Suitability of soluble transferrin receptor for the clinical diagnosis of different types of anaemia in children

L. Ragab,[1] H.A. Ibrahim,[2] A.S. Eid,[2] T. Kotb[1] and M.F. Konsowa[3]

ملاءمة مستقبلات الترانسفيرين الذوّابة للتشخيص السريري (الإكلينكي) لمختلف أنماط فقر الدم لدى الأطفال

لميس رجب، حنان عبد العزيز إبراهيم، عائشة عيد صالح، طارق قطب، منى فريد قنصوه

الخلاصة: قمنا بتقييم قدرة مستقبلات الترانسفيرين الذوابة في كشف مختلف أنماط فقر الدم لدى الأطفال. وقد شملت الدراسة 150 طفلاً مصرياً يعانون من فقر الدم (عوز الحديد أو المرافق لمرض مزمن أو الثلاسيميا-بيتا) إلى جانب 50 طفلاً شاهداً. وقد لوحظ وجود ازدياد ملحوظ لدى المجموعة المدروسة في مستويات وسطي مُستقبلات الترانسفيرين الذوابة في المصابين بفقر الدم بعوز الحديد والثلاسيميا مع نقص يُعتدُّ به في مستويات وسطي مُستقبلات الترانسفيرين الذوابة في المصابين بفقر دم مصاحب للأمراض المزمنة. وقد شوهد ازدياد ملحوظ في فيرِّيتين المصل لدى مجموعة المرضى باستثناء مجموعة المصابين بفقر الدم بعوز الحديد. وكان هناك علاقة ترابط يُعتدُّ به بين مُستقبلات الترانسفيرين الذوابة والنسبة بين هذه المستقبلات وبين لوغاريتم الفيريتين من جهة، وبين مختلف مناسب حالة الحديد وتكوُّن الكريات الحمر من جهة أخرى، بحيث يمكن استخدام منسب المستقبلات الذوابة للترانسفيرين إلى لوغاريتم الفريتين كأداة للتشخيص وللتحرِّي عن فقر الدم بعوز الحديد، وعن فقر الدم المرافق للأمراض المزمنة وعن الثلاسيميا.

ABSTRACT We evaluated the ability of serum transferrin receptor (sTFR) to identify different types of anaemia in children. Thus 150 Egyptian children suffering from anaemia (iron deficiency anaemia, anaemia of chronic disease and β-thalassaemia) were enrolled, together with 50 controls. There was a significant increase in the mean levels of sTFR in the groups with iron deficiency anaemia and thalassaemia, and a significant decrease in mean sTFR levels in the group with anaemia of chronic disease. Serum ferritin levels were significantly higher in all patient groups except the group with iron deficiency anaemia. There were also significant correlations between the sTFR and sTFR/log ferritin ratio (sTFR-F index) and different indices of iron status and of erythropoiesis. The sTFR-F index could be used as a diagnostic or screening tool for iron deficiency anaemia, anaemia of chronic disease and thalassaemia.

Applicabilité du récepteur soluble de la transferrine pour le diagnostic clinique de différents types d'anémie chez l'enfant

RESUME Nous avons évalué la capacité du récepteur de la transferrine sérique (sTFR) à identifier différents types d'anémie chez l'enfant. Cent cinquante (150) enfants égyptiens souffrant d'anémie (anémie ferriprive, anémie liée à une maladie chronique et bêta-thalassémie) ont donc été inclus, avec 50 témoins. Il y avait une augmentation significative du taux moyen de sTFR dans les groupes présentant une anémie ferriprive et une thalassémie, et une diminution significative du taux moyen de sTFR dans le groupe présentant une anémie liée à une maladie chronique. Le taux de ferritine sérique était accru de manière significative dans tous les groupes de patients sauf le groupe présentant une anémie ferriprive. Il y avait également une corrélation significative entre le sTFR et le rapport sTFR/log ferritine (rapport STFR-F) et différents indices du bilan en fer et l'érythropoïèse. Le rapport sTFR/ferritine pourrait être utilisé comme moyen de diagnostic ou de dépistage de l'anémie ferriprive, de l'anémie liée à une maladie chronique et de la thalassémie.

