Original Article

Soluble Transferrin Receptors in the diagnosis of iron deficiency anemia, ß thalassemia minor and ß thalassemia minor with concomitant iron deficiency anemia

Muhammad Saboor[1], Moinuddin[2]

ABSTRACT

Objective: Iron deficiency anemia and ß thalassemia minor are two important causes of microcytic and hypochromic anemia. ß thalassemia minor with concomitant iron deficiency anemia make the diagnosis difficult through conventional laboratory tests. Determination of soluble transferrin receptors is a helpful laboratory test for the diagnosis with certainty. Purpose of this study was to evaluate the role of soluble transferrin receptors in the differentiation of iron deficiency anemia from ß thalassemia minor.

Methodology: A total of 80 subjects were enrolled in this study. They were divided into four groups i.e. 20 normal adult male, 20 normal adult female, 20 patients with iron deficiency anemia group and 20 patients with ß thalassemia minor. Patients with ß thalassemia minor were further sub grouped in ß thalassemia minor with and without concomitant iron deficiency anemia. Soluble transferrin receptors were determined by ELISA technique using Quantikine IVD kit (R & D Systems).

Results: Levels of sTfR in individuals with ß thalassemia minor were increased but these were lower than in iron deficiency anemia group. Mean sTfR levels were higher in patients with ß thalassemia minor and concomitant iron deficiency anemia than in normal subjects as well as in patients with ß thalassemia minor alone. Their levels were similar to those in patients with iron deficiency anemia.

Conclusion: sTfR can be used as a discriminating marker between patients with iron deficiency anemia and ß thalassemia minor alone. Care must be taken while dealing the patients with ß thalassemia minor and concomitant iron deficiency anemia.

KEY WORDS: sTfR, Iron deficiency anemia, ß thalassemia minor.

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1. Muhammad Saboor, M.Sc (Microbiology), M.Sc, M.Phil (Hematology),
2. Dr. Moinuddin, FRCP (C), FRCP (E),
Director, Dean, Faculty of Medicine and Dentistry,
1-2: Baqai Institute of Hematology, Baqai Medical University, Karachi, Pakistan.

Correspondence:

Muhammad Saboor,
Assistant Professor,
Baqai Institute of Hematology,
Baqai Medical University,
Karachi - Pakistan.
E-mail: mujhgan_16@hotmail.com

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INTRODUCTION

Iron in the plasma is transported by transferrin which delivers iron to the cells through the intermediation of membrane associated transferrin receptors.[1] Soluble transferrin receptors (sTfR) are the proteolytic products of transferrin receptors; their concentration is proportional to the amount of transferrin receptors expressed on the cell surface. Increased cellular requirement for iron increases the concentration of transferrin receptors on the cell membrane. Individuals with iron deficiency anemia have elevated sTfR levels.[2] Increased concentration of

soluble transferrin receptors is therefore a reliable indicator of iron deficiency anemia. However sTfR levels may also be increased in non sideropenic erythroid hyperplasia.[3]

Iron deficiency and ß thalassemia minor are the two most important causes of microcytic and hypochromic anemia.[4] In most cases it is easy to diagnose these conditions through conventional laboratory tests. These tests however have limitations due to their poor sensitivity or specificity.[5] Difficulty may also arise when ß thalassemia minor is associated with iron deficiency anemia.

In this study sTfR levels were measured in patients with ß thalassemia minor and iron deficiency anemia to ascertain whether this parameter can be used as a diagnostic tool for their accurate diagnosis.

METHODOLOGY

A total of 80 whole blood samples (10cc each) were collected i.e. 20 samples each from normal male and female for establishing the reference ranges of sTfR while 40 samples were obtained from patients with hypochromic and microcytic blood picture. All individuals were divided into four groups' i.e. normal, iron deficiency anemia, ß thalassemia minor alone and ß thalassemia minor with concomitant iron deficiency anemia.

Each sample was divided into two portions; 3 ml of blood was added to a tube containing EDTA (ethylene diamine tetra acetic acid) as an anticoagulant while 7 ml of blood was placed in a glass tube without anticoagulant to obtain serum.

