ORIGINAL ARTICLE

SOLUBLE TRANSFERRIN RECEPTOR: A DIFFERENTIATING MARKER BETWEEN IRON DEFICIENCY ANAEMIA AND ANAEMIA OF CHRONIC DISORDERS

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Background: Iron deficiency anaemia and anaemia of chronic disorders are the two major causes of microcytic and hypochromic anaemia. Many times the diagnosis of these conditions becomes difficult through conventional laboratory tests. Determination of soluble transferrin receptors is a helpful laboratory test for the differential diagnosis of these conditions. The study was conducted to evaluate the role of soluble transferrin receptors in the differential diagnosis between iron deficiency anaemia and anaemia of chronic disorders. **Methods:** A total of 80 blood samples were evaluated, i.e., 20 samples from normal adult male, 20 samples from normal adult female, 20 samples from iron deficiency anaemia group and 20 samples from patients with anaemia of chronic disorders. Soluble transferrin receptors were determined by ELISA technique using Quantikine IVD kit (R and D Systems). **Results:** There was significant difference in the levels of sTfR in iron deficiency anaemia and anaemia of chronic disorders. Statistically non-significant difference was observed between the levels of sTfR in patients with anaemia of chronic disorders as compared to normal control group. **Conclusion:** The sTfR determination can be used as a reliable differentiating marker in the diagnosis of iron deficiency anaemia and anaemia of chronic disorders.

Keywords: sTfR, iron deficiency anaemia, anaemia of chronic disorders

INTRODUCTION

Soluble transferrin receptors (sTfR) are the truncated form of the intact transferrin receptors found in the soluble form in human serum.[1,2] They lack the first 100 amino acids and circulate in the form of a complex of transferrin and its receptor.[3] Their molecular mass is 85 kDa.[4] sTfR is produced by proteolysis which is mediated by a membrane associated serine protease. Cleavage is affected between amino acids Arg-100 and Leu-101.[4] It takes place mostly on the surface of the exosomes within the multivesicular bodies prior to exocytosis. Factors which determine the targeted receptors for proteolytic cleavage are unclear.[5]

Virtually all cells except mature red cells have transferrin receptor (TfR) on their surface; largest numbers are in the erythron, placenta and liver. In normal adults, about 80% of TfR are in the erythroid marrow. Receptor density on proliferating cells is related to the availability of iron; deprivation of iron results in prompt induction of TfR synthesis whereas excess iron suppresses their synthesis. Therefore total mass of cellular TfR depends both on the number of erythroid precursors in the bone marrow and on the number of molecules per cell which is a function of the iron status of the cells.[6–8]

Evaluation of body's iron status in patients with acute and chronic inflammation may be difficult. This is because conventional laboratory parameters of iron status often fail to differentiate between iron deficiency anaemia and anaemia of chronic disorders. This makes it necessary to examine bone marrow to evaluate iron stores and to establish a definite diagnosis. Bone marrow examination may not be performed routinely since it is an invasive, expensive, painful and time consuming procedure.[9,10]

MATERIAL AND METHODS

This study was conducted at the Baqai Institute of Haematology, Baqai Medical University, Karachi, from 2007–2009. For the evaluation of soluble transferrin receptors (sTfR) as a differentiating marker in the diagnosis of iron deficiency anaemia and anaemia of chronic disorders, certain haematogical and biochemical parameters were determined; these include: complete blood counts, morphology of stained peripheral blood smear, identification of any abnormal haemoglobin on Hb electrophoresis, estimation of serum iron profile and quantitation of soluble transferrin receptors.

Criteria observed for the inclusion of healthy individuals and patients in this study were:

- Healthy normal individuals with no history of recent illness or medication during the past six weeks.
- Patients with hypochromic and microcytic blood picture (with or without anaemia).

A total of 80 whole blood samples (10 cc) were collected as follows:

Forty samples from normal individuals both male and female for establishing the reference ranges of sTfR and 40 samples from patients with hypochromic and microcytic blood picture. Each sample was divided into two portions; 3 ml of blood were added to a tube containing EDTA as anticoagulant while the other 7 ml

was placed in a glass tube without anticoagulant to obtain serum.

Complete blood counts including Hb, RBC count, TLC, platelet count, PCV, MCV, MCH, MCHC, and red cell distribution width (RDW) were determined by automated cell analyser (Sysmex PO H 100i). Morphology of blood cells was studied on blood smears stained with Leishman's stain. Blood samples collected in EDTA tubes were used for the diagnosis of variants of haemoglobin by cellulose acetate haemoglobin electrophoresis at *p*H 8.6. Serum was divided into 3 parts, two of them were stored at -20 ºC for determination of serum ferritin and soluble transferrin receptors while the third aliquote was used for determination of serum iron and total iron binding capacity.

Serum iron was determined spectro-photometrically using Point Scientific, Inc Kit. Unsaturated iron binding capacity (UIBC) was determined using Point Scientific, Inc Kit. TIBC is the sum of iron concentration and UIBC. Transferrin saturation was calculated from the serum iron and TIBC concentration according the following formula:

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

Serum ferritin was determined by ELISA method using Point Scientific, Inc Kit. Soluble transferrin receptors were determined by ELISA using Quantikine IVD Kit (R and D Systems). The data was analysed using the statistical package SPPS-13.

