

# Soluble Transferrin Receptors in Malaria

Muhammad Saboor, Moinuddin, Muhammad Abdul Razzaq and Naeem Tahir

## ABSTRACT

**Objective:** To determine the concentration of soluble transferrin receptors (sTfR) in patients with malaria.

**Study Design:** Cross-sectional, analytical study.

**Place and Duration of Study:** Baqai Institute of Haematology, Baqai Medical University, Karachi, from December 2009 to April 2010.

**Methodology:** Twenty samples from normal male and female subjects each were drawn for establishing the reference range while 38 from patients with malaria (with or without anaemia) sTfR concentration was determined. Descriptive statistics were used for data analysis.

**Results:** Out of 38 patients, 4 had iron deficiency anaemia while 34 patients were without anaemia. Mean sTfR level in the control group was  $33.53 \pm 4.38$  nmol/l. In patients with malaria without iron deficiency anaemia, mean sTfR concentration was  $30.84 \pm 5.40$  nmol/l. Patients with malaria and concomitant iron deficiency anaemia had mean sTfR level of  $101.67 \pm 11.69$  nmol/l. Comparison of sTfR in normal subjects and in patients with malaria showed no statistically significant difference ( $p = 0.208$ ). Statistically significant difference ( $p < 0.001$ ) was observed in patients with malaria and concomitant IDA as compared to normal control group.

**Conclusion:** Malaria without concomitant iron deficiency anaemia had near normal sTfR levels. While those with concomitant iron deficiency anaemia had significant higher level of sTfR. This concludes that these receptors are not affected in malaria alone.

**Key words:** sTfR. Malaria. Iron deficiency anaemia.

## INTRODUCTION

There are five species of the malarial parasites of the genus *Plasmodium* (*P.*) that are known to transmit malaria to Man; these are *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *P. knowlesi*.<sup>1</sup> Iron deficiency is the most common nutritional problem all over the world.<sup>2</sup> Malaria affects the conventional laboratory tests of iron status such as serum iron, ferritin, total iron binding capacity (TIBC) and % saturation of transferrin.<sup>3</sup> Increase in serum ferritin during malarial infection has been reported.<sup>4</sup> Being an acute phase protein, serum ferritin concentration increases during acute infections or inflammation.<sup>5</sup> Damage to the liver and spleen may also be a cause of elevated serum ferritin concentrations during malarial infection.<sup>6</sup>

Soluble transferrin receptors (sTfR) level has been suggested to be a sensitive indicator of iron status in adults.<sup>6</sup> Human transferrin receptor (TfR) is transmembrane dimeric glycoprotein. It is composed of two identical 95 kDa sub-units linked by disulphide bonds.<sup>7</sup> sTfR is a truncated form of the intact transferrin receptor and circulating as a complex of transferrin and its

receptors. Their molecular mass is 85 kDa. About 80% of the circulating sTfR originate from erythroid precursors.<sup>8</sup> Unlike other laboratory tests of iron status (serum iron, TIBC, % transferrin saturation and serum ferritin), sTfR levels are not affected by infections and inflammation in the absence of iron deficiency.<sup>9</sup> sTfR levels constitute a reliable test for the diagnosis of iron deficiency in patients with concurrent malarial infection. This test, therefore, has an edge over the conventional serum iron profile which fails to give the expected results in this scenario. There are only a few reports on the application of sTfR levels in the diagnosis of iron deficiency and concomitant malaria in patients living in endemic areas.<sup>10,11</sup>

The purpose of this study was to determine the level of soluble transferrin receptors in patients with malaria and to evaluate its role in the assessment of iron status in these patients.

## METHODOLOGY

A total of 78 whole blood samples (10 cc each) were collected. Forty samples, 20 from male and 20 from female subjects with no history of illness or medication during the past 6 weeks were drawn for establishing the reference range of sTfR, while 38 samples were obtained from patients with documented malarial infection (with or without anaemia).

Each blood sample was divided into two portions; 3 ml of blood was added to a tube containing ethylene diamine

Department of Haematology, Baqai Institute of Haematology, Baqai Medical University, Karachi.

**Correspondence:** Dr. Muhammad Saboor, House No. 149, DOHS, Phase I, Malir Cantt, Karachi.

E-mail: mujhgan\_16@hotmail.com

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tetra-acetic acid (EDTA) as an anticoagulant while 7 ml of blood was placed in a glass tube without anti-coagulant to obtain serum.

Serum was separated from 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.

Complete blood counts (CBC), morphology of the stained peripheral blood smear, estimation of serum iron profile and quantitation of soluble transferrin receptors parameters were determined on all blood samples.

CBC of all samples was determined by automated cell analyzer (Sysmex poch-100i). This included Hb estimation, red blood cell count, total leukocyte count, platelet count, packed cell volume (PCV), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW). Morphology of blood cells were observed on Leishman's stained blood smears under high power and oil immersion lens of light microscope (Nikon, Japan). Peripheral smears were examined by a well-trained and experienced medical laboratory technologist and reviewed by a haematologist.

Serum iron was determined spectrophotometrically using Point Scientific and Inc kit. UIBC was determined using Point Scientific and Inc kit. TIBC is the calculated sum of serum iron concentration and unsaturated iron binding capacity (UIBC). Transferrin saturation was calculated from serum iron and TIBC concentration according to the following formula:

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

Serum ferritin was determined by enzyme linked immunosorbent assay using Point Scientific and Inc kit. Soluble Transferrin receptors were determined by ELISA method using Quantikine IVD kit (R & D systems).

Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 13.0. Descriptive statistics was used for the calculation of mean  $\pm$  SD. Independent sample t-test was used for comparing the groups. A p-value of < 0.05 (two tailed) was considered statistically significant.

## RESULTS

A total of 78 individuals were recruited in this study i.e. 38 patients with malaria and 40 normal subjects as control. Based on the haematological and biochemical parameters, all individuals included in this study were divided into three groups' i.e. normal controls, malaria positive without iron deficiency anaemia (IDA) and malaria positive with iron deficiency anaemia.

Twenty female and 20 male subjects served in the control (group A); 15 male and 19 female patients had malaria without iron deficiency (group B), while 4 patients had malaria with iron deficiency anaemia (group C).

Table I shows results of haematological parameters and serum iron profile in all groups. Mean sTfR value in the normal control (group A) was  $33.53 \pm 4.38$  nmol/l. In patients with malaria without IDA (group B), mean sTfR concentration was  $30.84 \pm 5.40$  nmol/l. Mean sTfR level in patients with malaria and concomitant iron deficiency anaemia (group C) was  $101.67 \pm 11.69$  nmol/l.

Comparison of three groups i.e. normal controls, patients with malaria without IDA and patients with malaria with concomitant IDA was done. Comparison of sTfR in normal and patients with malaria without IDA (groups A and B) showed no statistically significant difference ( $p = 0.208$ ). However, statistically significant difference  $p < 0.001$  was observed in patients with malaria and concomitant IDA (groups A and C) as compared to normal control group.

**Table I:** Haematological parameters and serum iron values in mean  $\pm$  SD among the groups..

Parameters	Normal control (Group A, n = 40)	Malaria without IDA (Group B, n = 34)	Malaria with IDA (Group C, n = 4)
Hb (g/dl)	13.55 $\pm$ 1.15	11.05 $\pm$ 1.95	9.80 $\pm$ 1.15
RBC(Mil / $\mu$ l)	4.66 $\pm$ 0.38	3.86 $\pm$ 0.55	3.44 $\pm$ 0.28
MCV (fl)	84.55 $\pm$ 13.31	82.30 $\pm$ 6.26	74.00 $\pm$ 5.09
MCH (pg)	28.86 $\pm$ 2.37	27.08 $\pm$ 2.39	23.50 $\pm$ 2.08
MCHC (%)	32.87 $\pm$ 1.26	32.77 $\pm$ 1.70	32.00 $\pm$ 0.81
IRON ( $\mu$ g/dl)	94.87 $\pm$ 25.33	140.47 $\pm$ 46.85	45.50 $\pm$ 14.27
TIBC ( $\mu$ g/dl)	302.94 $\pm$ 32.29	332.80 $\pm$ 51.99	403.25 $\pm$ 34.46
sTfR (nmol/l)	33.53 $\pm$ 4.38	30.84 $\pm$ 5.40	101.67 $\pm$ 11.69

$\pm$  SD

## DISCUSSION

Effect of malaria on soluble transferrin receptors remains incompletely understood although a number of studies have been conducted to establish the significance of sTfR levels in this disease.

In this study, the diagnostic value of sTfR levels was evaluated in patients with malaria alone and also in patients with malaria and concomitant iron deficiency anaemia. It was observed that patients with malaria but without an associated iron deficiency anaemia had normal sTfR levels. Our findings are consistent with those of Kuvibidila *et al.*<sup>11</sup> Patients with malaria and concomitant iron deficiency anaemia had significantly high sTfR levels in this study. Eighty percent of the sTfR relate to the erythroid precursors. Decreased availability of iron to the erythroid precursors causes increased level of sTfR. Hence, in iron deficient individuals sTfR levels are significantly increased.<sup>12</sup>

The present findings are at variance with those of Mockenhaupt *et al.*, Stoltzfus *et al.*, Menendez *et al.* and

Verhoef *et al.* who found increased sTfR level in patients with malaria.<sup>13-16</sup> Wiwanitkita *et al.* 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.<sup>17</sup>

Prevalence of iron deficiency, type of malaria and age group of the subjects may be the proposed mechanisms of these discrepant findings. Most of the studies conducted on sTfR levels in malaria are African based. This is supported by a study in the African children who showed increased sTfR levels in severe malarial anaemia. Iron deficiency anaemia is common in African children (15-44%). Since, iron deficiency anaemia results in increased synthesis of sTfR, high level of sTfR in these children may have been caused by the associated iron deficiency anaemia.<sup>18</sup> Another factor of this controversy may be the infestation of *P. falciparum* which is more prevalent in those areas whereas *P. vivax* is most common in Pakistan. Haemoglobinopathies, a leading cause of erythroid hyperplasia, are also common in Africa that also cause increased sTfR concentration. Differences in the age of the subjects recruited in these studies may also contribute to discrepancies. Findings of this study are different from others because only adults were recruited in the study with no history of haemoglobinopathies and haemolytic anaemias in their families.

Reduced sTfR concentrations in acute malaria were reported by Beesley *et al.*<sup>19</sup> and Williams *et al.*<sup>10</sup> Reduced bone marrow response to erythropoietin or its deficiency was suggested as one cause of erythropoietic suppression in the pathogenesis of anaemia in malaria.<sup>20,21</sup> This was proposed to explain the decreased sTfR levels in individuals with malaria.<sup>22</sup>

## CONCLUSION

It appears that acute malaria does not affect bone marrow activity as indicated by normal sTfR level. The number of patients with malaria and concomitant iron deficiency anaemia is small and further investigations are needed to establish the role of sTfR as a diagnostic marker of iron deficiency in patients with malaria.

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