Reference limits and behaviour of serum transferrin receptor in children 6–10 years of age

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SUMMARY

Serum transferrin receptor (sTfR) originates mostly from erythroblasts and lesser from reticulocytes. The usefulness of sTfR has been implicated in several clinical situations, mainly as a marker of accelerated erythropoiesis or iron deficiency. The assessment of sTfR may be useful in the period of rapid growth during infancy, childhood and adolescence. We evaluated sTfR and the other quantitative and qualitative parameters of the erythropoiesis (Hb, MCV, CHr, Ret-He) and of the iron storage (serum ferritin, sTfR/ferritin index) in a total of 916 children aged 6–10 years. Children were divided into three groups: (A) healthy children, (B) with storage iron deficiency (serum ferritin < 12 μg/l) and (C) Beta trait carriers (HbA2 > 3.3). We determined reference intervals by sex and by age in healthy children . sTfR showed a slight but statistically significant age related increase but did not show significant sex differences. We compared sTfR and the other parameters investigated in the three groups of children. sTfR is not a decisive parameter that can be utilized alone in discriminating the border-line situations between normal and pathologic ones but can help in completing the panel of tests in iron deficiency and in thalassaemia Beta trait carriers.

INTRODUCTION

Iron transport in the plasma is carried out by transferrin, which donates iron to cells through its interaction with specific membrane receptor. Virtually all cells, except mature red cells, have transferrin receptor (TfR) on their surface, but the largest numbers are in the erythron, placenta and liver. In a normal adult, about 80% of TfR are in the erythroid marrow (Feelders, Kulperkramer & Vaneijk 1999). In normal subjects the number of transferrin receptors (CD 71+) on nucleated red blood cell (NRBC) is within very narrow limits either in the single sample or in various persons (Ahluwalia 1998; Beguin *et al*. 1993).

The presence of TfR on NRBCs determine that a little amount of this receptor circulates. In fact, Kohgo *et al*. (1986) reported that transferrin receptors were detectable in plasma by immunoassay. Serum TfR (sTfR1) is a soluble truncated monomer of tissue receptor, lacking its first 100 amino acids, which

circulates in the form of a complex of transferrin and its receptor (Kohgo *et al.* 1987; R'Zik & Beguin 2001; Shih *et al.* 1990). The bulk of sTfR measured in serum is proportional to the mass of cellular TfR and originates mostly from erythroblasts and to a lesser extent from reticulocytes (Baynes, Shih & Cook 1994).

Early identification of iron deficiency in children is essential to prevent the damaging long-term consequences of this disease. Investigations regarding sTfR as a measure of iron status in infants and children have provide promising results, including evidence that, in infants, sTfR concentrations may be superior to ferritin measurements in diagnosing iron deficiency (ID) (Olivares *et al.* 2000).

In the early postnatal period, sTfR levels are comparable with those of adult with marrow suppression, but they soon increase again so that infants and adolescents tend to have a slightly higher sTfR levels than adults (Virtanen *et al.* 1999). Age-specific reference limits for children aged 0–4, 4–10 and 10–16 years have been derived in a carefully selected group of healthy children (Suominen *et al.* 2001), but these age-specific reference intervals should be implemented.

The purpose of this study was to establish appropriate reference limits of sTfR in healthy children 6–10 years of age and to evaluate the behaviour of sTfR related to the other quantitative and qualitative parameters of the erythropoiesis (Hb, MCV, CHr, RET-He) and of the iron storage (serum ferritin and sTfR/ferritin index) in the population studied.

MATERIALS AND METHODS

Patient groups

A total of 916 children from Costa of Amalfi aged 6–10 years were investigated (492 males and 424 females).The children examinated were divided into three groups: group A (851 children) were healthy children, group B (53 children) had storage iron deficiency (serum ferritin < 12 μg/l) and group C (12 children) are Beta trait carriers (HbA2 > 3.3). In all cases, parents gave their informed consent. The selection of children included in the healthy population of this study was made on the basis of detailed anamnesis and laboratory tests. The criteria for exclusion from this study population were: febrile episodes during the preceding 4 weeks, haematological diseases, recent iron supplementation, or drugs, anaemia or abnormal red cell indices analysed in this study and low serum ferritin concentration (<12 μg/l).

