

Evaluation of soluble transferrin receptor levels in children with iron deficiency and beta thalassemia trait, and in newborns and their mothers

Aziz Polat¹, Bünyamin Kaptanoğlu², Kemal Aydın¹, Ali Keskin³

Departments of ¹Pediatrics, ²Biochemistry, and ³Internal Medicine, Pamukkale University Faculty of Medicine, Denizli, Turkey

SUMMARY: Polat A, Kaptanoğlu B, Aydın K, Keskin A. Evaluation of soluble transferrin receptor levels in children with iron deficiency and beta thalassemia trait, and in newborns and their mothers. Turk J Pediatr 2002; 44: 289-293.

In this study we first aimed to investigate the value of soluble transferrin receptor levels (sTfR) in healthy, iron deficient and beta thalassemia trait children and to determine whether sTfR is a useful indicator of iron deficiency. Secondly, we investigated the effects of iron supplementation of sTfR levels in a group of iron deficient children. Third was to describe sTfR in newborn infants and determine whether or not maternal iron deficiency is an important predictor of infant sTfR. Six groups were formed: Children with iron deficiency (n=22), post-iron therapy (n=16), beta thalassemia traits (n=19), healthy children (n=19), full-term newborns (n=20), and their mothers (n=19). Complete blood count (CBC), serum iron, iron-binding capacity, ferritin and sTfR levels were measured. sTfR/log ferritin indexes were calculated. sTfR levels of children with iron deficiency and with beta thalassemia trait were found to be significantly higher than those of healthy children ($p<0.0001$ and $p<0.001$). Children with iron deficiency showed a greater increase in the levels of sTfR than those with beta thalassemia traits ($p=0.008$). Although sTfR levels of subjects having iron therapy decreased, the levels still remained high compared to controls ($p=0.002$). Newborns had significantly higher levels of sTfR than controls ($p<0.0001$). Although sTfR levels of mothers with iron deficiency were higher than those of mothers having no iron deficiency ($p=0.009$), there was no difference in the levels of sTfR between newborns of both groups of mothers ($p=0.790$). sTfR is a useful parameter which shows body iron status as well as erythropoietic activity in children. It is independent of mother's iron status, and is due to erythropoietic activity in newborns.

Key words: soluble transferrin receptor, iron deficiency, beta thalassemia trait, newborn, children.

Iron plays an essential role in the body. Cellular iron uptake is achieved by transferrin receptors on the membrane. Transferrin receptor (TfR) is a transmembrane dimeric glycoprotein composed of two identical 95 kDa subunits, linked by disulfide bonds. In the human body, 80% of the transferrin receptors are located in the erythroid marrow¹. Although TfR can be identified on nearly every cell type, it is predominantly expressed by maturing cells of the erythroid lineage, which have high iron requirements for heme synthesis, and syncytiotrophoblasts. TfR expression increases with cell proliferation, differentiation and iron need.

Th TfR is found in a soluble form in serum. Serum TfR (sTfR) is a truncated form of the intact TfR and has been identified as a

monomeric fragment of the extracellular domain with a molecular mass of 85 kDa. sTfR levels are not affected by age, sex and pre- or postmenopausal status of adults or by acute or chronic infections¹⁻³. In one report, sTfR concentration in 485 healthy infants between 9 and 15 months of age was not related to age or sex⁴. With these features sTfR seems to be a new and useful parameter in the diagnosis and differentiation of iron deficiency¹⁻³.

Studies on sTfR levels of children are few. In this study we first aimed to investigate the value of sTfR in healthy, iron deficient and beta thalassemia trait children and to determine whether or not sTfR is a useful indicator of iron deficiency. Second, we investigated the effects of iron supplementation of sTfR levels in a

group of iron deficient children. Third was to describe sTfR in newborn infants and determine whether or not maternal iron deficiency is an important predictor of infant sTfR.

