Serum soluble transferrin receptor in hypochromic microcytic anaemia

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ABSTRACT

Introduction: The objective of this study was to assess the clinical significance of soluble transferrin receptor (sTfR) in hypochromic microcytic anaemia.

Methods: Serum sTfR was determined on 91 blood samples with haemoglobin less than 110 g/L and MCV less than 76 fl or MCH less than 27.0 pg. The samples were classified as iron deficiency anaemia (IDA), anaemia of chronic disease (ACD) and thalassaemia. ACD was further divided into groups 1 and 2, based on the serum ferritin level.

Results: The sTfR level in the control was 1.53 +/- 0.8 mg/L. In IDA, the sTfR level was 5.53 +/- 8.76 mg/L and this was significantly higher compared with the control (p-value is less than 0.0001). sTfR levels in ACD (3.32 +/- 3.94 mg/L) and the thalassaemia group (1.64 +/- 1.02 mg/L) were not significantly different from that of the control (p-value is more than 0.05). In ACD, the sTfR level in group 1 was significantly higher when compared with the control (p-value is less than 0.001) and group 2 (p-value is less than 0.01). A significantly higher sTfR/ferritin ratio was observed in IDA (2,368.98 +/- 7,236.4 μg/μg) compared to the control (37.6 +/- 43.7 μg/μg) (p-value is less than 0.001). No significant difference was noted between ACD, thalassaemia and control (p-value is greater than 0.05). sTfR/ferritin ratio was significantly higher in group 1 of ACD when compared with that of control and group 2 (p-value is less than 0.001).

Conclusion: Serum sTfR and sTfR/ferritin ratio are useful parameters in hypochromic microcytic anaemia to diagnose iron deficiency particularly when associated with chronic inflammation. sTfR can be done selectively in ACD patients when ferritin levels are more than 60 μg/L and there is a diagnostic dilemma. If sTfR level is raised, a trial of iron therapy is suggested for these patients.

Keywords: anaemia, hypochromic microcytic anaemia, iron deficiency anaemia, thalassaemia, transferrin receptor

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INTRODUCTION

Iron deficiency is a common clinical problem that, in most instances, is relatively simple to diagnose using such conventional laboratory tests of iron status as serum iron, total iron binding capacity, transferrin saturation and ferritin. However, these tests are considerably influenced by acute phase responses, making difficult the distinction between iron deficiency anaemia and anaemia that accompanies infection, inflammation or malignancy. The latter is commonly termed anaemia of chronic disease. The anaemia of chronic disease is the most common cause of anaemia in hospitalised patients. This form of anaemia typically develops in patients suffering from chronic inflammatory disorders that involve activation of cellular immunity.

The detection of iron deficiency in the presence of chronic disease is an important diagnostic challenge because of the frequency of the problem and its direct effect on patient management. For patients with anaemia of chronic disease associated with chronic infection or malignancy, inappropriate iron supplementation should be strictly avoided. Firstly, supplementation of iron may counteract the iron-withholding strategy of the body and favour the growth and proliferation of microbes and tumour cells. Secondly, iron therapy may weaken cell-mediated immune effector mechanisms and promote progression of the underlying disease. In contrast, iron supplementation could conceivably benefit patients with anaemia of chronic disease associated with autoimmune or rheumatic disorders. In this instance, an iron-induced weakening of cell-mediated immunity may help reduce disease

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Fig. 1 Serum transferrin receptor levels in different study populations.

