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Serum transferrin receptor levels are increased in asymptomatic and mild *Plasmodium falciparum*-infection

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ABSTRACT

Background and Objectives. **The serum transferrin receptor (sTfR) concentration in an individual reflects the extent of erythropoietic activity and is considered a useful marker of iron deficiency independent of concurrent inflammation or infection. However, data on the impact of malaria on this parameter are ambiguous. We examined potential associations of asymptomatic and mild *Plasmodium falciparum*-infections and of several erythrocyte variants with sTfR values in South West Nigeria.**

Design and Methods. **In a cross-sectional study among 161 non-hospitalized children, sTfR concentrations and *P. falciparum* parasitemia were assessed. In addition, hemoglobin (Hb) and serum ferritin values, Hb-types, glucose-6-phosphate dehydrogenase (G6PD) deficiency and α-globin genotypes were determined and the effects of these factors on sTfR levels were analyzed by univariate and multivariate statistical methods.**

***Results. P. falciparum*-infection was present in 77% of the children. Mean sTfR levels were higher in infected than in non-infected children (geometric mean, 3.68, 95% confidence interval [3.5-3.9] vs. 2.99 [2.7-3.3] mg/L; $p = 0.0009$). There was a significant trend for higher sTfR values with increasing parasite density. sTfR values decreased continuously with age. Hb-types, G6PD-, and α-globin genotypes did not correlate with sTfR levels. In the multivariate analysis, age, Hb and log ferritin values, and parasite density of *P. falciparum* were independently associated with log sTfR values.**

Interpretation and Conclusions. **sTfR concentrations are increased in asymptomatic and mild *P. falciparum*-infections suggesting adequate bone marrow response in this condition. The diagnostic value of sTfR levels for iron deficiency may be impaired in areas where stable malaria occurs.**

Key words: serum transferrin receptor, anemia, *Plasmodium falciparum*, malaria, Nigeria

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In Sub-Saharan Africa, infection with *Plasmodium falciparum* and iron deficiency are predominant risk factors of childhood anemia.[1,2] The pathogenic factors involved in malarial anemia are incompletely understood. In acute and severe malaria, inhibition of erythropoiesis may aggravate anemia due to hemolysis of parasitized and non-parasitized erythrocytes.[3-6] In highly endemic areas, malaria commonly presents as a chronic rather than an acute disease.[7] In these infections with low parasite densities, erythrocyte destruction at schizont rupture and hemolysis are considered to be the predominant causes of anemia.[2] Iron deficiency may be equally important for anemia in malarial regions, however, the contribution of this factor cannot easily be estimated. In Africa, the characteristic changes of red cell indices in iron deficiency, i.e. microcytosis and hypochromia, may also reflect α^+-thalassemia.[8] Levels of serum transferrin and ferritin can be misleading in diagnosis since both parameters are affected by inflammatory processes and malaria.[9-11] The examination of bone marrow aspirates or a trial with oral iron substitution are not appropriate for routine diagnosis of iron status in developing countries.

Transferrin receptors are transmembrane glycoproteins that mediate the internalization of transferrin and iron into cells. The receptors are primarily expressed in tissues of high iron requirement such as the erythroid marrow.[12] During the development of red cell precursors to erythrocytes, serum transferrin receptor (sTfR) is released. sTfR levels have been shown to reflect the extent of erythropoetic activity[12] and to be increased in iron deficiency,[12-14] sickle cell anemia,[15] β-thalassemia[16] and α-thalassemia.[17] Unlike common indices of iron deficiency (e.g. transferrin, ferritin), sTfR is thought not to be affected by inflammation or infection.[10,11] Previous results indicated that sTfR levels are not altered in *P. falciparum*-infection, suggesting that this marker might be useful to assess the extent of iron-deficiency in malaria-endemic regions.[10] A recent study from Vanuatu, however, demonstrated decreased sTfR levels in acute malaria arguing for inhibition of ery-

thropoiesis in manifest disease.[18] Data on the impact of asymptomatic or mild *P. falciparum*-infection on sTfR concentrations are scarce. Such information could provide further understanding of the pathogenesis of malarial anemia and help to estimate the diagnostic value of sTfR levels for iron deficiency in Africa.