[1]*Department of Paediatrics;* [2]*Department of Medical Biochemistry, Faculty of Medicine, University of Cairo, Cairo, Egypt.*
[3]*Department of Paediatrics, Faculty of Medicine, University of Beni Suef, Beni Suef, Egypt.*
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Introduction

Iron deficiency (ID) is generally acknowledged to be the single most common worldwide nutritional deficiency. In industrialized countries, the prevalence of iron deficiency anaemia (IDA) has fallen rapidly over the past few decades, while the prevalence of subclinical ID has remained substantial [*1–3*]. Subclinical ID is particularly common in children aged 1–3 years, in adolescents of both sexes, in women of childbearing age, and in the elderly population [*4–8*]. Because current methods permit the accurate diagnosis of uncomplicated IDA, the focus of clinical interest has shifted to the distinction of ID from other entities in the differential diagnosis of anaemia [*9*].

The transferrin receptor (TFR) is a key protein in the cellular uptake of transferrin iron. The released iron is used for haemoglobin synthesis. The greatest density of transferrin receptors is found on the surface of erythroblasts. Regulation of TFR synthesis depends on the intracellular iron concentration, and the synthesis of ferritin and TFR are regulated in an antagonistic manner. Serum TFR (sTFR) is a truncated monomeric form of the cellular receptor. Most of the circulating receptors come from erythroid marrow precursors. sTFR levels mirror the total tissue receptor mass, and depend on the rate of erythropoiesis and on the iron status. Unlike ferritin, the concentration of sTFR is unchanged in inflammatory diseases, infections, malignancies or cytolysis [*10*].

Serum ferritin concentration is particularly informative in estimating the amount of storage iron available to a particular individual. The sTFR concentration, in contrast to serum ferritin, provides direct information about any deficit in the iron supply for erythropoiesis. The combination of serum TFR and serum ferritin provides complete information about the storage and functional iron compartments. Using this combination, it is possible to define the iron nutritional status of an individual [*11*].

This study was performed to evaluate the usefulness of sTFR as a diagnostic tool for IDA, and to assess its efficiency in the diagnosis of iron depletion in patients with anaemia of chronic disorders such as inflammation, infection or malignancy. sTFR may also reflect hyperplastic erythropoiesis in patients with haemolytic anaemia [*12*], as its level is influenced by the erythroid marrow activity (and therefore by congenital and acquired erythroid disorders) [*13*].

Methods

This study included 200 Egyptian infants and children selected from patients attending the Haematology Outpatient Clinic at the Paediatric Hospital, University of Cairo. These children were classified into five groups: IDA (50 patients), anaemia of chronic disease (ACD) (50 patients), β-thalassaemia major (25 patients), β-thalassaemia minor (25 patients), and finally a control group (50 normal children). Their ages ranged from 6 months to 16 years with a mean value of 7.3 ± 5.9 years. The male to female ratio in different patient groups was as follows: in the ACD group 1.5:1, in the IDA group 1:1, in the thalassaemia major group 2.5:1, in the thalassaemia minor group 1:4, and in the control group 1:1.5.

All patients had anaemia, defined according to World Health Organization (WHO) recommendations as a haemoglobin concentration lower than 11 g/dL.

The ACD group included anaemic children with liver cirrhosis, systemic lupus erythematosus, leukaemia, rheumatic heart disease, chronic renal failure or cardiomy-

opathy, and children receiving chemotherapy.

None of the control subjects or those in the IDA or ACD groups had received blood transfusions before assessment, while the mean value of number of blood transfusions per patient in the case of thalassaemia patients was 3.47 ± 3.0 per year.

Sample collection

Fasting blood samples were drawn from each child. Each sample was divided into two portions: the first part was collected on EDTA and used for the complete blood count (performed on an automated Coulter cell counter T540), while the second sample was collected in a plain tube, left to clot, centrifuged and the serum separated and stored at –80 °C for later use.

sTFR was quantitatively determined by enzyme immunoassay using two different monoclonal antibodies specific for sTFR [*14*]. Serum ferritin levels were determined by a one-step enzyme immunoassay [*15*] and the sTFR/log ferritin ratio was calculated [*16*].

