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Original Article

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Soluble transferrin receptor in sickle cell diseases: correlation with spleen function

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ABSTRACT

Objective: To correlate spleen function with soluble transferrin receptor (sTfR) levels and red cell ferritin (RCF) values in patients with sickle cell diseases.
Design: Prospective study.
Location: University Hospital, School of Medical Sciences, State University of Campinas; a tertiary hospital.
Participants: 60 patients with sickle cell diseases, in a steady state, who had not received blood transfusions for 3 months; 28 normal individuals with no clinical or laboratory signs of anemia.
Measurements: Determination of serum iron, transferrin iron-binding capacity, serum ferritin, RCF and sTfR. Evaluation of spleen function: erythrocytes with pits were quantified.
Results: Patients with sickle cell anemia had sTfR levels significantly higher than in normal individuals or those with HbSC (p=0.0001) and there was an inverse correlation between sTfR and fetal Hb (p=0.0016). RCF values were significantly higher in sickle cell anemia patients than in normal individuals or those with HbSC (p=0.0001), and there was a correlation between RCF and pitted erythrocytes (p=0.0512).
Conclusion: The association between sTfR and fetal Hb confirms the contribution of fetal Hb to improving the hemolytic state by minimizing the consequent reactive erythrocyte expansion. High sTfR levels are not related to the degree of spleen function deficiency seen in sickle cell disease patients. The deficiency in the exocytosis process of the spleen occurring in sickle cell anemia patients may contribute to their accumulation of RCF.
Key words: Hemoglobinopathies. Pitted erythrocytes. Red cell ferritin. Soluble transferrin receptor. Sickle cell disease.

INTRODUCTION

The transferrin receptor, a glycoprotein present on the surface of most cells, is responsible for binding transferrin during the endocytosis of iron. The soluble form of the receptor (sTfR) was initially described by Kohgo et al and is derived from the cleavage of the extracellular portion of the receptor.[1] The expression of sTfR is regulated by the availability of iron. Thus, an iron deficiency rapidly induces synthesis of the receptor and an excess of iron suppresses its synthesis.[1-3] High levels of sTfR have been reported in immune hemolytic anemia, hereditary spherocytosis, Hb H disease, β thalassemia intermedia and sickle cell syndromes. The reported correlation between the degree of hemolysis and sTfR levels supports the hypothesis that the determination of sTfR concentrations could be a useful indicator of the degree of erythrocyte expansion.[4-6]

Singhal et al[5] analyzed the clinical significance of sTfR levels in patients with S hemoglobinopathy and found that there was no alteration in the serum concentrations of this receptor during infectious situations or painful crises. However, they observed high sTfR levels in patients with hypersplenism, which returned to normal values after splenectomy. These

changes most likely reflect the extent of erythrocyte expansion before and after splenectomy. In individuals with sickle cell disease, repeated infarctions resulting from vascular occlusion by erythrocytes leads to deficient functioning of the spleen, and hence, to a greater susceptibility to infections.[7] Deficient functioning of the spleen may also be related to high levels of red cell ferritin (RCF) in patients with hemoglobinopathies, probably due a deficiency in the exocytosis of excess ferritin, a process which occurs preferentially in the spleen.[8]

To investigate the relation between sTfR levels and the degree of spleen function deficiency, we have examined the correlation between the serum concentrations of the sTfR and the number of erythrocytes with membrane irregularities ("pits") in patients with S hemoglobinopathy. Pitted erythrocytes are a well-established indicator of spleen function.[9]

METHODS

Patients. Sixty patients with sickle cell diseases seen at the Hemocentro-Unicamp were studied. Forty-three had sickle cell anemia (SS), seven had Sβ thalassemia (Sβthal), nine had hemoglobin SC disease and one was CC homozygous (SC+CC group). All patients were adults in a steady state and had not received a blood transfusion for 3 months. They all gave their informed consent to participate in this study which was approved by the hospital Ethics Committee. Twenty-eight normal individuals (N) with no clinical or laboratory signs of anemia served as the control for this study.