To evaluate the role of soluble transferrin receptors as a differentiating marker between iron deficiency anemia and ß thalassemia minor, certain hematogical and biochemical parameters were determined; these include:

1. complete blood counts
2. morphology of the stained peripheral blood smear
3. Hb electrophoresis for the identification of any abnormal hemoglobins
4. estimation of serum iron profile
5. quantitation of soluble transferrin receptors

Complete blood counts, serum iron profile and sTfR determination: CBC of all samples was determined by automated cell analyzer (Sysmex PO H 100i). This included Hb estimation, red blood cell count, total leukocyte count, platelet count, packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). Morphology of blood cells was studied on blood smears stained with Leishman's stain under high power and oil immersion lens of light microscope (Nikon, Japan). Blood samples collected in EDTA tubes were used for the diagnosis of variants of hemoglobin by cellulose acetate hemoglobin electrophoresis at pH 8.6.

Serum was separated from the clotted blood after centrifugation. Serum was divided into three parts; two of these were stored at –20° C for the determination of serum ferritin and soluble transferrin receptors while the third aliquote was tested for serum iron and total iron binding capacity.

Serum iron was determined spectrophometrically using POINT SCIENTIFIC, INC kit. UIBC was determined using POINT SCIENTIFIC, INC kit. TIBC is the calculated sum of serum iron concentration and UIBC. Transferrin saturation was calculated from serum iron and TIBC concentration according the following formula:

$$\% \text{ Transferrin saturation} = \frac{\text{Serum iron}}{\text{TIBC}} \times 100$$

Serum ferritin was determined by Enzyme Linked Immuno Sorbant Assay method using POINT SCIENTIFIC, INC kit. Soluble transferrin receptors were determined by ELISA method using Quantikine IVD kit (R & D Systems).

Statistical analysis: All data of this study were analyzed using the statistical package SPPS Version 13.0.

RESULTS

Samples were divided into four groups based upon their laboratory findings i.e. normal, iron deficiency anemia, ß thalassemia minor and ß thalassemia minor with concomitant iron deficiency anemia. Results of complete blood counts, serum iron profile, hemoglobin electrophoresis and soluble transferrin receptors level of all four groups are shown in Table-I.

DISCUSSION

In this study, mean level of soluble transferrin receptors was 32.51±4.35 nmol/l in normal adult males and 34.56±4.27 nmol/l in normal adult female. In iron deficiency anemia group Hb, Hct, RBC, MCH, MCHC had a significant correlation with the sTfR levels (p<0.01). RDW in iron deficiency anemia group also showed a similar correlation with sTfR level (p<0.008). No correlation was observed between MCV and sTfR (p=0.161) as shown in Table-II.

Serum levels of soluble transferrin receptors were higher in patients with iron deficiency anemia than

Table-I: Consolidated result of red cell parameters and indices, hemoglobin electrophoresis, serum iron profile and soluble transferrin receptors of normal adult male, normal female, iron deficiency anemia group and ß Thalassemia minor group.