Material and Methods

Six study groups were formed. None of the subjects in these groups had an acute or chronic disease, or had used iron preparations within the last one year. Group 1 consisted of 22 children (12 male, 10 female) aged 2.2 ± 1.5 years old (10 months to 6.5 years old) and diagnosed as iron deficiency according to the criteria which included low serum ferritin (<10 ng/ml) with or without low transferrin saturation ($<14\%$) and low hemoglobin (<11 g/dl)⁵. Group 2 included 16 subjects from Group 1 who were appropriately treated with iron (6 mg/kg/d, peroral) for two months. All of the children in Group 2 had serum ferritin levels >10 ng/ml and hemoglobin >11 g/dl. Group 3 consisted of 19 beta thalassemia trait children (11 male, 8 female) aged 8.4 ± 3.6 years old (6 months to 14 years old) and diagnosed by hemoglobin electrophoresis as having $>3.5\%$ HbA₂. Group 4 (control group) included 19 healthy children (6 male, 13 female) aged 4.3 ± 2.4 years old (9 months to 8 years old). All of the healthy subjects had serum ferritin levels >10 ng/ml and hemoglobin >11 g/dl. Group 5 consisted of 20 healthy full-term

newborns with mean birth weight of 3,300 g. Within the first day of life blood samples were taken from the newborns. Group 6 were the mothers of newborns in Group 5, aged 26.3 ± 5.4 years old (17 to 35 years old). Group 6 was divided into those with iron deficiency (ferritin <12 ng/ml and/or Hb <10.5 g/dl) ($n=9$) and those without iron deficiency ($n=10$).

The subjects were outpatients. The parents were informed and consented to the study.

Cell blood counts, serum iron, total iron-binding capacity, ferritin and sTfR levels of the subjects were analysed. Cell blood counts were determined with Cell-DYN 3500R (Abbott) automatic cell counter. Serum iron, total iron-binding capacity and ferritin were measured by standard methods. sTfR levels were analysed with fluorescence polarization immunoassay (AIA-Pack-sTfR kit, Belgium) by an automated analyser (TOSOH, Japan). For the statistical evaluations, Mann-Whitney U and Pearson correlation tests were used.

Results

Mean hemoglobin, hematocrit, mean corpuscular volume (MCV), serum iron, iron-binding capacity, ferritin, sTfR levels and sTfR/log ferritin indexes of the groups are given in Table I. Results of sTfR levels are shown in Figure 1.

Table I. Hemoglobin, Hematocrit, MCV, Serum Iron, Total Iron-Binding Capacity, Ferritin, sTfR Levels and sTfR/log Ferritin Indexes of the Groups

Groups	Hemoglobin (g/dl)	Hematocrit (%)	MCV (fl)	Serum iron (μ /dl)	Total iron-binding capacity (μ /dl)	Ferritin (ng/ml)	STFR (U/ml)	STFR/log ferritin index
Group 1: Iron deficiency ($n=22$)	10.3 ± 1.3 (7.7-12.2)	31.8 ± 3.2 (26.7-36.5)	68.8 ± 6.9 (55.0-80.8)	42.5 ± 29.1 (7-118)	401 ± 55 (281-500)	5.0 ± 2.8 (1.5-9.7)	925 ± 268 (607-1500)	1198 ± 2553 (681-8158)
Group 2: After iron therapy ($n=16$)	11.9 ± 0.8 (11.0-13.3)	36.9 ± 2.4 (34.0-41.5)	74.7 ± 4.3 (67.3-82.7)	56.7 ± 20.2 (32-192)	353 ± 87 (220-503)	25.2 ± 11.6 (10.9-53.8)	686 ± 137 (557-1151)	485 ± 168 (358-1009)
Group 3: β -thalassemia traits ($n=19$)	10.6 ± 0.8 (9.5-12.4)	33.2 ± 2.6 (27.6-37.8)	58.7 ± 2.8 (53.3-67.4)	71.5 ± 23.0 (47-109)	303 ± 55 (216-399)	39.8 ± 19.1 (15.4-85.2)	725 ± 153 (488-1074)	488 ± 90 (332-667)
Group 4: Healthy children ($n=19$)	11.8 ± 0.5 (11.0-12.9)	35.5 ± 1.5 (33.0-38.3)	78.7 ± 4.4 (72.8-90.9)	75.4 ± 50.8 (7-229)	309 ± 50 (224-421)	41.8 ± 33.5 (10.3-151)	578 ± 80 (389-725)	391 ± 86 (269-585)
Group 5: Newborns ($n=20$)	17.6 ± 1.3 (14.6-19.5)	52.4 ± 4.3 (42.6-59.1)	100.3 ± 2.8 (96-105)	46.9 ± 26.5 (18-141)	215 ± 70 (108-403)	260.2 ± 142.0 (8.8-560)	849 ± 186 (575-1315)	359 ± 244 (224-1398)
Group 6: Mothers of newborns ($n=19$)	11.5 ± 1.0 (9.7-13.9)	35.4 ± 2.9 (30.2-41.5)	83.9 ± 6.1 (69.0-96.7)	57.4 ± 18.8 (30-98)	386 ± 103 (147-568)	20.8 ± 25.1 (6.5-101)	672 ± 278 (233-1500)	661 ± 358 (155-1704)
With ID ($n=9$)	10.9 ± 0.9 (9.7-12.3)	33.9 ± 2.5 (30.2-36.7)	82.4 ± 5.3 (69-87)	58.4 ± 21.5 (35-98)	387 ± 69 (292-505)	9.1 ± 1.4 (6.5-11)	836 ± 279 (536-1500)	829 ± 335 (525-1704)
Without ID ($n=10$)	11.9 ± 0.9 (10.7-13.9)	36.8 ± 2.6 (32.8-41.5)	85.3 ± 6.7 (76.4-96.7)	56.6 ± 17.1 (30-78)	385 ± 130 (147-568)	31.4 ± 31.7 (12.1-101)	524 ± 185 (233-101)	392 ± 201 (155-756)