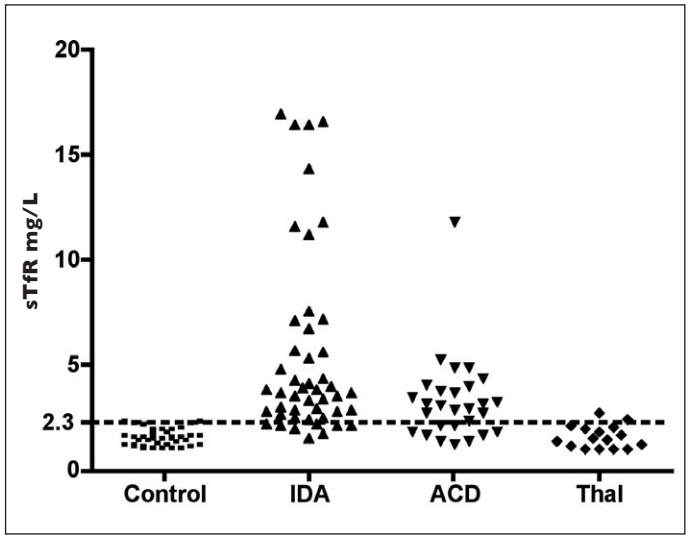


Fig. 2 sTfR/ferritin ratio in different study populations.

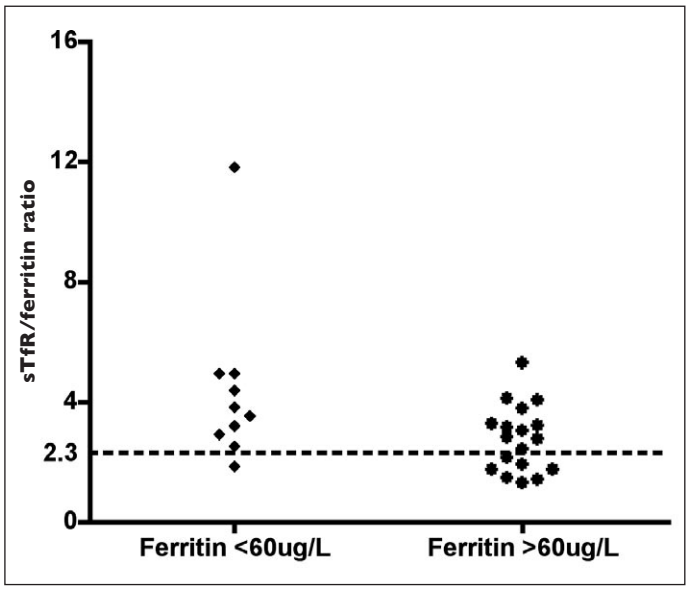
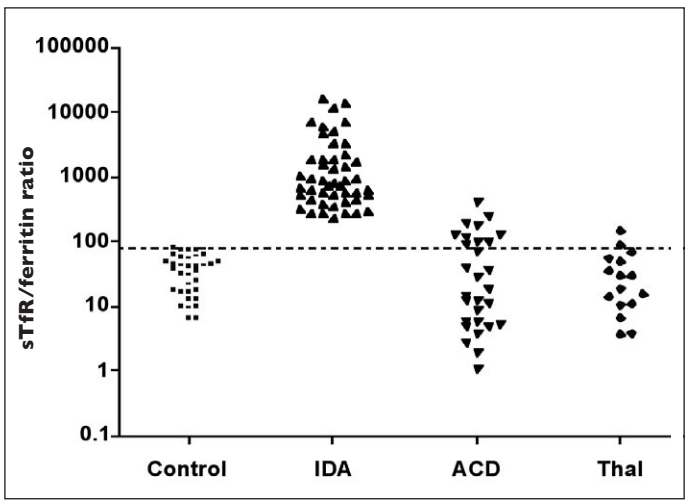


Fig. 3 sTfR/ferritin ratio in different study populations.



activity and improve the anaemia of chronic disease by counteracting TNF-α and IFN-γ activity[1].

Bone marrow examination is generally regarded as the definitive marker of iron deficiency. However, such examination is uncomfortable, cumbersome and impractical for routine use. There is a clinical need for noninvasive and sensitive means of detecting iron deficiency and a possible approach is the estimation of serum soluble transferrin receptor (sTfR). The plasma transferrin receptor concentration is not increased with infection or inflammation, unlike plasma ferritin. Hence, measurement of the plasma transferrin receptor concentration may be especially helpful in the task of differentiating between anaemia of iron deficiency and the anaemia associated with chronic inflammatory disorders.