The data were analysed using *SPSS* version 8.0. Both statistical analysis and tabulation were carried out according to Altman [*17*]. A one way analysis of variance (ANOVA) test was used to detect statistical significance between the different groups. Unequal number HSD (honestly significant difference) post-hoc tests were applied in post-hoc analysis where needed. The Pearson product moment correlation coefficient r between the different parameters was calculated. Lastly, the chi-squared test was used for analysis of categorical data.

Receiver operating characteristics (ROC) curves were visualized, and the corresponding areas under the curves (AUC^{ROC}) were calculated and compared with each other using Graph ROC for Windows software.

The level of significance was set at $P < 0.05$.

Results

The results are illustrated in Tables 1–5 and Figures 1 and 2.

Discussion

Both IDA and thalassaemia are major health problems in Egypt. Differentiation between the conditions is often troublesome [*18*]. Clinical and laboratory studies have shown that the concentration of sTFR provides a useful quantitative measure of the erythroid marrow mass, and thereby assists clinically in categorizing the type of anaemia and in determining the cause of iron-deficient erythropoiesis (that is, identifying IDA whether it occurs alone or in the presence of anaemia of chronic disease) [2].

In this study it was found that the mean serum ferritin level in the ACD group and the thalassaemia major and minor groups was significantly increased relative to the control group. This is in accordance with the findings of Rees and coworkers who reported a similar increase in children with thalassaemia [*19*]. On the other hand, in the case of IDA, the mean level of serum ferritin was significantly decreased compared to the controls.

Ferritin measurements have been used to differentiate between normality and IDA, and the reference limits have been designed accordingly. However, disagreement still remains on the level indicating clinically relevant storage iron depletion [5]. This is mainly due to the wide physiological variability in serum ferritin between individual patients. In addition, the fact that ferritin is a known acute phase reactant also complicates its use as a marker of ID in the pres-

Table 1 **Serum ferritin, serum transferrin receptor (sTFR) and sTFR-F index in different groups**

Parameter	Control ($n = 50$)	ACD ($n = 50$)	IDA ($n = 50$)	Thalassaemia major ($n = 25$)	Thalassaemia minor ($n = 25$)
Ferritin (µg/L)	28.93 ± 12.0 (a)	1009.4 ± 315.01 (b)	7.95 ± 2.79 (c)	1072.9 ± 341.7 (b)	145.5 ± 48.9 (d)
sTFR (nmol/L)	19.06 ± 2.76 (a)	11.72 ± 4.37 (b)	41.79 ± 7.1 (c)	106.33 ± 22.37 (d)	42.52 ± 17.24 (c)
sTFR-F index	13.59 ± 2.67 (a)	3.99 ± 1.59 (b)	50.46 ± 16.12 (c)	34.99 ± 6.97 (d)	19.78 ± 8.63 (a)

Values are expressed as mean ± standard deviation.
Different letters (a, b, c, d) indicate significant difference between groups, i.e. P < 0.05. Same letters indicate no significant difference between groups.
ACD = anaemia of chronic disease.
IDA = iron deficiency anaemia.

Table 2 **Levels of haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in different groups**

Parameter	Control ($n = 50$)	ACD ($n = 50$)	IDA ($n = 50$)	Thalassaemia major ($n = 25$)	Thalassaemia minor ($n = 25$)
Haemoglobin (g/dL)	12.73 ± 0.63 (a)	7.378 ± 1.84 (b)	8.34 ± 1.3 (c)	6.02 ± 2.1 (d)	9.71 ± 2.1 (e)
Haematocrit	41.53 ± 3.2 (a)	22.23 ± 0.83 (b)	27.75 ± 8.33 (c)	17.07 ± 16.12 (d)	27.68 ± 5.17 (c)
Mean corpuscular volume	82.6 ± 3.48 (a)	74.13 ± 0.79 (b)	70.1 ± 4.68 (c)	62.93 ± 7.94 (d)	68.4 ± 9.32 (c)
Mean corpuscular haemoglobin	32.06 ± 2.83 (a)	24.28 ± 0.43 (b)	25.54 ± 4.9 (b)	28.98 ± 9.9 (ab)	24.36 ± 3.97 (b)
Mean corpuscular haemoglobin concentration	34.06 ± 1.88 (a)	26.38 ± 0.67 (b)	32.15 ± 2.78 (a)	36.26 ± 5.2 (a)	35.6 ± 0.97 (a)