Instruments and parameters investigated

A complete blood cell counts and reticulocyte indices were measured with the automated analyzer Bayer ADVIA 120 (Bayer Diagnostics, Tarrytown, NY, USA). We measured Haemoglobin concentration (Hb), Mean Corpuscolar Volume (MCV), Reticulocyte Haemoglobin Content (CHr). We measured also Reticulocyte Haemoglobin (RET-He) with the automated analyzer Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan).

Serum ferritin were measured with the Abbott Architect Ferritin assay that is a two-step immunoassay to determine the presence of ferritin in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflel.

sTfR assay were carried out using an automated immunoturbidimetric assay (IEMA) using a monoclonal antibody (IDeA sTfR-IT, Orion Diagnostica, Espoo, Finland). The method has been described in detail elsewhere (Suominen *et al.* 1999). The sTfR/ferritin index were calculated dividing the value of sTfR for log of serum ferritin.

Statistical analysis

The reference intervals of sTfR by sex and by age were calculated using the nonparametric summary statistics. Differences between sex, age and between groups were evaluated using the Kruskall–Wallis ANOVA method for the parameters with a non-normal distribution (sTfR, serum ferritin, sTfR/ferritin index) and using the one-way ANOVA method with the Bonferroni test for the parameters with a Gaussian distribution (Hb, MCV, CHr, RET-He). Significance was assumed at $P < 0.01$. All statistical analysis was performed with Analyse-it software, version 1-73, Clinical Laboratory & General module (Analyse-it Software, Ltd, Leeds, England, UK).

RESULTS

sTfR reference intervals in healthy children (group A) divided by sex are shown in Table 1. sTfR reference

Table 1. sTfR reference intervals by sex in healthy children (group A)

	Healthy children (*N* = 851)	Males (*N* = 459)	Females (*N* = 392)	*P*-value
sTfR(mg/l)				
Median	1.50	1.50	1.50	0.1824
2.5°–97.5° percentile	0.93–3.00	1.00–3.00	0.90–3.10	

intervals of males and females did not show any significant differences ($P = 0.1147$). sTfR reference intervals in healthy children (group A) divided by age are shown in Table 2. Differences between different ages were weak but statistically significant ($P = 0.0004$) with a slight increase of lower and upper values of reference intervals from 6–9 years. Distribution of reference intervals are shown in Figure 1. Spearman correlation between sTfR and serum ferritin, sTfR/ferritin index, Hb, MCV, CHr and RET-He are shown in Table 3.

In healthy children, sTfR was inversely correlated ($P < 0.0001$) with CHr and RET-He and, as expected, was directly correlated ($P < 0.0001$) with Hb. sTfR did not show significant correlation with serum ferritin ($P = 0.0142$) and with MCV ($P = 0.885$). The correlation of sTfR with serum ferritin, Hb, CHr and RET-He was significantly increased in children with storage iron deficiency (with r values higher than in healthy and P-values <0.01), while was not significantly correlated in Beta trait carriers children (P-values >0.01). Finally, in a preliminary study, we compared reference intervals of healthy children (group A) with values obtained from children with storage iron deficiency (group B) and values obtained from children Beta trait carriers (group C) to evaluate the behaviour of the parameters investigated.

Results obtained are shown in Table 4. sTfR showed statistically significant differences between healthy children (group A) and both children with storage iron deficiency (group B) and Beta trait carriers (group C). Haematological parameters of iron status investigated (Hb, CHr and RET-He) showed

Table 2. sTfR reference intervals by age in healthy children (group A)

	Healthy children (*N* = 851)	6 years (*N* = 146)	7 years (*N* = 167)	8 years (*N* = 179)	9 years (*N* = 181)	10 years (*N* = 178)	*P*-value
sTFR (mg/l)							
Median	1.50	1.40	1.50	1.50	1.70	1.50	0.0003
2.5°–97.5° percentile	0.93–3.00	0.87–2.86	0.90–2.98	0.95–3.05	1.00– 3.14	1.00–2.95	6,7 *vs.* 9 years