The serum ferritin level varies with iron stores, while TfR is assumed to reflect the degree of tissue iron supply[2]. The two major determinants of the level of sTfR are body iron status and the bone marrow erythroid expansion and activity[3,4]. Hence, there will be an increased synthesis of sTfR in conditions associated with reduced iron supply to the bone marrow and increased erythropoietic activity. Cytokines such as tumour necrosis factor-α and interleukin-6 have been suggested to reduce transferrin receptor expression in in-vitro experiments[2]. In anaemia of chronic disease, sTfR level is not affected by the reduced plasma iron concentration, since there is a concurrent increase in the cytokine levels.

In a study by Skikne et al, results indicated that serum ferritin is the most sensitive index of iron status when there are residual iron stores, whereas the serum receptor is more sensitive when there is functional iron deficiency. Because of the reciprocal relationship between serum receptor and ferritin measurements, sTfR/ferritin ratio is inversely proportional to the body iron[5]. This ratio would also help in the differentiation of iron deficiency from the anaemia of chronic disease[6]. The purpose of this study was to evaluate the usefulness of sTfR and sTfR/ferritin ratio in the differential diagnosis of hypochromic microcytic anaemia, in addition to the conventional markers used for the diagnosis of iron deficiency anaemia.

METHODS

Full blood counts were determined using the Cell Dyn 4000 analyser. Serum sTfR was measured immunoturbidimetrically using IDeA®sTfR IT kits from Orion Diagnostica applied for Cobas

Mira Plus analyser. The reference range of the kit as mentioned by the manufacturer was 0.9-2.3 mg/L. Ferritin which had a reference range of 22-322.0 μg/L was measured using Advia Centaur Immunoanalyser (Bayer Healthcare, New York, USA). Serum iron (reference range 9.5-29.9 μmol/L) was analysed on Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany).

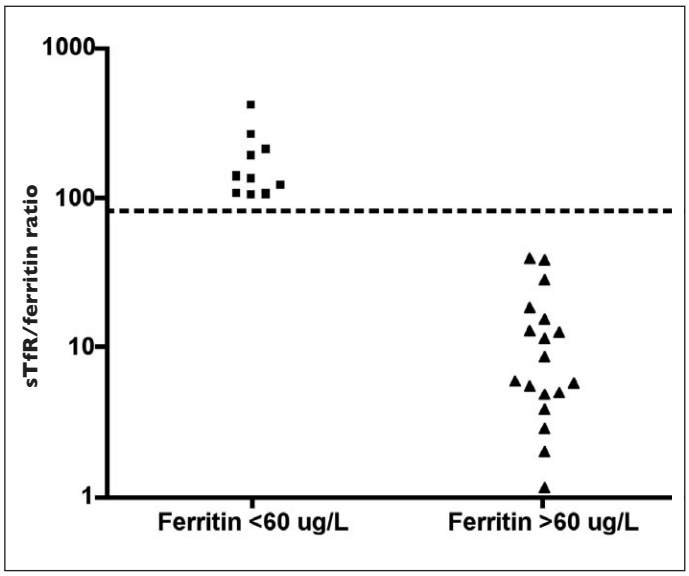
Serum transferrin receptor level was determined on 91 blood samples which had haemoglobin levels less than 110 g/L and MCV <76 fl or MCH <27.0 pg. Patients with abnormal renal function, haemolytic anaemia, vitamins B12 and folic acid deficiency, on iron therapy, or with a history of recent blood transfusion were excluded. These factors are known to affect the sTfR levels. The 91 blood samples were divided into three groups: iron deficiency anaemia, anaemia of chronic disease and thalassaemia. The case records were studied for demographical profile, clinical information and diagnosis. The average age and age range of the study group were 37 and 17 to 69, respectively.