Parameters	*N M(n 20)*	*N F(n 20)*	*IDA M (n 03)*	*IDA F(n 17)*	*Thal M (n 11)*	*Thal F (n 05)*	*Thal with IDA M (n 02)*	*Thal with ID A F (n 02)*
Hb (g/dl)	14.36±0.95	12.73±0.64	8.0±3.38	9.61±0.96	12.0±1.1	10.7±0.9	12.0±0.4	9.9±0.21
	(12.5-15.9)	(11.9-14.3)	(4.1- 10.1)	(6.2-12.0)	(9.2-13.1)	(9.8-12.2)	(11.7-12.4)	(9.8-10.1)
PCV (%)	42.56±2.52	37.70±2.50	26.73±7.40	29.87±5.13	40.7±4.9	36.2±2.6	40.5±1.6	33.9±0.21
	(38.2-47.1)	(32.3-43.0)	(18.2-31.5)	(20.3-36.9)	(31.8-52)	(32.6-40.1)	(39.3-41.7)	(33.8-34.1)
RBC (Mil/µl)	4.91±0.7	4.4±0.30	3.62±0.75	4.33±0.66	6.1±0.4	5.5±0.5	6.2±0.4	5.13±0.11
	4.38-.30	3.98-.04	2.76-.15	3.10-5.56	(5.46-6.92)	(5-6.4)	(5.93-6.52)	(5.05-5.13)
MCV (fl)	86.32±5	86.39±5.24	64.33±7.7	66.21±6.25	65.2±4.3	65.0±3.9	65.1±1.6	66.2±0.98
	(75.495.9)	(74.3-92.7)	(56.1-71.0)	(52.8-75.0)	(57.6-74.2)	(61.7-71.2)	(64-66)	(65.5-66.9)
MCH (pg)	29.02±2.06	28.71±2.69	19.33±5.58	20.98±2.77	19.8±1.5	21.3±3.7	19.3±0.4	19.0±0.56
	(24.1-32.1)	(22.1-36.8)	(14.9-25.5)	(14.4-25-2)	(16.7-22.9)	(18.8-27.6)	(19-20)	(18.6-19.4)
MCHC (%)	33.39±1.31	32.36±1.00	27.63±4.87	30.44±1.58	30.3±1.0	29.6±0.9	29.7±0.07	28.7±1.2
	(30.3-36.0)	(29.7-33.6)	(22.5-32.2)	(27-33)	(28.9-32.8)	(28.5-30.4)	(29.7-29.8)	27.8-29.6)
RDW (%)	14.26±0.90	14.49±1.46	19.0±2.55	17.60±2.44	19.9±1.2	18.9±1	19.7±0.9	21.0±2.3
	(12.4-15.7)	(12.0-18.5)	(16.5-21.6)	(14.7-25.8)	(17-22)	(17.8-20.1)	(19.4-20.4)	(19.4-22.7)
Hb A	96.17±0.17	96.13±0.21	97.16±0.23	97.01±0.44	93.9±0.5	94.2±0.5	93.9±0.4	93.9±0.07
	(96.0-96.6)	(95.8-96.6)	(96.9-97.3)	(96.1-97.7)	(93.1-95)	(93.7-95)	(93.6-94.3)	(93.9-94)
HB A_2	3.13±0.21	3.11±0.21	2.06±0.37	2.29±0.40	5.4±0.5	5.0±0.4	5.4±0.2	5.1±0.2
	(3-3)	(2.7-3.4)	(1.8-2.5)	(1.7-3.0)	(4.5-6.7)	(4.5-5.6)	(5.2-5.6)	(5-5.2)
Hb F	0.70±0.11	0.76±0.10	0.77±0.15	0.69±0.13	0.6±0.1	0.6±0.1	0.7±0.1	0.6±0.06
	(0.5-0.9)	(0.5-0.9)	(0.6-0.9)	(0.5-0.9)	(0.2-0.9)	(0.5-0.8)	(0.6-0.8)	(0.6-0.7)
Iron (µg/dl)	104.65±26.12	85.11±20.83	23.67±6.02	23.92±11.52	108.6±33.3	90.2±23	34.7±9.8	34.7±9.8
	(60-141)	(56-125)	(18-30)	(10.28-47.61)	(55.5-147)	(69-125)	(27.7-41.6)	(27.7-41.66)
TIBC (µg/dl)	301.82±28.17	304.07±36.66	448.33±32.53	436.40±46.10	299.9±69.5	309.8±52.1	247.9±24.2	299.41±9.8
	(245.3-350.0)	(256-41)	(415-448)	(295-497)	(214.8-390.6)	(260-392)	(160.1-355.7)	(292.4-306.3)
% Sat of TIBC	35.30±10.39	28.32±7.70	5.36±1.75	5.93±3.4	36.1±8.1	30.0±10.2	14.87±3.4	11.54±2.9
	(17.65-50.0)	(16.96-46.99)	(3.75-7.22)	(2.072-11.75)	(23.9-46.2)	(17.6-45.8)	(12.4-17.3)	(9.49-13.59)
Ferritin (ng/ml)	147.50±48.19	75.73±21.88	8.30±4.09	7.21±2.12	38.9±11.0	37.2±7.7	9.1±2.9	12.7±7.7
	(46.14-220.60)	(40.3-110.5)	(4-12)	(2.8-11.0)	(24.6-58.5)	(29-48.2)	(7-11)	(7.2-18.2)
sTfR(nmol/l)	32.51±4.35	34.56±4.27	122.53±10.90	121.02±15.40	57.2±5.6	58.2±7.2	117.7±3.3	120.75±3.8
	(26.9-40.3)	(29.5-41.7)	(112.3-134)	(100-142)	(48.5-68.6)	(48.4-68.3)	(115.3-117.7)	(118-123.5)