Values are expressed as mean ± standard deviation.
Different letters (a, b, c, d, e) between groups indicate significant difference between them i.e. P <0.05. Same letters indicate no significant difference.
ACD = anaemia of chronic disease.
IDA = iron deficiency anaemia.

Table 3 **Sensitivity, specificity and positive predictive values of different cut-off points of ferritin, serum transferrin receptor (sTFR) and sTFR-F index in different groups**

Parameter	Cut-off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
Ferritin (µg/L)	5.41–52.4 (mean = 28.93)	74.00	64.00	86.05
sTFR (nmol/L)	13.64–24.49 (mean = 19.07)	86.67	98.00	99.24
sTFR-F index	8.34–18.85 (mean = 13.6)	93.33	100.00	100.00

ence of acute or chronic inflammatory diseases [*20*].

Mean sTFR levels recorded in this study showed a significant increase in the IDA group relative to the controls. This reflects a response to the cells' obligate need for iron [*21*]. Our results are in agreement with Cook [*22*] and Hou et al. [*23*], who stated that the two disorders that resulted in an elevation of sTFR were tissue iron depletion (such as occurs in IDA) and anaemia associated with enhanced erythropoiesis.

A more pronounced rise in the mean sTFR level was observed in the thalassaemia major group. This is again in accordance with Rees et al. [*19*] who attributed this increase to an imbalance in the globin chain, leading to ineffective erythropoiesis. Our findings are also consistent with Camaschella et al. in 1996 [*24*], and Rees et al. in 1999 [*12*] who attributed the increased sTFR level to the erythropoietic marrow expansion which takes place to compensate for the marked anaemia. In addition to the degree of anaemia, elevated levels of fetal haemoglobin (HbF) are an additional stimulus to erythropoiesis. This is due to the high oxygen affinity of HbF. The mean level of sTFR in the ACD group was significantly lower than that of the control group; this is consistent with the reports of Rees et al. [*19*].

sTFR in the current study correlated with indices of erythropoiesis as well as with biochemical indices of iron status in the enrolled children (Table 5), again in line with several earlier reports [*25–27*]. The fact that sTFR concentrations remain unaffected by any active acute phase reactant has made sTFR measurement an attractive tool for the differential diagnosis of IDA [*19*], distinguishing ACD from IDA [*27*] and assessing the state of erythropoiesis [*25*].

Although sTFR concentrations are known to reflect the degree of depletion of the functional compartment well before IDA develops, most clinical experience de-

Table 4 **AUC^{ROC} values for serum ferritin, serum transferrin receptor (sTFR) and sTFR-F index**

Parameter	AUC^{ROC}	
	ACD vs IDA	ACD vs thalassaemia
Ferritin (µg/L)	1.00 (0.00)	0.63 (0.061)
sTFR (nmol/l)	1.00 (0.00)	0.996 (0.003)
sTFR-F index	1.00 (0.00)	1.00 (0.00)

Using AUC^{ROC}, the diagnostic accuracy of sTFR-F index was higher than that of ferritin or sTFR in discrimination between ACD and thalassaemia.
ACD = anaemia of chronic disease.
IDA = iron deficiency anaemia.