This study was performed to assess possible associations of asymptomatic and mild *P. falciparum*-infection with sTfR levels among non-hospitalized Nigerian children living in an area of intense malaria transmission. In addition, associations of sTfR values with age, serum ferritin concentrations, HbS and HbC traits, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and α-thalassemia were looked for.

Design and Methods

Out of 695 subjects enrolled in a survey on hematologic indices in the city of Ibadan, Nigeria, and the neighboring village of Abanla,[8] sTfR levels were determined in 161 randomly selected children (86 male, 75 female; aged 0.8-7 year). Asymptomatic children were recruited from schools and vaccination programs in Abanla (n = 126) and children presenting with fever or a history of fever were enrolled at health posts in Ibadan (n = 35). There were no clinical cases of severe malaria among the children. Parasitological indices of the study group have been described in detail.[19] Children were recruited with informed consent of their guardians. Ethical approval was obtained from the *Joint Ethical Committee of the University of Ibadan/University College Hospital*.

Blood was collected into EDTA and serum tubes. Hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV) values and red blood cell counts (RBC) were determined within 24 hours using a cell counter (HC 555, Clinicon, Mannheim, Germany). Malaria parasites were microscopically counted per 100 high power fields of Giemsa-stained thick blood films. In addition, after extraction of DNA from blood, a nested *P. falciparum*-specific polymerase chain reaction (PCR) assay was performed for all samples.[20] Parasite densities were categorized into *negative*, *submicroscopic* (positive PCR result but negative on blood film), *low* ($\leq$ 1 parasite/high power field [P/F]), and *moderate* (> 1 P/F).[19] The hemoglobin variants HbS and HbC were identified by hemoglobin electrophoresis.[21] G6PD variants were assigned by hybridization with mutation-specific oligonucleotides after PCR-based amplification of sequences of the G6PD-gene.[22] G6PD-genotypes were categorized as G6PD-normal (Gd^B/Gd^B, Gd^B/Gd^A, Gd^A/Gd^A, Gd^B, Gd^A), -heterozygous (Gd^B/Gd^{A-}, Gd^A/Gd^{A-}) and -deficient (Gd^{A-}/Gd^{A-}, Gd^{A-}). The $-\alpha^{3.7}$ type of α^+-thalassemia was screened for by two separate PCR assays.[23] Serum was separated from blood by centrifugation and stored at –20°C. Serum ferritin values were determined using a chemoluminescence immunoassay (Architect i4000, Abott, Illinois, USA). sTfR concentrations were measured by a commercially available ELISA kit (R&D Systems, Wiesbaden, Germany).

Statistics

Considering the age-dependent increase of Hb values in children, anemia was defined as: Hb < 110 g/L in children < 5 years old and < 115 g/L in those $\geq$ 5 < 8 years.[24] Individuals were subdivided into the age-groups of 0-1, 2-3, 4-5, and 6-7 years. Evaluation of data included only children whose sTfR levels were within the range of the standard curve of the assay (0.26-6.80 mg/L). Proportions were compared by χ^2-tests. sTfR and ferritin values were normalized by log10 transformation. Associations of log sTfR and log ferritin levels with age, sex, anemia, *P. falciparum*-infection, Hb-types, G6PD- and α-globin genotypes were determined by analysis of variance. Correlation coefficients (R) between log sTfR values and hemoglobin and log ferritin concentrations, respectively, were calculated. To adjust for potential confounding effects, multiple linear regression analysis was performed. Variables were kept in the multivariate model if they were independently associated with log sTfR levels ($p < 0.05$).

Results

Data from 158 children were analyzed. The hematologic data are summarized in Table 1. Anemia was observed in 57% (90/158) and was most prevalent in subjects with homozygous α^+-thalassemia, in those with parasite densities > 1 P/F, and in children younger than two years (Table 2). Seventy-seven percent (121/158) of the children were infected with *P. falciparum*. Of these, 24% (29/121) had submicroscopic parasite densities detectable only by PCR, 35% (42/121) exhibited low parasite densities of $\leq$ 1 P/F (median 0.45 P/F, range [0.01-1]) and 41% (50/121) had a moderate parasitemia of more than one P/F (median: 2.65 [1.1-48] P/F). Infection rates and parasite densities did not differ with age-groups, sex, Hb-types, G6PD- or α-globin genotypes (data not shown). Sixty-four percent (78/121) of *P. falciparum*-infected children were anemic as compared to 32%

Table 1. Hematologic data of the study group.