Sera obtained from 35 healthy blood donors were used as control. 47 patients who had iron <9.5 μmol/L and ferritin <12 μg/L were considered to have iron deficiency anaemia (IDA). 28 patients with a history of chronic illness associated with increased ESR or C-reactive protein, who had normal or low iron, and ferritin >12 μg/L, were presumed to have anaemia of chronic disease (ACD). 16 patients who had hypochromic microcytic anaemia, but with normal iron and ferritin were diagnosed to have α- or β-thalassaemia by HPLC Biorad Variant II and Sebia Hydrasys Electrophoresis System. Statistical evaluations were performed by analysis of variance and simple regression analysis.

RESULTS

A summary of the results of the control and the study group is presented in Table I. The serum sTfR level in the control was 1.53 ± 0.8 mg/L which agreed with the manufacturer's stated normal range of 0.9 to 2.3 mg/L. Patients with IDA had a mean sTfR level of 5.53 mg/L (range 1.61-17 mg/L) and a statistically significant increase ($p<0.001$) was observed compared with the control group (Fig. 1). The serum sTfR level was more than 2.33 mg/L in 39 out of 47 IDA patients. The mean level of sTfR in ACD patients was 3.32 mg/L and no statistically significant difference was noted, compared with the control group ($p>0.05$). However, a significant difference was present between the ACD and IDA patients ($p<0.01$). 19 out of 28 patients with ACD were noted to have serum sTfR levels of more than 2.33 mg/L (Fig. 1).

Fig. 4 sTfR/ferritin ratio in the two groups of anaemia of chronic disease.



Using serum ferritin level of 60 μg/L as a cut-off value, patients with ACD were divided in to two groups (Table II and Fig. 2). Ten patients had serum ferritin levels of less than 60 μg/L (Group 1) and 18 had more than 60 μg/L (Group 2). Nine in group 1 had sTfR level >2.33 mg/L and it was statistically significant when compared to the controls ($p<0.01$). 11 out of 18 patients in group 2 also had sTfR levels of more than 2.33 mg/L, and statistically significant difference was noted when compared with the control ($p<0.01$). The serum sTfR levels in group 1 were significantly higher ($p<0.01$) compared to that in group 2. The mean sTfR level in the thalassaemic group was 1.64 mg/L and not significantly different from the control group ($p>0.05$). Only two in this group were noted to have high sTfR levels (Fig. 1). One of them had a history of bleeding haemorrhoids and other was a double heterozygote for HbE and β-thalassaemia trait.

The sTfR/ferritin ratio of the IDA group (2,368.9 ± 7,236.472 μg/μg) was significantly higher than the control (37.6 ± 43.7 μg/μg) ($p<0.05$). There was no significant difference between the control and the ACD group (71.01 ± 193.58 μg/μg) ($p>0.05$) as well as between the control and the thalassaemic group (38.17 ± 79.02 μg/μg) ($p>0.05$). In patients with ACD, the sTfR/ferritin ratio was higher in group 1 (176.31 ± 197.12 μg/μg) compared with that of control and group 2 ($p<0.001$). In group 2, the sTfR/ferritin ratio

Table I. Various parameters in the different study populations.

Parameter	Control (n=35)	IDA (n=47)	ACD (n=28)	Thalassaemia trait (n=16)
HGB (g/L)	138.7 ± 29	81.54 ± 34.02	92.2 ± 23.8	108 ± 29.2
MCV fl	85.1 ± 20.8	63.4 ± 12.36	72.14 ± 14.6	64.4 ± 10.2
MCH pg	29.3 ± 3.4	19.4 ± 5.12	22.7 ± 5.012	20.49 ± 3.5
Serum iron μmol/L	15.08 ± 9.8	3.58 ± 3.32	5.23 ± 6.76	13.5 ± 11.84
Serum ferritin μg/L	60.69 ± 84.88	4.63 ± 5.0	305.5 ± 784.2	129.27 ± 291
STfR mg/L	1.53 ± 0.8	5.53 ± 8.76	3.32 ± 3.94	1.64 ± 1.02
sTfR/ferritin ratio (ug/ug)	37.6 ± 43.7	2368.98 ± 7236.4	71.01 + 193.58	38.17 + 79.02

Values are expressed as mean ± 2 SD.