Table 5 **Correlation between indices of erythropoiesis and of iron status and both of serum transferrin receptor (sTFR) and sTFR-F index**

Parameter	ACD ($n = 50$) r	IDA ($n = 50$) r	Thalassaemia ($n = 50$) r
sTFR			
Haemoglobin	0.2	–0.49*	–0.67*
Haematocrit	0.11	–0.13	–0.59*
Mean corpuscular volume	–0.12	–0.09	–058*
Mean corpuscular haemoglobin	–0.09	–0.56*	–0.09
Mean corpuscular haemoglobin concentration	0.49*	–0.03	–0.01
Ferritin	0.13	–0.51*	0.85*
sTFR-F index	0.99*	0.75*	0.94*
sTFR-F index			
Haemoglobin	0.20	–0.54*	–0.64
Haematocrit	0.11	–0.53*	–0.53*
Mean corpuscular volume	–0.12	–0.52*	–0.49*
Mean corpuscular haemoglobin	–0.05	–0.63*	–0.19
Mean corpuscular haemoglobin concentration	0.52*	0.02	–0.13
Ferritin	0.13	–0.76*	0.65*

**Significant at $P < 0.05$.*
ACD = anaemia of chronic disease.
IDA = iron deficiency anaemia.

rives from settings where reference limits have been set to diagnose ID in patients who are already anaemic. Trials have been made to modify sTFR measurement to permit the detection of ID erythropoiesis more precisely [25].

The sTFR/log ferritin ratio (TFR-F index) has been shown to distinguish between iron-replete and iron-deplete anaemic patients. It was suggested that in borderline cases, when the results of sTFR and ferritin assays are ambiguous, the TFR-F index is more sensitive in the detection of iron-deficient states [25]. Other recent studies elucidate the role of the TFR-F index in the differential diagnosis of IDA and ACD, and provide direct evidence that this parameter is useful in detecting functional iron deficiency, irrespective of the concurrent iron storage status [28].

Our findings are thus also consistent with the above-mentioned studies as the calculated TFR-F index showed significant differences between each of the studied patient groups and the control group.

Our results (Table 3) revealed that serum ferritin alone was sensitive (74.00%) but less specific (64.00%), with a positive

