who had received blood transfusion the 3 months prior to the study were excluded from the study.

Statistical Analysis

The results were analyzed statistically using SPSS software (version 20; SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ SD. Statistical analysis was performed using ANOVA test for comparison among the different groups and Pearson correlation for determining the relationship between the variables. Receiver Operating Characteristic curve (ROC curve) analysis is a graph of sensitivity against specificity at different cutoff points. The optimal cutoff point is that gives the highest sensitivity and specificity. Significance was considered at P value <0.05 .

Results

From the history, physical examination and hematological and biochemical assays, children were classified into the following groups:

Group I (control): included 34 healthy children with normal iron status (66.7% males, age 4.2 ± 1.2).

Group II (IDA): included 38 children diagnosed as iron deficiency anemia (47.4% males, age 4.9 ± 1.6).

Group III (IDA + infection): included 26 children with infectious disease (upper respiratory tract infection, otitis media, pneumonia, stomatitis, and urinary tract infection) and anemia meeting criteria of IDA (50% males, age 4.2 ± 0.7).

Group IV (anemia + infection): included 33 children with infectious anemia without iron deficiency (56.2% males, age 5.06 ± 1.4).

In Table 1, the mean values of the hematologic and biochemical iron parameters, sTfR, and indices were presented in the studied groups. There was no significant difference when HB, MCV, MCHC and RDW were compared in groups (II, III and IV) in a general evaluation. CRP was significantly higher in group III and IV when compared with group II. Even though, these values did not facilitate interpretation. In acute infection, while the ferritin level increase, its diagnostic value decrease. In such cases, the high level of sTfR indicates association of iron deficiency in some cases. sTfR values were significantly more elevated in children suffering from IDA than the control group and the association of concomitant infection (IDA + infection) did not affect them. Children having infectious anemia (anemia + infection) their values were similar to the control group. All IDA and (IDA + infection) children had higher values than 1.41 mg/L and none of (anemia + infection) group.

sTfR-F index was very high in children with IDA. In the presence of infection (IDA + infection), sTfR/log ferritin decreased. Although the difference between normal and IDA + I children was significant. Both IDA and (IDA + infection) children had higher values than 1.41.

In the IDA group, a statistically significant relationship was found between both sTfR and the sTfR/log ferritin and

TIBC and Serum ferritin (proportional for TIBC and inversely proportional for serum ferritin) (Table 2).

In order to evaluate the clinical value of serum soluble transferrin receptor concentrations and of the sTfR/log ferritin values to differentiate IDA from (anemia + infection), the sensitivity, specificity and accuracy were studied (Fig. 1).

STFR (mg/l) at cutoff 5.45 has 80.9, 81.8, 63, 91.8% sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) and with 81.6% accuracy for prediction of iron deficiency anemia.

STFR (log) at cutoff 8.7 has 100, 96.4, 91.3, 100% sensitivity, specificity, PPV and NPV and with 97.3% accuracy for prediction of iron deficiency anemia.

Table 3 showing the types of infection in groups III and IV.

Discussion

There is a substantial difficulty in the interpretation of the iron status indices in patients with infection, because of the presence of a continuous inflammatory process, which interferes with the value of some established markers, such as ferritin and transferrin. sTfR seems particularly useful for diagnosis of IDA in patients with infection, inflammation or malignancy where serum ferritin is not a good indicator of iron deficiency [12].

Table 1 Comparison of mean values of laboratory parameters between different groups