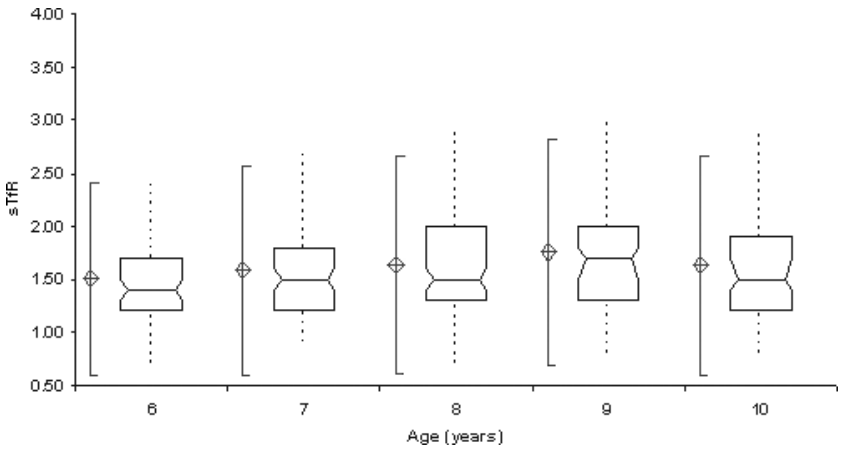

Figure 1. Distribution of reference intervals of sTfR by age in healthy children (group A).

Table 3. Spearman correlation between sTfR and other haematological and biochemical iron parameters investigated (*$P < 0.01$)

	Group A (healthy subjects)	Group B (iron storage deficiency)	Group C (beta trait carriers)
sTFR *vs.* serum ferritin			
r-value	−0.08	−0.36	0.35
P-value	0.0142	0.0081*	0.2634
sTFR *vs.* Hgb			
r-value	+ 0.19	−0.36	−0.25
P-value	<0.0001*	0.0088*	0.4349
sTFR *vs.* RET-He			
r-value	−0.15	−0.47	−0.36
P-value	<0.0001*	0.0014*	0.2435
sTFR *vs.* CHr			
r-value	−0.21	−0.45	−0.40
P-value	<0.0001*	0.0007*	0.2024
sTFR *vs.* MCV			
r -value	+ 0.00	−0.08	−0.24
P-value	0.8849	0.5650	0.4620

statistically significant difference between healthy children (group A) and both children with storage iron deficiency (group B) and Beta trait carriers (group C). MCV values were not statistically significant different ($P = 0.4129$) between healthy children and children with storage iron deficiency, while the difference between healthy children and children Beta Trait carriers ($P < 0.0001$) was significant.

DISCUSSION

The usefulness of sTfR has been implicated in several clinical situations, mainly as a marker of accelerated erytropoiesis or iron deficiency (Ahluwalia, 1998; Ferguson *et al.* 1992; Huebers *et al.* 1990; Malope *et al.* 2001; Skikne, Flowers & Cook 1990): in pregnancy, in the period of rapid growth during infancy, childhood and adolescence, and anaemic conditions complicated by co-existent chronic inflammatory diseases, or malignancies.

Infants, children and adolescents are known to be particularly prone to iron deficiency. There are few reports of reference ranges for haematological values in school age children. Investigations regarding sTfR in infants and children have been made (Dimitriou *et al.* 2000; Kuiper-Kramer *et al.* 1998; Lockitch *et al.* 1988; Skikne 1998), but there are few data about sTfR concentrations reference limits (Suominen *et al.*, 2001).

In our experience we determined reference intervals by sex and by age in healthy children aged 6–10 years (group A). No significant between-gender difference in sTfR concentrations was observed. sTfR showed a weak but statistically significant age related increase between children aged 6, 7 and 9 years, but this increase did not show clinical significance. Values of median (1.50) and reference intervals in children (2.5°–97.5° percentile, corresponding to 0.93-3.00) were higher than sTfR values in adults (0.9–2.3 mg/l; Suominen *et al.*, 1999).

We evaluated also the correlation between sTfR and the other haematological and biochemical iron parameters. In healthy children, the absence of correlation with serum ferritin showed that sTfR has an independent behaviour as a marker of iron status in subjects with normal iron status compared with other parameters. The weak inverse correlation with CHr and RET-He, the weak direct correlation with Hb and any correlation with MCV did not allow us to understand if there is a correlation between sTfR and quantitative and qualitative parameters of the erythropoiesis. In subjects with storage iron deficiency, sTfR showed a dependent behaviour with a significant inverse correlation with serum ferritin, Hb, CHr and RET-He.