Age-group (yrs)	*No.*	*Hb* (g/L)*	*PCV* (L/L)*	*RBC (x 10^{12}/L)*	*MCV* (fL)*
0-1	41	102.5±13	32.8±3.6	4.37±0.7	74.8±6.9
2-3	62	108.1±10	34.5±3.9	4.36±0.6	79.1±5.2
4-5	38	110.7±13	35.2±3.9	4.33±0.6	81.6±6.2
≥6	17	113.2±14	35.1±4.7	4.35±0.6	81.1±5.2
total	158	108±13	34.3±3.9	4.36±0.6	78.9±6.4

**Increase with age, p (ANOVA) < 0.05; for PCV and MCV: n = 150. Data are given as means ± standard deviations.*

Table 2. Factors influencing ferritin and serum transferrin receptor levels; univariate analysis.

	No.	anemic (%)	Ferritin°	sTfR°
Hb-type				
AA	119	67 (56)	54.1 (46-63)	3.56 (3.3-3.8)
AS	26	15 (58)	70.1 (47-105)	3.29 (2.9-3.7)
AC	13	8 (62)	91.8 (53-160)	3.49 (2.8-4.3)
			F = 2.7, p = 0.07	*F = 0.6, p = 0.58*
G6PD-genotype*				
normal	118	67 (57)	55.6 (47-65)	3.45 (3.3-3.7)
heterozygous	18	9 (50)	67.2 (43-106)	3.48 (2.9-4.2)
deficient	20	12 (60)	73.9 (45-122)	3.78 (3.1-4.5)
			F = 1.1, p = 0.35	*F = 0.6, p = 0.54*
α-globin genotype				
αα/αα	85	39 (46)	55.2 (46-67)	3.53 (3.3-3.8)
-α/αα	59	39 (66)	67.4 (53-86)	3.37 (3.1-3.7)
-α/-α	14	12 (86)^	50.4 (33-76)	4.00 (3.3-4.9)
			F = 1.1, p = 0.34	*F = 1.5, p = 0.23*
Anemia				
none	68	–	43.3 (36-52)	3.34 (3.1-3.6)
anemia	90	90 (100)	74.6 (61-91)	3.64 (3.4-3.9)
			F = 15.6, p = 0.0001	*F = 2.6, p = 0.11*
Sex				
male	84	47 (56)	58.3 (46-73)	3.66 (3.4-3.9)
female	74	43 (58)	59.8 (51-70)	3.34 (3.1-3.6)
			F = 0.03, p = 0.87	*F = 3.0, p = 0.09*
Age (years)				
0 –1	41	29 (71)	43.6 (31-61)	4.04 (3.6-4.5)
2 – 3	62	33 (53)	52.5 (44-63)	3.50 (3.2-3.8)
4 – 5	38	20 (53)	82.0 (62-109)	3.30 (3.0-3.7)
≥ 6	17	8 (47)	89.5 (61-131)	2.88 (2.5-3.4)
			F = 5.2, p = 0.002	*F = 5.0, p = 0.002*
P. falciparum-infection				
none	37	12 (32)	34.0 (25-46)	2.99 (2.7-3.3)
PCR only	29	16 (55)	51.4(35-76)	3.48 (3.0-4.0)
≤ 1 P/F#	42	22 (52)	76.1 (59-98)	3.48 (3.1-3.9)
> 1 P/F	50	40 (80)^	77.6 (65-93)	4.00 (3.7-4.3)
			F = 8.6, p < 0.0001	*F = 5.7, p = 0.001*

*°Geometric mean (95% confidence interval); *for G6PD-genotyping: n = 156; αα/αα, normal α-globin genotype; –α/αα, heterozygous and –α/–α, homozygous α*-thalassemia; #P/F, parasites/high power field; ^prevalence of anemia differs with groups, p (χ^2) < 0.01.*