Parameter	Group			
	Group I	Group II	Group III	Group IV
Hb (g/dl)	12.2 $\pm$ 2.9	8.8 $\pm$ 0.4 ^a	8.6 $\pm$ 0.4 ^b	8.7 $\pm$ 0.4 ^e
MCV (fL)	79.58 $\pm$ 1.40	67.48 $\pm$ 0.87 ^a	66.52 $\pm$ 1.33 ^b	66.68 $\pm$ 1.34 ^e
MCHC (pg)	27.34 $\pm$ 0.70	21.44 $\pm$ 0.40 ^a	20.00 $\pm$ 0.82 ^b	20.48 $\pm$ 0.74 ^e
RDW (%)	13.62 $\pm$ 1.22	18.18 $\pm$ 0.34 ^a	18.36 $\pm$ 0.69 ^b	18.24 $\pm$ 0.72 ^e
CRP (mg/l)	0.18 $\pm$ 0.48	0.12 $\pm$ 1.40	45.00 $\pm$ 30.98 ^{bc}	48.6 $\pm$ 27.66 ^{ce}
Serum iron (mg/dl)	83.8 $\pm$ 12.8	54.1 $\pm$ 5.2 ^a	54.6 $\pm$ 6.9 ^b	54.6 $\pm$ 2.7 ^e
TIBC (mg/dl)	309.6 $\pm$ 50.7	468.8 $\pm$ 12.2 ^a	378.5 $\pm$ 112.3 ^b	215.5 $\pm$ 49.8 ^e
Serum ferritin (ng/ml)	28.5 $\pm$ 0.6	7.5 $\pm$ 1.4 ^a	34.6 $\pm$ 3.6 ^{bd}	84.6 $\pm$ 42.8 ^{ce}
Transferrin saturation	6.5 $\pm$ 1.3	5.2 $\pm$ 0.6 ^a	17.2 $\pm$ 3.2 ^{bd}	20.1 $\pm$ 2.1 ^{ce}
sTfR (mg/l)	1.8 $\pm$ 0.3	7.7 $\pm$ 2.8 ^a	5.4 $\pm$ 1.9 ^{bd}	1.7 $\pm$ 0.3 ^e
sTfR-F index	0.5 $\pm$ 0.4	12.2 $\pm$ 2.7 ^a	4.1 $\pm$ 2.3 ^{bd}	1.1 $\pm$ 2.4 ^e

Hb hemoglobin, MCV mean corpuscular volume, MCHC mean corpuscular hemoglobin concentration. RDW random distribution width, CRP C-reactive protein, TIBC total iron binding capacity, sTfR soluble transferrin receptor, sTfR-F index soluble transferrin receptor-ferritin index

^a Significant difference between group I&II

^b Significant difference between group I&III

^c Significant difference from group II

^d Significant difference between group III&IV

^e Significant difference between group I&IV

Table 2 Correlation coefficient between sTfR (mg/l), sTfR-F index and different patients parameters

Parameter	Group I		Group II		Group III		Group IV	
	sTfR (mg/l) r	sTfR-F index r	sTfR (mg/l) r	sTfR-F index r	sTfR (mg/l) r	sTfR-F index r	sTfR (mg/l) r	sTfR-F index r
Age	−0.38	0.064	−0.309	0.064	−0.38	0.152	−0.046	0.429
Hb (g/dl)	−0.43	0.146	0.343	0.275	−0.43	0.146	−0.199	−0.006
Serum iron (mg/dl)	0.439	0.326	0.224	0.323	0.439	0.326	0.181	0.059
TIBC (mg/dl)	0.167	−0.058	0.087*	0.033*	0.167	−0.058	−0.086	−0.007
Serum ferritin (ng/ml)	0.553	−0.27	−0.077*	−0.052*	0.553	−0.273	−0.274	0.018
Transferin saturation	0.37	0.347	0.321	−0.116	0.37	0.347	0.35	0.376

r Spearman correlation

sTfR soluble transferrin receptor, *sTfR-F index* soluble transferrin receptor-ferritin index

* Significant correlation

Fig. 1 ROC curve (receiver operating curve) for prediction of iron deficiency anemia using STFR (mg/l) and sTfR-F index shows AUC (area under the curve) 0.913 and 0.986 respectively. *sTfR* soluble transferrin receptor, *sTfR-F index* soluble transferrin receptor-ferritin index

Table 3 Types of infection in groups III and IV

Parameter	Group	
	Group III	Group IV
Upper respiratory tract infection	10 (38.5%)	13 (39.4%)
Otitis media	5 (19.3%)	4 (12.1%)
Pneumonia	6 (23%)	8 (24.2%)
Stomatitis	2 (7.7%)	3 (9.2%)
Urinary tract infection	3 (11.5%)	5 (15.1%)

In children, it has some advantages which make it a potentially useful parameter: it is sensitive [13] and specific [14] and it shows little biological variability [15].