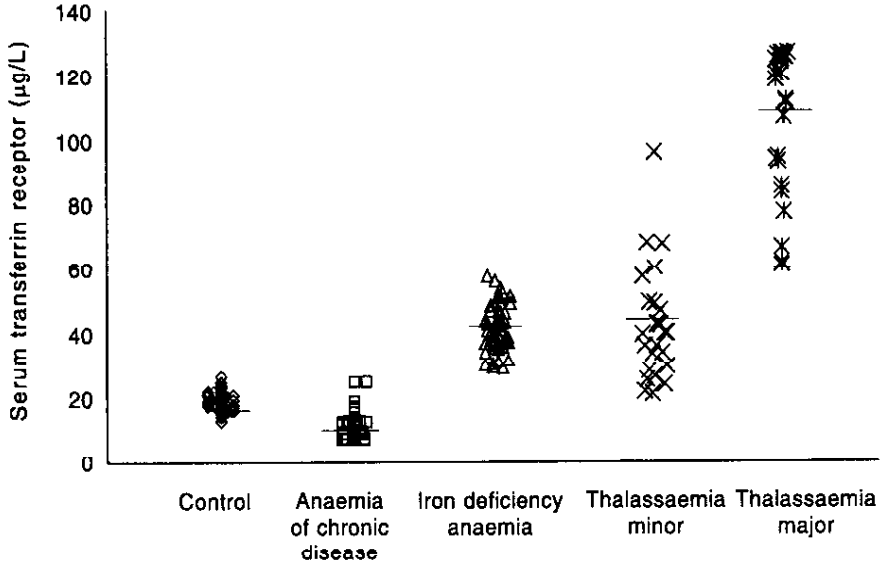

Figure 1 **Serum transferrin receptor levels (µg/L) in the studied groups**

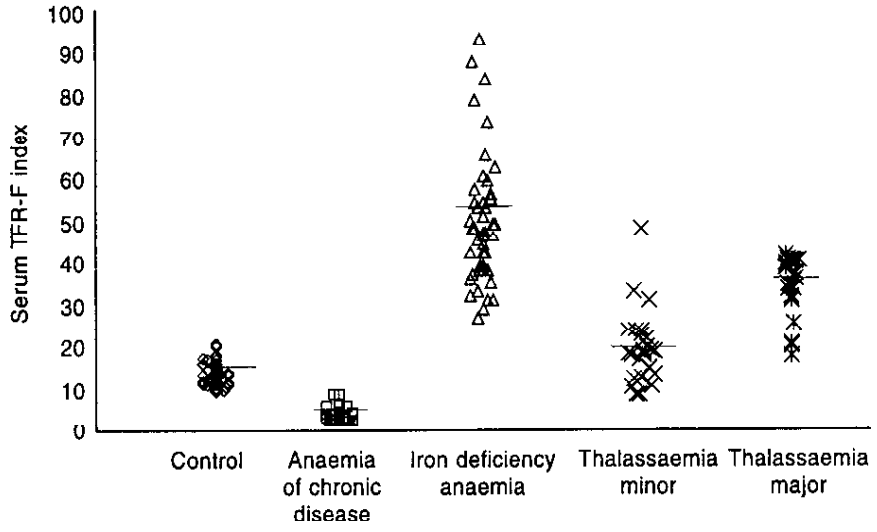

Figure 2 **Serum transferrin receptor-ferritin (TFR-F) index in the studied groups**

predictive value (PPV) of 86.05%, which determines the efficacy of the test. On the other hand sTFR was more specific (98.00%) but less sensitive (86.67%), with a PPV of 99.24%. Our data are in accordance with those of Vernet [*10*] and Vernet and Doyen [*29*], who reported that the concentration of sTFR and the TFR-F index were more precise indicators and, unlike serum ferritin, were unaffected by confounding pathologies. The calculated TFR-F index of our study was both sensitive (93.33%) and highly specific (100%) with a PPV of 100%. Thus combining serum ferritin and sTFR results in one parameter introduced an index that was as specific as sTFR and as sensitive as serum ferritin and possessed the best efficacy (PPV = 100%). Means and coworkers have previously presented similar results [*30*].

In this study sample, serum ferritin measurements distinguished effectively between patients with uncomplicated IDA and ACD (AUC^{ROC} 1.00). The sTFR and sTFR-F index had the same diagnostic accuracy in discriminating between IDA and ACD (AUC^{ROC} 1.00). This is consistent with earlier reports [*14*]. Using AUC^{ROC} the diagnostic accuracy of the sTFR-F index was higher than that of either sTFR or of serum ferritin alone in discrimination between ACD and thalassaemia (Table 4).

It is evident that the ability of ferritin to distinguish between IDA and ACD is due not only to the decrease in serum ferritin level in IDA patients, but to a considerable extent also to the increase in serum ferritin caused by the acute phase responses associated with chronic inflammatory disease. A major advantage of sTFR over serum ferritin measurements is the apparent specificity of the biological response to changes in iron status and erythropoiesis. Compared with ferritin, the clinical interpretation of sTFR measurement is simpler. When haemolysis or megaloblastosis can be excluded, the sTFR measurement provides an attractive alternative to more conventional tests of iron status [*19*].

The serum ferritin level varies with iron stores, while sTFR is assumed to reflect the degree of tissue iron supply [*31*]. The sTFR/ferritin ratio has been proposed as a better estimate of body iron in individual subjects [*32*]. The sTFR-F index takes advantage of the relationship between two phenomena, i.e. an increase in sTFR and a decrease in ferritin concentration. This parameter consists of two variables which in general are influenced by the body iron stores, the availability of iron for erythropoiesis, and the total mass of erythroid bone marrow [*33*]. Thus calculation of the sTFR/log ferritin ratio (sTFR-F index) provides an indicator of iron depletion [*29*], as demonstrated by the corresponding ROC curve [*19*]. Furthermore, the sTFR-F index differentiated well between IDA and thalassaemia minor (Table 1, Figure 2).

Finally, it can be concluded that the combined use of ferritin and sTFR along with the TFR-F index provides maximum sensitivity and specificity, facilitating the accurate determination of the iron status of any given patient, and differentiating between various types of anaemia. The ability of the TFR-F index to differentiate between IDA and thalassaemia minor has a clear clinical application in the Egyptian population where both conditions are common.

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