Data are means ± SD, Hb= Hemoglobin, PCV= Packed Cell Volume, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, RDW= Red Cell Distribution Width, Fe= Serum iron, TIBC= Total Iron Binding Capacity, % Sat= % Saturation of Transferrin, Fer= Ferritin, N= Normal, M= Male, F= Female, IDA= Iron Deficiency Anemia, Thal= ß Thalassemia Minor.

in control group; mean values being 122.53±10.90 nmol/l in males and 121.02±15.40 nmol/l in females as compared to normal controls (32.51±4.35 nmol/l and 34.56±4.27 nmol/l in male and female groups respectively). Our results correlated well with those reported in the literature.[6-12] Increased level of sTfR thus reflects iron deficiency anemia or iron deficient erythropoiesis.[13,14]

Correlation between sTfR, serum iron, TIBC and % transferrin saturation in this group was statistically significant (p<0.01) in both sexes as shown in Table-II. Correlation between sTfR and serum ferritin was insignificant (p>0.01).

Correlation of soluble transferrin receptors with packed cell volume, red cell count and mean cell hemoglobin concentration in ß thalassemia minor was significant (p<0.01). In this group hemoglobin level, mean cell volume, mean cell hemoglobin and red cell distribution width showed no correlation

Table-II: Pearson correlation between sTfR, complete blood counts and serum iron profile.

Parameters	*Iron Deficiency Anemia*		*ß Thalassemia Minor*	
	r	p	r	p
Hb.sTfR	-.688*	.000	-.246	.296
Hct.sTfR	.977*	.000	.884*	.000
RBC.sTfR	.807*	.000	.764*	.000
MCV.sTfR	.326	.161	.316	.174
MCH.sTfR	.671*	.001	.090	.705
MCHC.sTfR	.750*	.000	.624	.003
RDW.sTfR	-.577*	.008	.410	.073
Sfe.sTfR	-.825**	0.000	-.704**	0.001
TIBC.sTfR	.469*	0.037	-.274	0.242
% Sat.sTfR	-.782**	0.000	-.665**	0.001
Sfer.sTfR	-.325	0.162	-.731**	0.000

** correlation is significant at the 0.01 level.
*correlation is significant at the 0.05 level.
r= Pearson correlation, p=Sig.

(p>0.01) with the level of soluble transferrin receptors as shown in Table-II.

In these patients serum iron, % saturation of transferrin and serum ferritin showed significant correlation (p<0.01) while total iron binding capacity showed no correlation (p>0.01) with soluble transferrin receptors as shown in Table-II. High levels of soluble transferrin receptors in ß thalassemia minor compared with the control group probably reflect increased bone marrow activity as in iron deficiency anemia. Level of sTfR in iron deficiency anemia was much higher than in patients with ß thalassemia minor. These findings are supported by DHYPERLINK "/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Du ru%20F%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus"Duru et al[15], Ragab et al[16] & Polat et al.[17]

In iron deficiency anemia and ß thalassemia minor the concentration of soluble transferrin receptors is increased but there is a wide variation in their level in ß thalassemia minor group as shown in Table-I. This can be explained on the basis of serum iron profile in ß thalassemia minor. Based upon serum iron profile, ß thalassemia minor group was further divided into two subgroups i.e. ß thalassemia minor with normal iron profile and ß thalassemia minor with iron profile reminiscent of iron deficiency anemia. Patients with ß thalassemia minor with normal serum iron profile had increased sTfR levels but their level was significantly lower than those in ß thalassemia minor with concomitant iron deficiency. Mean sTfR levels were similar in patients with iron deficiency anemia and in patients with ß thalassemia minor with concomitant iron deficiency. This is listed in Table-III. Care must therefore be taken while dealing with patients who have ß thalassemia minor lest they have an underlying iron deficiency state.

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Table-III: Comparison of sTfR levels in the studied groups.

	N	*Minimum*	*Maximum*	*Mean*	*Std. Deviation*
Male thal	11	48.5	68.6	57.22	5.67
Female thal	05	48.45	68.30	58.24	7.28
Male thal with IDA	02	115.3	120.1	117.70	3.39
Female thal with IDA	02	118	124	120.75	3.88
Male with IDA	03	112.3	134.0	122.53	10.90
Female with IDA	17	100	142	121.02	15.40

thal= ß Thalassemia Minor, IDA= Iron deficiency anemia

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Authors Contribution:

Muhammad Saboor carried out the research work, statistical analysis and writing of manuscript. Dr. Moinuddin supervised, reviewed and finalized the research the article.

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