RESULTS

Based on the haematological and biochemical parameters, all individuals included in this study were divided into six groups, i.e., normal adult male, normal adult female, iron deficiency anaemia male, iron deficiency anaemia female, anaemia of chronic disorder male and female groups respectively. Results of CBC, serum iron profile and soluble transferrin receptor is given in Table-1.

Table-1: Results of CBC, iron profile and sTfR in all groups included in the study

Parameters	N M (n=20)	N F (n=20)	IDA M (n=3)	IDA F (n=17)	ACD M (n=2)	ACD F (n=18)
Hb (g/dl)	14.36±0.95	12.73±0.64	8.0±3.38	9.61±0.96	10.85±0.07	10.98±1.30
PCV (%)	42.56±2.52	37.70±2.50	26.73±7.40	29.87±5.13	31.35±0.91	33.13±3.04
RBC (M/µl)	4.91±0.7	4.4±0.30	3.62±0.75	4.33±0.66	4.26±0.48	4.56±0.33
MCV (fl)	86.32±5	86.39±5.24	64.33±7.7	66.21±6.25	68.5±6.34	33.13±3.04
MCH (ρg)	29.02±2.06	28.71±2.69	19.33±5.58	20.98±2.77	23.45±5.86	24.36±3.41
MCHC (%)	33.39±1.31	32.36±1.00	27.63±4.87	30.44±1.58	32.7±3.53	30.37±2.93
RDW (%)	14.26±0.90	14.49±1.46	19.0±2.55	17.60±2.44	17.6±0.55	16.55±1.240
Hb A (%)	96.17±0.17	96.13±0.21	97.16±0.23	97.01±0.44	96.10±0.23	96.26±0.25
HB A_2 (%)	3.13±0.21	3.11±0.21	2.06±0.37	2.29±0.40	3.15±0.07	3.04±0.17
Hb F (%)	0.70±0.11	0.76±0.10	0.77±0.15	0.69±0.13	0.75±0.07	0.69±0.15
Iron (µg/dl)	104.65±26.12	85.11±20.83	23.67±6.02	23.92±11.52	90.97±16.92	102.28±53.11
TIBC (µg/dl)	301.82±28.17	304.07±36.66	448.33±32.53	436.40±46.10	328.47±23.29	283.68±70.21
% Sat	35.30±10.39	28.32±7.70	5.36±1.75	5.93±3.4	27.58±3.19	41.02±34.33
Ferritin (ηg/ml)	147.50±48.19	75.73±21.88	8.30±4.09	7.21±2.12	41.95±18.45	107.02±100.44
sTfR (ηmol/l)	32.51±4.35	34.56±4.27	122.53±10.90	121.02±15.40	35.15±3.18	33.72±5.74

Data are Mean±SD, Hb= Hemoglobin, PCV= Packed cell Volume, RBC= Red Cell Count, 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, ACD= Anemia of Chronic Disorders

In the study of soluble transferrin receptors in normal individuals the mean concentration of soluble transferrin receptors in normal adult male group was 32.51±4.35 ηmol/l (Range 26.9–40.3 ηmol/l) while mean level in normal adult female was 34.56±4.27 ηmol/l (Range 29.5–41.7 ηmol/l). In patients with iron deficiency anaemia haemoglobin concentration was significantly reduced in both male and female groups. Packed cell volume, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration percent (MCHC %) were also reduced in these patients as compared with normal samples. Red cell distribution width (RDW) was increased in iron deficiency anaemia as compared with control samples.

Correlation between red cell indices and soluble transferrin receptors was also evaluated. Correlation between soluble transferrin receptors with haemoglobin concentration (r= -0.688), PCV (r= 0.977), RBC count (r= 0.807), MCH (r= -0.671), MCHC (r= 0.750) and RDW (r= 0.750) was significant (p<0.01) in both sexes. However, correlation with mean cell volume (r= 0.326) was non-significant (p>0.01). Low levels of haemoglobin were associated with increased levels of soluble transferrin receptors. In this group sTfR level were also noted to have an inverse correlation with PCV, RBC count, MCH, MCHC and RDW (p<0.01) but not with MCV (p>0.01).

Iron is the only micronutrient that influences sTfR concentrations. In this study correlation of iron profile with soluble transferrin receptors was also carried out. Relationship between sTfR with serum iron (r= -0.825), TIBC (r= -0.469) and % transferrin saturation (r= -0.782) was highly significant (p<0.01) in both sexes.