Hematological profile. The hematological measurements were obtained using a Cobas Argos (ABX - Horiba, France) analyzer. The levels of fetal hemoglobin (HbF) were determined by an alkaline denaturation method.[10]

TABLE I - Iron status of the various groups studied

	N			SS			SβThal†			SC+CC		
Parameters	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
SI	28	88.18	30,0	43	129.53*	59.0	7	137.14	59.4	10	105.7	27.85
(μg/dL)		(38-155)			(41-254)			(75-253)			(65-144)	
TIBC	28	300.93	59.1	43	287.14	69.7	7	296.71	5.71	10	303.6	35.0
(μg/dL)		(218-394)			(174-454)			(245-389)			(242-364)	
SF	28	63.23	46.9	43	608.95*	1171.5	7	985.79	1249	10	291.1	385.7
(ng/ml)		(11-162)			(12-5870)			(36-3020)			(36-1280)	
RCF	28	11.47	7.48	41	50.29*	45.9	7	54.76	37.1	10	18.78**	10.9
(att/cell)		(3.22-35.3)			(9.58-213.3)			(8.7-128.4)			(2.8-33.3)	
sTfR	28	2.10	0.37	42	13.69*	5.23	5	13.04	3.95	9	6.58*/**	1.36
(μg/ml)		(1.3-2.83)			(2.54-29.44)			(8.88-17.6)			(4.76-8.08)	

[(range)]

SI: serum iron; TIBC: transferrin iron binding capacity; SF: serum ferritin

RCF: red cell ferritin; sTfR: soluble transferrin receptor

* significantly different from N ($p<0.05$)

** significantly different from SS ($p<0.05$)

† no comparison made (n=7)

Evaluation of the iron state. Determination of serum iron (SI) and the transferrin iron-binding capacity (TIBC) was done with a Mira Plus Cobas analyzer (Roche - Switzerland) using Unimate 5 Iron and Unimate 7 UIBC kits (Roche Diagnostic Systems - Switzerland). Serum ferritin (SF) was determined by a fluorometric immunoenzymatic test (Stratus-Dade International Inc. - Miami, USA) and hemolyzed RCF levels were quantified by a rapid freeze-thaw method followed by a fluorometric immunoenzymatic assay.[11] The serum concentration of sTfR was measured by an immunoenzymatic technique (Quantikine R&D Systems - USA). The samples from SS and Sβthal patients were diluted 1:400 prior to assaying. All samples were kept at -80 ^{0}C.

Evaluation of spleen function. Erythrocytes with pits were quantified by interference contrast microscopy.[12]

Statistical methods. For comparison between the groups, the Mann-Whitney or Kruskal-Wallis tests were employed for variance analysis and the Spearman correlation coefficient test was used for assessing the association between variables, with level of significance set at <0.05.

RESULTS

Evaluation of the sTfR levels. The serum concentrations of sTfR were statistically different when the N and SS, N and SC+CC, and SS and SC+CC groups were compared ($p<0.05$) (Table I). In the Sβthal group, only five patients were evaluated for sTfR levels, all of whom showed extremely high values (Figure 1). Two SS individuals had laboratory profiles compatible with iron deficiency anemia: microcytic hypochromic anemia, reduced levels of SI and SF, high TIBC, transferrin saturation (TS) <15% and a normal level of Hb A_2. The sTfR concentrations for these patients did not differ from those observed in other members of the same group. However, the receptor / ferritin ratio was >500 in both cases, a finding compatible with iron depletion.[2] A significant inverse correlation between sTfR levels and the HbF concentration was seen in SS patients, but not in the SC+CC group (Table III).

sTfR levels and spleen function. All patients with sickle cell diseases presented some degree of spleen function deficiency based on how much above normal values (>2%) the pitted erythrocyte percentage was.[12] The degree of variability in this parameter was considerable (Table II). No correlation was observed between the number of pitted erythrocytes and the sTfR values. To determine whether an elevated RCF could have resulted from a malfunction in spleen exocytosis capacity, we examined the correlation between the number of pitted erythrocytes and RCF levels. A positive correlation was observed ($p=0.051$) between these two variables in SS patients, but not in SC or CC individuals (Table III).