* ID: iron deficiency; MCV: mean corpuscular volume; STFR: serum transferrin receptor.

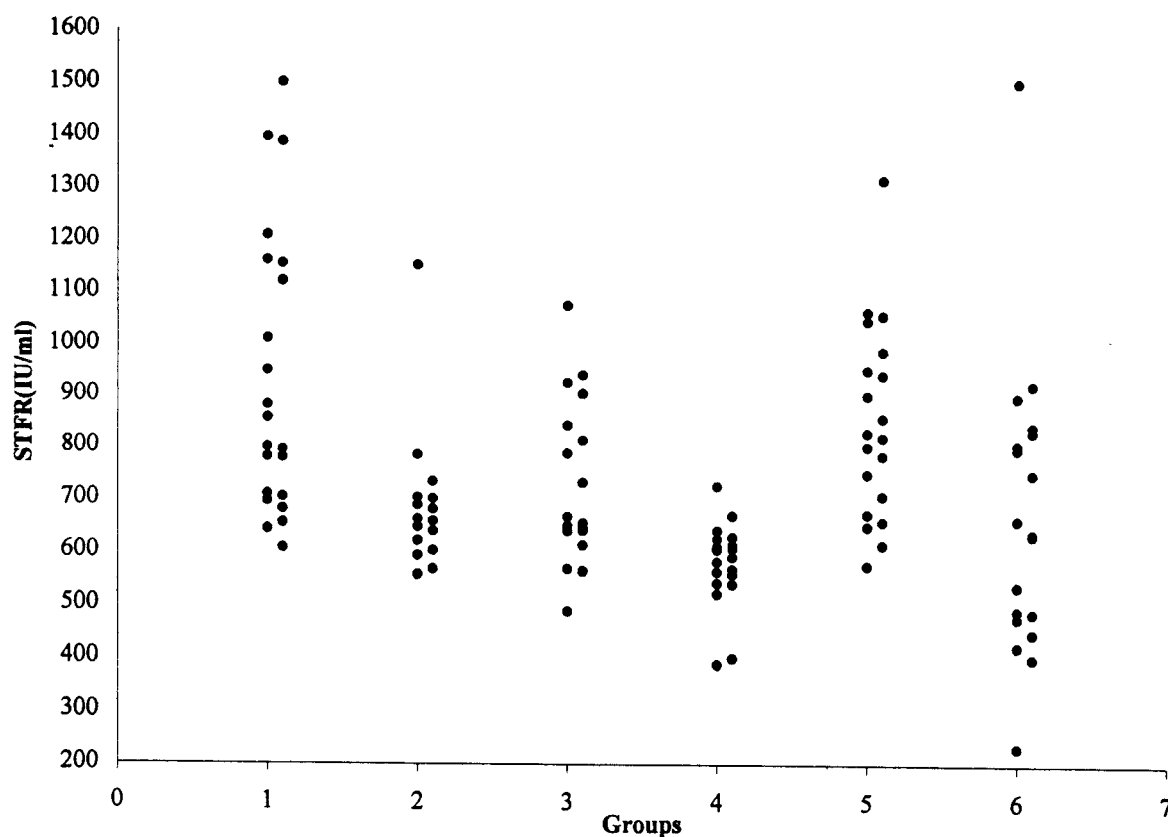


Fig. 1. Results of sTfR levels. Group 1: iron deficiency; Group 2: after iron therapy; Group 3: beta thalassemia trait; Group 4: healthy children; Group 5: newborns; Group 6: mothers of newborns.