(12/37) of non-infected ones (χ^2 = 11.9, *p* = 0.001).

sTfR concentrations ranged from 1.55 to 6.65 mg/L (geometric mean: 3.51 mg/L). There was no significant effect of Hb-types, G6PD-, or α-globin genotypes on sTfR levels (Table 2). Log sTfR correlated negatively with hemoglobin concentrations (R = -0.28, *p* = 0.0004). A trend only for increasing log sTfR values with decreasing log ferritin concentrations was observed (R = -0.126, *p* = 0.1). Ferritin concentrations were increased in *P. falciparum*-infection (Table 2). In non-infected individuals, log sTfR and log ferritin values were significantly correlated (R = -0.497, *p* = 0.002).

With increasing age, mean sTfR levels decreased significantly from 4.04 mg/L (95% confidence interval [3.6-4.5]) in the age group of 0-1 year to 2.88 [2.5-3.4] mg/L in children of 6 years and older (F = 5.04, *p* = 0.002). Males were younger than females (median age: 2.6 vs. 3.1 years, *p* [Mann-Whitney U test] = 0.01) and tended to have slightly higher sTfR values (F = 3.0, *p* = 0.09).

Mean sTfR values were higher in *P. falciparum*-infected than in non-infected children (3.68 [3.5-3.9] vs. 2.99 [2.7-3.3] mg/L, F = 11.53, *p* = 0.0009). Although there was a considerable overlap of sTfR concentrations between non-infected and infected children grouped by parasite densities (Table 2), a significant trend for higher sTfR levels with increasing parasite density (F = 5.67, *p* = 0.001) was observed.

In the multivariate analysis, hemoglobin concentrations (regression coefficient b = -0.023, standard error [SE] = 0.01, *p* = 0.02), log ferritin values (b = - 0.094, SE = 0.032, *p* = 0.003), age-groups (b = - 0.027, SE = 0.013, *p* = 0.03) and the categorized *P. falciparum*-densities (b = 0.042, SE = 0.01, *p* < 0.0001) were independently associated with log sTfR levels (equation: sTfR (log) = 0.945 + 0.042 × *P. falciparum* density (categ.) – 0.027 × age-group (categ.) – 0.094 × ferritin (log) – 0.023 × Hb). Thus, in the multivariate model, the adjusted geometric means of sTfR values decreased by a factor of 1.1 with each age-group, whereas they increased by the same factor with each category of *P. falciparum*-infection. Age, hemoglobin, log ferritin and parasite density accounted for 24% of the variation in log sTfR values in the study group (R^2 = 0.24).

Three children had sTfR concentrations above the highest control value (6.8 mg/L). All three subjects were ≤ 2 years of age, infected with *P. falciparum*, anemic, and had normal ferritin values (31, 124, and 177 ng/mL).

Discussion

Factors involved in malarial anemia include hemolysis of parasitized erythrocytes and increased clearance of non-parasitized ones[3] as well as an inadequate bone marrow response. In several studies on human and murine malaria, inhibition of erythropoiesis and dyserythropoiesis have been described[4,5] and attributed to inhibitory cytokines released during the course of infection.[6, 25]

The degree to which hemolysis and bone marrow depression contribute to malarial anemia is not well understood. In this study, sTfR levels were significantly raised in asymptomatic or mild *P. falciparum*-infection and increased with parasite density. This is in contrast to a possible impairment of erythropoiesis due to malarial infection in these children as sTfR levels have been shown to reflect the extent of erythropoietic activity.[12] Hemolysis has been suggested to be the main factor responsible for the anemia of *P. falciparum*-infection in children with low parasite den-