DISCUSSION

We found a significant increase in sTfR levels in all of the patients studied, with significantly higher levels in the SS group than in the SC+CC group. These findings agree with those in the literature.[5,14-16]

The transferrin receptor is a membrane glycoprotein responsible for internalizing transferrin in the erythrocyte cell. Following the formation of a vesicle, fusion of the endosomes and acidification, iron is released for heme synthesis, while the receptor returns to the cell membrane and the apotransferrin to the

TABLE 2 - Evaluation of spleen function - % of erythrocytes with pits

Group	n	mean (range)	SD
N	28	0.66 (0 to 1.8)	0.54
SS	43	26.51 (5 to 41.8)	7.98
Sßthal	7	21.74 (2.9 to 44.6)	15.68
SC+CC	10	22.17 (5 to 47.2)	12.16

plasma.[4,17] The principal factors regulating the density of these receptors in the cells are the quantity of iron, stimulation by erythropoetin and the cell cycle.[18] The sTfR is found in human plasma and appears be a truncated form of the tissue receptor generated by a proteolytic mechanism that is still not well understood.[17] It has been suggested that the serum concentration of sTfR may be an accurate indicator of iron depletion, especially in distinguishing between iron-deprivation anemia and chronic disease anemia.[19] In situations where erythropoiesis is threatened, such as in aplastic anemia, the sTfR levels are significantly lower than in patients with iron deficiency anemia, hemolytic anemia or in normal individuals.[20]

In hemoglobinopathy, an elevation in sTfR levels may result from the high degree of erythrocyte expansion found in this illness. Corroborating this hypothesis is the demonstration of an inverse relationship between sTfR levels and the age of the patients, as well as between sTfR levels and HbF rates.[5] Serjeant et al[16] studied the possible determinants of HbF levels in SS patients in Jamaica and found that HbF had no influence on sTfR levels, although there was a tendency for sTfR concentrations to decrease as the Hb level increased. In our subjects, there was an inverse correlation between the sTfR and HbF levels in the SS, but not the SC+CC group. This negative correlation in SS patients supports the hypothesis that high HbF levels inhibit sickling and hemolysis, thereby minimizing erythropoietic activity.[5]

The results obtained in the present study have confirmed those reported in the literature, indicating that patients with sickle cell anemia have some degree of spleen function deficiency.[7] Whilst there may be great variability in the pitted erythrocyte counts in these cases, they are always higher than in normal individuals.[12,21,22] In none

TABLE 3 - Correlation between variables

Variables	Group	n	Correlation coefficient (r)	p value
sTfR x HbF	SC+CC	9	0.4667	0.2054
	SS	41	-0.4765	0.0016*
sTfR x PITS	SC+CC	9	-0.2833	0.4600
	SS	42	-0.0550	0.7292
sTfR x RCF	SC+CC	9	0.3000	0.4328
	SS	40	-0.1298	0.4248
RCF x PITS	SC+CC	9	0.0546	0.8810
	SS	41	0.3066	0.0512
sTfR x HbF	SC+CC	9	0.4667	0.2054
	SS	41	-0.4765	0.0016*
sTfR x HbF	SS, RPI<2	11	-0.8767	0.0004*
	SS, RPI>3	23	-0.2347	0.2810
RCF x SF	SS	41	0.4774	0.0016*
	SC+CC	10	-0.4128	0.2291
TS x RCF	SS	41	0.3645	0.0191*
	SC+CC	10	0.8182	0.0038

* statistically significant

HbF: fetal hemoglobin

RCF: red cell ferritin

TS: transferrin saturation

RPI: reticulocyte production index

of our patients was there any spleen hyperactivity, nor had any of them undergone splenectomy. Our analysis therefore refers to sTfR behavior in patients with hypoactive spleens in whom erythropoietic activity was not exacerbated. In these cases, the abnormally high sTfR values result from chronic hemolysis and from erythropoietic expansion at the level of the marrow with a greater release of red cells into the circulation. Crosby,[23] commenting on hematopoiesis in the human spleen, indicated that in diseases that require a high reactive production of red blood cells by the bone marrow, small clusters of hematopoietic cells may be found in the spleen. This possibility may be considered to make some contribution to the elevation of sTfR levels in sickle cell disease patients.