Finally, we compared the difference between sTfR and the other parameters investigated in healthy children (group A), in children with storage iron deficiency (group B) and Beta trait carriers (group C). We found that in children with storage iron deficiency (group B) all these parameters (except MCV) showed difference statistically significant with healthy children (group A). sTfR, Hb, MCV, CHr and RET-He were significant different between healthy children (group A) and children Beta trait carriers (group C). All parameters, except sTfR, showed differences between children with storage iron deficiency (group B) and children Beta trait carriers (group C).

In conclusion, sTfR is not a decisive parameter that can be utilized alone in discriminating the border-line situations between normal and pathologic ones, but seems to be a parameter that can help in completing

Table 4. Comparison between reference intervals of healthy children (group A) and values obtained for children with storage iron deficiency (group B)and beta trait carriers (group C)

	Group A (healthy)	Group B (iron storage deficiency)	Group C (beta trait carriers)	*P*-value (comparison between Groups)
sTFR (mg/l)				
Median	1.50	1.90	2.10	A *vs.* B $P < 0.0001$*
2.5°–97.5° percentile	0.93–3.00	1.00–4.69	n.a. – n.a.	A *vs.* C $P = 0.0019$*
Range (min–max)	0.70–3.80	1.00–4,90	1.30–4.10	B *vs.* C $P = 1.000$
Serum ferritin (μg/l)				
Median	31.00	10.00	45.50	A *vs.* B $P < 0.0001$*
2.5°–97.5° percentile	14.00–85.10	3.35–12.00	n.a. – n.a.	A *vs.* C $P = 0.0741$
Range (min–max)	13–157	3.00–12.00	22.0–87.0	B *vs.* C $P = <0.0001$*
sTfR/ferritin Index				
Median	1.04	1.92	1.37	A *vs.* B $P < 0.0001$*
2.5°–97.5°percentile	0.55–2.09	1.16–6.44	n.a. n.a.	A *vs.* C $P = 0.1935$
Range (min–max)	0.43–2.88	1.15–7.14	0.72–2.28	B *vs.* C $P = 0.0013$*
Hb (g/dl)				
Median	12.70	12.40	10.95	A *vs.* B $P = 0.0003$*
2.5°–97.5°percentile	11.40–14.17	10.73–13.30	n.a. – n.a.	A *vs.* C $P < 0.0001$*
Range (min–max)	10.60–15.00	10.70–13.30	9.70–12.40	B *vs.* C $P = 0.0019$*
MCV (fl)				
Median	78.60	77.50	59.70	A *vs.* B $P = 0.4129$
2.5°–97.5°percentile	71.80–85.64	69.00-85.29	n.a.–n.a.	A *vs.* C $P < 0.0001$*
Range (min–max)	64.70–95.50	68.5–85.50	55.0–71.0	B *vs.* C $P < 0.0001$*
CHr (pg)				
Median	29.00	28.10	19.70	A *vs.*B $P = 0.0017$*
2.5°–97.5°percentile	25.10–31.40	23.44–31.09	n.a.–n.a.	A vs C $P < 0.0001$*
Range (min–max)	22.70–33.50	22.60–31.30	18.70–25.90	B *vs.*C $P = 0.0002$*
RET-He				
Median	29.56	28.81	19.66	A *vs.*B $P = 0.0008$*
2.5°–97.5°percentile	25.94–31.84	23.39–31.40	n.a.–n.a.	A *vs.* C $P < 0.0001$*
Range (min–max)	23.30–33.30	23.06–31.46	17.64–25.97	B *vs.* C $P = 0.0008$*

Values obtained for group C (2.5°–97.5° percentile) were not assigned (n.a.), because of the sample size lower than 40. *P*-values with asterisk (*) highlight significant differences between groups ($P < 0.01$).

the panel of the test in these situations. It is therefore important to establish reference intervals and to study the behaviour of sTfR in the three groups analyzed because these data can improve the use of this parameter in children age 6–10 years.

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