Serum transferrin receptor levels of children with iron deficiency and with beta thalassemia trait were found to be significantly higher than those of healthy children ($p < 0.0001$ and $p < 0.001$). Children with iron deficiency showed a greater increase in the levels of sTfR than those with beta thalassemia traits ($p = 0.008$). Although sTfR levels of subjects having iron therapy decreased, the levels still remained high compared to controls ($p = 0.002$). Newborns had significantly higher levels of sTfR than controls ($p < 0.0001$). Although sTfR levels of mothers with iron deficiency were higher than those of mothers having no iron deficiency ($n = 10$) ($p = 0.009$), there was no difference in the levels of sTfR between newborns of both groups of mothers ($p = 0.790$).

A significant negative correlation was found in Group 1 (iron deficiency) between sTfR and hemoglobin, hematocrit, MCV and ferritin levels. This correlation disappeared after iron therapy. sTfR levels of newborns and their mothers showed a correlation with hemoglobin

levels and hematocrit but no correlation with iron parameters (serum iron, total iron-binding capacity and ferritin levels). Iron parameters also showed no correlation with hemoglobin levels, hematocrit or MCV in newborns and their mothers.

Discussion

Transferrin receptor is responsible for the transfer of iron bound to the transferrin into the cell. Serum TfR is a truncated form of the intact TfR and can be determined by immunoassay methods¹⁻³. sTfR levels increase specifically in tissue iron deficiency and hyperplastic erythropoiesis. Because sTfR is not an acute phase reactant like ferritin, is a more sensitive parameter compared to other iron parameters, and is a more stable analyte against physiologic changes, it has recently become a more popular diagnostic tool⁶⁻⁹. Use of sTfR has become wide spread because of its easier determination in a small amount of serum and its close correlation with TfR¹⁻³.

Generally studies on sTfR have been performed in adults. Little information has been reported on children in the literature. We hope that this study can make some contribution to this issue because the subjects chosen were children. The most comprehensive study so far has been the work of Young et al.⁴ on 485 infants investigating normal percentile estimates for sTfR. Virtanen et al.¹⁰ reported that sTfR levels were higher in infants and prepubertal children than in adults.

When iron deficiency occurs in tissues, TfR expression increases rapidly. This is achieved directly by the effects of iron regulatory proteins (IRP) and erythropoietin^{1,11}. Many studies have shown that in patients with iron deficiency anemia, sTfR levels rise 3 to 5 fold, related to the severity of iron deficiency, whereas in iron overload sTfR levels are normal or slightly decreased^{4,6-9}. In the absence of a hyperplastic erythropoiesis, the sTfR is a sensitive marker of early tissue iron deficiency¹. sTfR reflects the tissue iron requirements. The only parameter reflecting the tissue iron deficiency exactly within the period between the beginning of anemia and consumption of iron stores are sTfR levels. In our study, sTfR levels of children with iron deficiency were also significantly higher than those of healthy children. This marked rise almost recovered after iron therapy.

The amount of TfR on erythroblasts with high iron need and turnover is higher than that of the mature erythrocyte. Because of this, sTfR may be used as a non-invasive method in the evaluation of erythropoiesis¹². In some studies, decreased levels of sTfR have been reported in cases with decreased erythropoiesis such as in aplastic anemia and renal failure, while an increase in sTfR has been showed in cases with increased erythropoiesis such as in haemolytic anemia and thalassemia^{3,13}. In our study, we found higher levels of sTfR in children with beta thalassemia traits than in controls.

Prevalence of beta thalassemia trait is high in the southern and western parts of Turkey like in other Mediterranean countries. Differential diagnosis of iron deficiency from beta thalassemia is essential in these areas. Ferritin levels as well as sTfR levels have to be analysed since sTfR levels are high in both situations. Dimitriou et al.¹⁴ reported then necessity of the use of sTfR-ferritin index for the differentiation

of beta thalassemia trait from those with iron deficiency. In that report the sTfR values, but not the sTfR/log ferritin index values, were found increased in children with beta thalassemia trait. However, in our study the sTfR/log ferritin indexes were found increased in children with iron deficiency and with beta thalassemia trait.