In our study, high RCF levels were seen in SS and Sβthal patients; patients with C hemoglobinopathy had values approximating those of normal individuals. The possible causes of high RCF in hemoglobinopathy include greater capture of iron by erythrocyte tissue, inadequate utilization of iron in Hb synthesis, an increase in apoferritin synthesis subsequent to greater release of iron due to intracellular denaturation, increased erythrocyte destruction and deficiency in the process of exocytosis or "pitting" resulting in the inefficient removal of excess ferritin by the spleen.[8,9,13]

We studied the latter aspect by quantifying the number of pitted erythrocytes. Although the correlation coefficient was not significant, its value suggests a possible contribution of spleen function deficiency to RCF accumulation in patients with sickle cell anemia.

We conclude that patients with sickle cell diseases show important alterations relevant to sTfR behavior. In SS patients, the association between sTfR and HbF confirms the contribution of HbF to improving the hemolytic state by minimizing the consequent reactive erythrocyte expansion. RCF levels as an indicator of iron accumulation have a limited value and need to be analyzed together with other pertinent laboratory parameters. Spleen function deficiency apparently does not interfere with or participate in alterations of iron metabolism, although deficiencies from "pitting" could contribute to a reduced ability to remove ferritin from erythrocytes.

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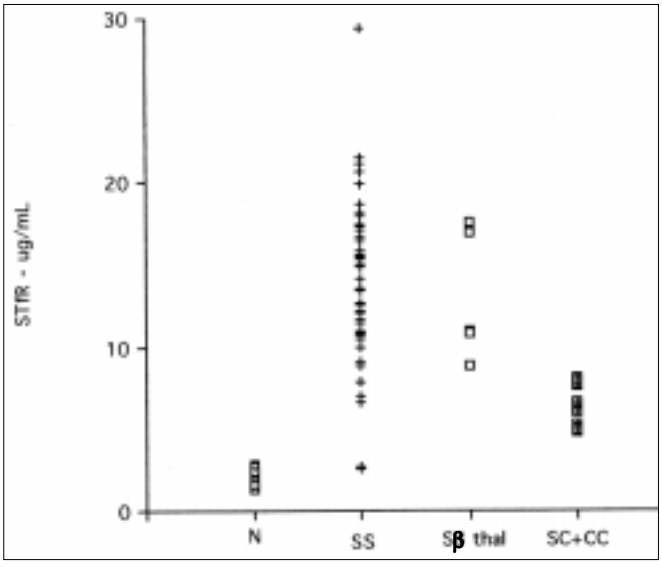

Figure 1 - Distribution of STfR levels in sickle cell diseases.

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RESUMO

Objetivo: Relacionar a função esplênica com os níveis do receptor solúvel da transferrina (sTfR) e com os valores da ferritina intra-eritrocitária (RCF) em pacientes com doenças falciformes. **Tipo de Estudo:** Estudo prospectivo. **Local:** Hospital das Clínicas da Faculdade de Ciências Médicas da UNICAMP, atendimento terciário. **Participantes**: 60 pacientes portadores de doenças falciformes, na fase estável da doença, sem transfusão de sangue nos últimos 3 meses; 28 indivíduos normais, sem sinais clínicos e laboratoriais de anemia. **Variáveis estudadas**: Determinação do ferro sérico, capacidade de ligação do ferro à transferrina, ferritina sérica, RCFe sTfR. Avaliação da função esplênica: quantificação de hemácias com "pits". **Resultados:** Pacientes com anemia falciforme: os níveis de sTfR foram significativamente mais elevados do que nos indivíduos normais e com Hb SC (p=0,0001); correlação inversa entre sTfR e Hb Fetal (p=0,0016); valores de RCF significativamente mais elevados do que nos indivíduos normais e pacientes SC (p=0,0001); correlação entre RCF e hemácias com "pits" (p=0,0512).**Conclusões:** A associação entre sTfR e Hb Fetal confirma a contribuição da Hb Fetal na melhora do quadro hemolítico, o que, conseqüentemente, minimiza a reativação da expansão eritróide. Os altos níveis de sTfR observados nos pacientes com doença falciforme não estão relacionados com o grau de hipofunção esplênica. A deficiência do processo de exocitose exercido pelo baço pode contribuir para o acúmulo de RCF nos pacientes com anemia falciforme.