The aim of this work was to evaluate the usefulness of serum sTfR concentrations and sTfR/log F in diagnosis of iron deficiency accompanied by acute infection in children.

In the procedure of IDA diagnosing, we used the standard analyses which include the complete blood count, as well as additional biochemical analyses. We obtained significantly lower values of Hb, MCV, MCHC, iron and ferritin in group II, III and IV compared to the control group and significantly higher value of TIBC.

All these parameters were not sufficient to establish an IDA diagnosis in each individual group. Even with the added ferritin, that was significantly decreased in group II, III and IV, with no significant difference among the three different anemic groups. These results come in line with Marković et al. [16]. Also, Skikne et al. [17], found that, using ferritin alone for differential diagnosis of anemia would have resulted in approximately half of the patients misclassified.

Olivers et al. [18] said that, in the presence of infection, ferritin is elevated and, by acting as an acute phase reactant, loses sensitivity as a marker of iron status.

The sTfR and sTfR/log ferritin were useful in differentiating IDA in this study as a significant increase in sTfR and sTfR/log ferritin values in both of IDA group and mixed group when compared with both of pure infectious anemia and control groups, this comes in line with Marković et al. [16], who found that, most of the patients in the infectious anemia group had sTfR concentrations within the reference limits, unaffected by infection. This result is in agreement with Infusino et al. [19], who found that, the overall probability of a patient with IDA to have a positive sTfR test result is approximately 23-fold higher than for a patient with infectious anemia.

Serum transferrin receptor (sTfR) concentration, has been proven to be unaffected by acute phase reactant; consequently, providing superiority over other laboratory methods [16]. Moreover, Koşan Çulha and Uysal [20], concluded that, sTfR/log ferritin is more sensitive and

specific in diagnosis of iron deficiency in early stages and in the presence of infection.

A correlation analysis showed statistically significant relationships between both sTfR and the sTfR/log ferritin and TIBC and Serum ferritin in IDA children. No such relationship was found in other groups, in accordance to the results of Kamer et al. [8]. Other authors did not show any statistical significant correlations between sTfR and biochemical iron indice [21]. These relationships indicate that soluble transferrin receptor increases with growing iron deficiency, but remains unchanged with normal iron stores.

In an attempt to investigate clinical utility of sTfR concentrations and sTfR-F index values to differentiate IDA from infectious anemia, our study demonstrated that at cutoff point of 5.45 mg/l sTfR has 80.9, 81.8, 63, 91.8 and 81.6% for sensitivity, specificity, PPV, NPV and accuracy respectively for prediction of iron deficiency anemia, while at cutoff point of 8.7 sTfR (log) has 100, 96.4, 91.3, 100 and 97.3% for sensitivity, specificity, PPV, NPV and accuracy respectively for prediction of IDA. These results are in accordance with many previous studies Punnonen et al. [22], Matsuda et al. [23], Beguin [24] and Koulaouzidis et al. [2] that found sTfR index may improve diagnostic accuracy, providing higher sensitivity and specificity than sTfR determination alone.

The sTfR Index may improve the ability to accurately classify anemia in diseases with active inflammation. The sTfR Index is superior to sTfR or ferritin alone. sTfR Index takes advantage of the reciprocal relationship between two variables influenced by iron deficiency, an increase in sTfR and a decrease in ferritin concentration. The use of all three parameters in combination more than doubles the detection of IDA, from 41% (ferritin alone) to 92% (ferritin, sTfR, and sTfR Index) [17].

Nadeem et al. [25] stated that estimation of sTfR or index may offer a new and surrogate marker to estimate body iron stores in adults with IDA.

Conclusion

The use of sTfR and sTfR/log ferritin improves the diagnosis of IDA in pediatric patients, especially in the presence of coexisting acute infection.

Author Contribution Fady M El-Gendy: Study design, follow up. Mahmoud A. El Hawy: Interpretation of data and paper writing. Mohamed S Rizk: Lab work. Sally M El-Hefnawy: Lab work. Mohamed Z Mahmoud: Data collection.

Compliance with Ethical Standards

Conflict of interest None.

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