Serum transferrin receptor levels of newborns do not change with sex, gestational age or birth weight, and are independent of iron parameters. However, there have been controversial results in these situations. In our study there were no sex differences in sTfR levels of newborns. Rusia et al.¹⁵ found inversely related results between sTfR and serum hemoglobin, MCV, iron, and total iron-binding capacity in newborns. In this study, it was found that sTfR levels of newborns of anemic mothers were increased while iron parameters were normal. Kuiper-Kramer et al.¹⁶ reported that sTfR levels of newborns were independent of iron parameters. Choi et al.¹⁷ showed that iron parameters of newborns were not affected unless mothers had marked iron deficiency. In that study, sTfR levels of newborns whose mothers had iron deficiency without anemia were found to be elevated. In our study, we found increased levels of sTfR in newborns. sTfR level showed correlations with hematocrit and hemoglobin but no correlation with iron parameters in newborns. We found significantly increased levels of sTfR in mothers with iron deficiency compared to others. This finding correlates with the literature showing that the sTfR level is the best parameter reflecting iron deficiency during pregnancy^{18,19}. However we could not find any difference in the levels of sTfR between newborns of mothers with or without iron deficiency. Although the number of cases in our study was small, these results suggest that iron is consumed for the benefit of newborns.

REFERENCES

1. Feelders RA, Kuiper-Kramer EP, Eijk HG. Structure, function and clinical significance of transferrin receptors. *Clin Chem Lab Med* 1999; 37: 1-10.
2. Cook JD. The measurement of serum transferrin receptor. *Am J Med Sci* 1999; 318: 269-276.
3. Kohgo Y, Niitsu Y, Kondo H, et al. Serum transferrin receptor as a new index of erythropoiesis. *Blood* 1987; 70: 1955-1958.
4. Young GS, Zlotkin SH. Percentile estimates for transferrin receptor in normal infants 9-15 mo of age. *Am J Clin Nutr* 1997; 66: 342-346.

5. Dallman PR, Yip R, Oski FA. Iron deficiency and related nutritional anemias. In: Nathan DG, Oski FA (eds). *Hematology of Infancy and Childhood* (4th ed) Vol. 1. Philadelphia: WB Saunders Co; 1993: 413-450.
6. Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. *Ann Rev Med* 1993; 44: 63-74.
7. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* 1990; 75: 1870-1876.
8. Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* 1997; 89: 1052-1057.
9. Suominen P, Punnonen K, Rajamaki A, Irjala K. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. *Blood* 1998; 92: 2934-2939.
10. Virtanen MA, Viinikka LU, Virtanen MK, et al. Higher concentrations of serum transferrin receptor in children than in adults. *Am J Clin Nutr* 1999; 69: 256-260.
11. Weiss G, Houston T, Kastner S, et al. Regulation of cellular iron metabolism by erythropoietin: activation of iron-regulatory protein and upregulation of transferrin receptor expression in erythroid cells. *Blood* 1997; 89: 680-687.
12. Huebers HA, Beguin Y, Poortrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood* 1990; 75: 102-107.
13. Beguin Y, Clemons GK, Poortrakul P, Filled G. Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. *Blood* 1993; 81: 1067-1076.
14. Dimitriou H, Stiakaki E, Morkaki EA, Bolonaki I, Giannakopoulou C, Kalmanti M. Soluble transferrin receptor levels and soluble transferrin receptor/log ferritin index in the evaluation of erythropoietic status in childhood infections and malignancy. *Acta Paediatr* 2000; 89: 1169-1173.
15. Rusia U, Flowers C, Madan N, Agarwal N, Sood SK, Sikka M. Serum transferrin receptor levels in the evaluation of iron deficiency in the neonate. *Acta Paediatr Jpn* 1996; 38: 455-459.
16. Kuiper-Kramer EP, Baerts W, Bakker R, van Eyck J, van Raan J, van Eijk HG. Evaluation of the iron status of the newborn by soluble transferrin receptors in serum. *Clin Chem Lab Med* 1998; 36: 17-21.
17. Choi JW, Kim CS, Pai SH. Erythropoietic activity and soluble transferrin receptor level in neonates and maternal blood. *Acta Paediatr* 2000; 89: 675-679.
18. Akesson A, Bjellerup P, Berglund M, Bremme K, Vahter M. Serum transferrin receptor: a specific marker of iron deficiency in pregnancy. *Am J Clin Nutr* 1998; 68: 1241-1246.