(16.02 ± 28.97 μg/μg) was not statistically different from that of the control (p>0.05).

DISCUSSION

Evaluation of anaemia in patients with inflammation may be difficult because conventional laboratory measurements of iron status are often unable to differentiate between iron deficiency and anaemia of chronic disorders, making it necessary to do a bone marrow examination to evaluate iron stores and to establish a definitive diagnosis. Nevertheless, this examination cannot be routinely performed since it is invasive, painful, expensive and time consuming. Studies have shown sTfR to be a better indicator of iron deficiency when associated with inflammation[6,9]. The main value of sTfR assay is in the differential diagnosis of microcytic anaemia[8]. Circulating transferrin receptor concentrations do not increase in anaemia secondary to inflammatory disorders[10]. In situations where iron deficiency anaemia co-exists with anaemia of chronic disease, transferrin receptor concentrations increase secondary to the underlying iron deficiency[10].

Soluble transferrin receptor is a trans-membrane glycoprotein that controls the uptake of circulating iron into cells. sTfR can be detected in serum and is reported to be an excellent marker for erythropoiesis[1]. Serum sTfR levels are high in patients with IDA and in conditions with erythroid hyperplasia, such as haemolytic anaemia and polycythaemia, and reduced levels are noted in conditions with erythroid hypoplasia such as aplastic anaemia[1,6]. Increases in serum transferrin receptor reflect cellular iron deficit and its measurement is able to distinguish the anaemia of inflammation from that of iron deficiency[7]. In our study, we noted that sTfR level for the control group was the same as quoted by the manufacturers and increased levels were found in IDA patients (mean 5.53 mg/L). Our results corroborate with those of previous reports[5,6] that sTfR concentrations are higher in patients with iron deficiency.

Table II. Various parameters in the two groups of anaemia of chronic disease.

Parameter	Group 1 Ferritin <60 μg/L	Group 2 Ferritin >60 μg/L
HGB (g/L)	91.15 ± 27.76	92.76 ± 22.86
MCV fl	70 ± 19.72	73.3 ± 11.62
MCH pg	21.14 ± 4.94	23.64 ± 4.34
Serum iron μmol/L	3.99 ± 4.44	5.92 ± 7.66
Serum ferritin μg/L	24.7 ± 10.98	461.49 ± 849.15
STfR mg/L	4.36 ± 5.64	2.74 ± 2.18
sTfR/ferritin ratio (ug/ug)	176.31 ± 197.12	16.02 +28.97

Values are expressed as mean ± 2 SD

Serum sTfR level is inversely correlated with the serum ferritin level in IDA group (p<0.001) and an increased level of sTfR thus reflects iron deficiency. In addition, in this group the sTfR level was noted to have an inverse correlation with Hb, MCV and MCH (p<0.05) but not with serum iron (p>0.05), similar to the findings observed by Pettersson et al[6]. In a study by Skikne et al[5], the mean sTfR/ferritin ratio increased from <100 in the presence of adequate iron stores to over 2000 at the time of significant iron depletion. In our study, the sTfR/ferritin ratio in the control was 37.6 ± 43.7 μg/μg and in the IDA group 2,368.98 ± 7,236.4. indicating significant functional iron depletion. In our study, there was no statistical difference in sTfR concentrations between the control group and ACD, but we noted that 19 of the 28 patients had high sTfR levels (>2.33mg/L). As sTfR is usually not elevated in ACD, the

higher value in these patients suggests the presence of concurrent iron deficiency.