Correlation between sTfR and serum ferritin (r= -0.325) was statistically non-significant (p>0.01). In anaemia of chronic disorders haemoglobin concentration (r= 0.651), packed cell volume (r= 0.849), mean cell volume (r= 0.695), mean cell haemoglobin (r= 0.774), mean cell haemoglobin concentration (r=

0.618) and red cell distribution width (r= -0.643) showed significant correlation (p<0.01) with the soluble transferrin receptors. Correlation between soluble transferrin receptors and serum iron (r= -0.747) and % saturation of transferrin (r= -0.642) was significantly negative (p<0.01) while correlation between total iron binding capacity (r= 0.289) and serum ferritin (r= -0.233) with soluble transferrin receptors was statistically insignificant (p>0.01) (Table-2).

sTfR in normal control group and ACD groups were compared; there was no significant difference in the levels of sTfR in the two groups (Table-3). Effect of increased ferritin and normal ferritin level on soluble transferrin receptors was studied in anaemia of chronic disorders. Results showed some difference in their values but the difference was statistically insignificant (p>0.01) (Table-4).

Comparison of sTfR values in anaemia of chronic disorders of diverse aetiologies was made. There was no significant difference in mean levels of sTfR among various etiological groups of this disorder except malaria (Table-5).

Table-2: Correlation of sTfR vs red cell parameters, indices and iron profile

Parameters	IDA		ACD	
	r	p	r	p
Hb . sTfR	-0.688*	0.000	0.651*	0.002
Hct . sTfR	0.977*	0.000	0.849*	0.000
RBC . sTfR	0.807*	0.000	0.019	0.937
MCV . sTfR	0.326	0.161	0.695*	0.001
MCH . sTfR	0.671*	0.001	0.774*	0.000
MCHC . sTfR	0.750*	0.000	0.618*	0.004
RDW . sTfR	-0.577*	0.008	-0.643*	0.002
Sfe . sTfR	-0.825**	0.000	-0.747**	0.000
TIBC . sTfR	0.469*	0.037	0.289	0.217
% Sat . sTfR	-0.782**	0.000	-0.642**	0.002
Sfer . sTfR	-0.325	0.162	-0.233	0.323

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level, r= Pearson Correlation

Table-3: Comparison of sTfR (ηmol/l) in normal adults and ACD group

sTfR level	Mean	Minimum	Maximum	*p*
Normal group	34.56	32.55	36.56	0.19
ACD group	33.87	31.29	36.44	

Table-4: Comparison of sTfR (ηmol/l) in ACD with normal and increased serum ferritin level

sTfR in ACD with	Mean	Minimum	Maximum	*p*
Increased ferritin	30.05	25.96	35.111	0.112
Normal ferritin	36.09	33.26	38.921	

Table-5: Compression of sTfR (ηmol/l) in subgroups of ACD

sTfR level	Mean	Minimum	Maximum
Normal controls	34.56	32.55	36.56
Rheumatoid arthritis	37.20	32.08	42.31
Renal disease	33.48	30.39	36.58
Chronic Infections	30.48	25.02	35.94
Malaria	22.95	13.42	32.48

DISCUSSION

In this study high concentration of soluble transferrin receptors was observed in patients with iron deficiency (male and female) subjects as compared to normal control groups (male and female), validating the previous reports.[11,12]

Soluble transferrin receptors test can be used to discriminate iron deficiency anaemia from anaemia of chronic disorders and inflammation. sTfR level appears to be a specific and sensitive marker of iron deficiency and enjoys the advantages over serum ferritin as serum ferritin, being an acute phase protein, is increased in inflammatory disorders.[13] Serum ferritin level may be disproportionately elevated in relation to iron stores in patients with inflammation. Soluble transferrin receptor level is not affected by these disorders and is therefore a reliable laboratory index of iron deficiency anaemia. This is supported by the observations made by Cook *et al*.[14] Negative correlation of sTfR with red blood cell count, serum iron level and haemoglobin concentration was shown by Chijiwa *et al*.[15]

The determination of soluble transferrin receptors is not only helpful in differentiating iron deficiency anaemia from anaemia of chronic disorders. It may also identify the group of combined patients with iron deficiency and anaemia of chronic disorders. This is because sTfR levels in these patients remain in normal reference range.[16–18]

Mean sTfR level in rheumatoid arthritis, chronic renal disease and chronic infections was 30.72 ηmol/l compared to normal control 34.56 ηmol/l (p<0.01). Mean sTfR levels were on the lower side compared to normal controls yet the values were statistically significant (p<0.01). sTfR levels in malaria were lower in comparison with normal control group. Bone marrow in patients with anaemia of chronic disorders is usually not hyper cellular and serum soluble transferrin receptors are also not increased in these disorders. This may be an explanation for normal sTfR in ACD. Mean level of sTfR in malaria in this study (22.95 ηmol/l) was significantly lower than normal values (34.56 ηmol/l). Reduced sTfR levels in malaria may be explained on the basis of suppression of erythropoietic activity by plasmodium.[11,19]

Our findings are different from other studies, Mockenhaupt *et al*[20], Stoltzfus *et al*[21], Menendez *et al*[22] and Verhoef *et al*[23] who found increased sTfR level in patients with malaria. Wiwanitkita *et al*[24] found increased level of sTfR during the infection period of *P. gallinaceum* in an animal study but this increased level was also statistically not significant.[24]

CONCLUSION

sTfR determination can be used as a reliable differentiating marker in the diagnosis of iron deficiency anaemia and anaemia of chronic disorders.

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