sities in Tanzania.[2] In murine models, marrow erythrophagocytosis and dyserythropoiesis were found in mice with severe illness due to *P. berghei*-infection, but not in infected asymptomatic controls.[6] Likewise, no signs of dyserythropoiesis were noticed among subjects with chronic *P. falciparum*-infection and low parasite densities in another study.[26] In our study, 77% of children were infected with *P. falciparum*, the majority of whom exhibited low or submicroscopic parasite densities. The increased sTfR levels in these children with mild or asymptomatic malaria indicate an adequate erythropoietic response to malarial anemia. This suggests that hemolysis rather than inhibition of erythropoiesis constitutes the leading cause of anemia in this group. Accordingly, children with subclinical malaria tended to have higher sTfR values than non-infected ones or those with clinical malaria in a previous report.[10] A recent study from Vanuatu has demonstrated reduced sTfR levels in children with acute malaria attacks underlining the role of dyserythropoiesis in clinical disease.[18] In contrast to our results, the authors reported similar sTfR levels in a small group of children with asymptomatic infections and in non-infected controls. There are several possible explanations for the contradictory findings. In Vanuatu, malaria epidemiology is markedly different from that in Nigeria. Prevalence rates of malaria are about 30% throughout the year.[27] Hence, asymptomatic infections are less likely to occur and may be of shorter duration. In the study in Vanuatu, the asymptomatic malaria group consisted of 12 and 6 patients infected by *P. falciparum* and *P. vivax*, respectively. It has been shown that different strains of *P. falciparum* vary considerably in their ability to induce production of tumor necrosis factor, a cytokine thought to be involved in inhibition of erythropoiesis.[6,28] It is, therefore, conceivable that geographical diversity of parasite strains and species may account for different effects on erythropoiesis. Finally, in Vanuatu, asymptomatically infected children were younger than controls, which again might be responsible for the conflicting results.

In the multivariate analysis, age was shown to be independently associated with sTfR levels. Highest mean values were observed in infants younger than two years. A similar continuous decline of sTfR concentrations with age is seen in healthy European children (*Suominen et al., personal communication*). This finding may reflect a high turn-over of erythrocytes and raised iron requirement during growth. In Nigeria, as has been shown in other developing countries,[29,30] iron deficiency can be expected to be particularly common in early childhood. Correspondingly, ferritin values increased with age in our study. Ferritin concentrations were higher in anemic than in non-anemic children. This finding may result from the predominant role of *P. falciparum*-infection in anemia in this population and from the known bias of malarial infection on ferritin levels.[9,10]

Hb-types, G6PD-, and α-globin genotypes did not correlate with sTfR values. sTfR levels have been found to be markedly elevated in subjects with sickle cell disease (HbSS and HbSC).[15] In our study, children with HbAS and HbAC had sTfR values similar to those in subjects with HbAA. A slightly, but not significantly higher mean sTfR was seen in G6PD-deficient subjects. Reticulocytosis is commonly found in G6PD-deficiency during steady state due to moderate hemolysis and, subsequently, a young red cell population.[31] In contrast to the Mediterranean type of G6PD-deficiency, the Gd^{A-} type is characterized by far greater enzyme activity and, consequently, less hemolysis in the steady state.[32]

Recently, increased sTfR concentrations have been described in both heterozygous and homozygous α^+-thalassemia and attributed to an expansion of the erythron in these conditions.[17] Within the small range of values in our study, sTfR levels were higher, but not significantly, in homozygous carriers of α^+-thalassemia than in non-thalassemic children. This is consistent with an elevated pyruvate kinase activity, a surrogate marker of red cell age, in α^+-thalassemia suggesting an increased proportion of young red cells in this trait (*May, unpublished observation*).

In conclusion, sTfR levels are raised in children with asymptomatic or mild *P. falciparum*-infection. This finding suggests effective erythropoiesis in response to hemolysis of red blood cells in these children. Nevertheless, it is likely that in acute and severe malaria, erythropoietic depression may prevail and lead to decreased sTfR concentrations. *P. falciparum*-infection and age have to be regarded as confounding factors when assessing sTfR values. Due to the high prevalence of asymptomatic malaria, the value of sTfR concentrations in the diagnosis of iron deficiency in Africa is questionable.

Contributions and Acknowledgments

FPM, JM and UB conceived and designed the study. UB, AGF and CGM were responsible for recruitment, and parasitologic and hematologic measurements. PCR and ELISA assays were done by FPM and JM. FPM and KS did the statistical analysis and wrote the paper with major contributions from CGM, AGF, JM and UB. The order of authorship reflects the contribution to the study with UB being the principal investigator.

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Disclosures

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