In a study by Chijiwa et al[9], serum ferritin of 60 μg/L was used as a cut-off value between iron depleted and repleted states in patients with rheumatoid arthritis. Using the same cut-off value for ferritin, we noted that the sTfR level was significantly higher in the majority of patients in the iron depleted group (group 1) of ACD patients. In all these patients, sTfR/ferritin ratio was above the cut-off value of 81 (cut-off value = control mean ± 2 SD). According to Pettersson et al[6], a high TfR/ferritin ratio indicated iron deficiency, with or without inflammation. Our results appear to concur with this finding. 10 out of 18 patients in the iron repleted group (group 2) had high sTfR levels but sTfR/ferritin ratio was below the cut-off value. Chijiwa et al[9] noted similar findings in four of their patients in the iron repleted group who were then treated with oral iron supplementation for over three months. The sTfR levels decreased after iron supplementation. We therefore suggest a trial of iron therapy in this category of patients with elevated sTfR.

An increase in sTfR may be found in any condition where there is increased erythropoietic activity. Therefore raised sTfR levels may be seen in conditions associated with erythroid hyperplasia, such as β-thalassaemia and auto-immune haemolytic anaemia. Studies have shown that serum sTfR concentrations are elevated in heterozygous β-thalassaemia[11,12]. In our study, sTfR was not elevated in the thalassaemia group but for two patients. One of them had bleeding haemorrhoids and other was a double heterozygote for HbE and β-thalassaemia trait, which may account for their increased sTfR level. However, sTfR/ferritin ratio was not increased in these two patients. Raised sTfR level in patients with thalassaemia trait and bleeding haemorrhoids may be due to loss of iron, in which case a trial of iron therapy may be tried. However, the increased level of sTfR in double heterozygote for HbE and β-thalassaemia trait could be the result of increased erythropoietic activity. In such instances, iron therapy is not indicated.

In conclusion, we suggest sTfR may be a useful investigation in hypochromic microcytic anaemia to diagnose iron deficiency when associated with chronic inflammation. To be cost-effective, sTfR can be done selectively in ACD patients when ferritin levels are more than 60 μg/L and there is a diagnostic dilemma. It does not provide any additional information in pure iron deficiency anaemia where the routine laboratory investigations are sufficient to make the diagnosis.

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REFERENCES

1. Brittenham GM, Weiss G, Brissot P, et al. Clinical consequences of new insights in the pathophysiology of disorders of iron and heme metabolism. Hematology (Am Soc Hematol Educ Program) 2000: 39-50.
2. Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997; 89:1052-7.
3. Huebers H.A, Beguin Y, Pootraku P, et al. Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 1990; 75:102-7.
4. Kaltwasser JP, Gottschalk R. Erythropoietin and iron. Kidney Int Suppl 1999; 69:S49-56.
5. Skikne BS, Flowers CH, Cook JD. Serum transferring receptor: a quantitative measure of tissue iron deficiency. Blood 1990; 75:1870-6.
6. Pettersson T, Kivivuori SM, Siimes MA. Is serum transferrin receptor useful for detecting iron-deficiency in anaemic patients with chronic inflammatory diseases? Br J Rheumatol 1994; 33:740-4.
7. Kohgo Y, Niitsu Y, Kondo H, et al. Serum transferrin receptor as a new index of erythropoiesis. Blood 1987; 70:1955-8.
8. Baynes RD. Assessment of iron status. Clin Biochem 1996; 29:209-15.
9. Chijiwa T, Nishiya K, Hashimoto K. Serum transferrin receptor levels in patients with rheumatoid arthritis are correlated with indicators of anaemia. Clin Rheumatol 2001; 20:307-13.
10. Ferguson BJ, Skikne BS, Simpson KM, et al. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med 1992; 119:385-90.
11. Skarmoutsou C, Papassotiriou I, Traeger-Synodinos J, et al. Erythroid bone marrow activity and red cell hemoglobinization in iron suffcient beta-thalassemia heterozygotes as reflected by soluble transferrin receptor and reticulocyte hemoglobin in content. Correlation with genotypes and Hb A(2) levels. Haematologica 2003; 88:631-6.
12. Bianco I, Mastropietro F, D'Asero C, et al. Serum levels of erythropoietin and soluble transferrin receptor in the course of pregnancy in non beta thalassemic and beta thalassemic women. Haematologica 2000; 85:902-7.