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Short Report: Common Genotypic Polymorphisms in Glutathione S-Transferases in Mild and Severe Falciparum Malaria in Tanzanian Children

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Abstract. Malaria infection induces oxidative stress in the host cells. Antioxidant enzymes such as glutathione S-transferases (GSTs) are responsible for fighting reactive oxygen species and reduction of oxidative stress. Common GST polymorphisms have been associated with susceptibility to different diseases whose pathologies involve oxidative stress. In this study, we tested the hypothesis that GST polymorphisms that lead to reduced or lack of enzyme activity are associated with severe *Plasmodium falciparum* malarial anemia. We studied the genotypic distribution of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms between mild malaria ($N = 107$) and severe malarial anemia ($N = 50$) in Tanzanian children. We did not find a significant relationship with the *GSTT1* polymorphism. *GSTM1*-null was higher in the severe malaria anemia group but the difference was not significant ($P = 0.08$). However, a significant association of *GSTP1* I105V genotype with severe malarial anemia was discovered (26.0% against 10.3% mild malaria, $P = 0.004$). We concluded that *GSTP1* and possibly *GSTM1* may protect against severe falciparum malaria in children.

Oxidative stress plays an important role in malaria immunity and pathogenesis. Malaria-induced oxidative stress is thought to originate from immuno-defensive reactions of the host cells against the parasite and as a result of parasite metabolism. The parasite feeds on hemoglobin and releases the highly reactive and toxic heme. This can react with molecular oxygen to form hemin and superoxide radical (O_2^-), a highly reactive oxygen species. In the parasite's food vacuole heme is, however, rendered inert and nontoxic through conversion into hemozoin, the malaria pigment.[1] Most of the quinoline antimalarials interfere with the conversion of heme to hemozoin thereby inducing its accumulation inside the food vacuole and eventually killing the parasite.[2,3]

In severe malaria, parasite toxins may trigger the release of oxygen free radicals and stimulate a variety of pro-inflammatory cytokines, such as tumor necrosis factor-alpha, interleukins, gamma interferon, and nitric oxide.[4] These pro-inflammatory factors are believed to cause much of the clinical complications observed in severe malaria with multiple organ involvement. Several studies have implicated malaria-induced oxidative stress in complications such as reduced macrophage function, reduced erythrocyte deformability, and increased activation of pro-inflammatory cytokines.[5–7] In children with malaria, both blood plasma and erythrocytic lipid peroxidation are increased, whereas erythrocytic antioxidants such as glutathione (GSH) were shown to be lower in patients than in controls.[8] Polymorphisms resulting into absence or reduced enzyme activity have been identified and linked with pathogenesis in a number of disorders and diseases characterized with increased oxidative stress.[9–11] In a previous study, we observed that *GSTM1*-null genotype was associated with severe malaria in Cameroonian children.[12] In this study, we have investigated the genotypic distribution of human *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in mild versus severe malaria in Tanzanian children.

The clinical data and DNA samples of this study were collected in the period between July and September 2006, in a drug efficacy study of mild malaria cases[13] in Mnyuzi, Tanga Region, Tanzania. Briefly, children 3–15 years of age with a temperature > 37.5°C or a history of fever within the last 48 hours and with *Plasmodium falciparum* mono-infection at a density between 500 and 100,000 parasites/μL were eligible for recruitment. Children with a hemoglobin (Hb) concentration below 8 g/dL, as measured by HemoCue (HemoCue AB, Ängelholm, Sweden), were excluded. Children with severe malaria were included for the current study in the same study period. Severe anemia (Hb < 5 g/dL) was observed in all children who attended the clinic with severe malaria. Additional signs of severe disease that were examined: hyper parasitaemia (≥ 250,000 parasites/μL), metabolic acidosis manifested by respiratory distress as described by Marsh and others,[14] cerebral malaria presented as coma score ≤ 2 (Blantyre coma scale)[15] or impaired consciousness with Blantyre score < 3 and prostration or extreme weakness (e.g., inability to sit or stand). For severe cases treatment was initiated with quinine, according to Tanzanian National Guidelines and referred to the nearby district hospital in Korogwe in case the study physician considered this appropriate. There was no active follow-up of the outcome of severe malaria cases after the appropriate treatment was installed.

For all mild and severe malaria cases, a malaria blood slide, Hb measurement, and filter paper DNA sample were collected. A short questionnaire was used to obtain information on sex, age, disease presentation, and ethnicity of the patient. The ethical clearance for the collection of the mild malaria material was obtained from the Tanzanian National Institute for Medical Research (NIMR/HQ/R.8a Vol. XIII/446) and clearance for the collection of material of severe malaria cases was obtained from Kilimanjaro Christian Medical Center (KCMC 2006#28). In the informed consent obtained from the parents or guardians of the children, they approved the use of their children's DNA samples to study the relation between human genetic factors and malaria disease presentation. A total of 107 mild and 50 severe malaria cases were enrolled. Parasite density in the severe malaria group ranged from 4,640–174,000 parasites/μL and was higher than in the mild malaria group (Wilcoxon-rank sum test, $P < 0.001$). For more group characteristics see Table 1.

The DNA extracted from the dried filter papers was done using Nucleospin Tissue kits (Macherey-Nagel, Düren,

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TABLE 1

Characteristics of mild vs. severe malaria in falciparum malaria in Tanzanian children

	Mild	Severe malaria
N	107	50
Age, median (IQR)	5.0 (3.0–9.0)	8.0 (5.0–11.0)
Gender, % male (n/*N*)	51.4 (55/107)	60.0 (30/50)
Hemoglobin concentration, median g/dL (IQR)	10.6 (9.7–11.9)	4.3 (3.8–4.8)
Temperature, median (IQR)	37.3 (36.9–38.0)	38.5 (37.8–39.1)
Asexual parasite density, GM (IQR)	7,700 (1,120–23,480)	105,900 (57,480–121,040)
Signs of severe disease:		
Severe anemia, % (n/*N*)	–	100.0 (50/50)
Hyperparasitemia, % (n/*N*)	–	0.0 (0/50)
Respiratory distress, % (n/*N*)	–	6.0 (3/50)
Reduced consciousness, % (n/*N*)	–	4.0 (2/50)
Prostration, % (n/*N*)	–	8.0 (4/50)

IQR = interquartile range; GM = group median.

Germany), polymerase chain reaction (PCR) was performed using native, Taq polymerase (invitrogen) and all primers were purchased from Biolegio, Nijmegen, The Netherlands. Primers for *GSTT1, GSTM1,* and *GSTP1* were designed according to Pemble and others,[16] Bröckmoller and others,[17] and Watson and others,[18] respectively. The PCR conditions for *GSTM1*, *GSTT1,* and *GSTP1* were followed as previously described.[12] Samples that gave negative results for *GSTM1* and *T1* were measured again with β-globin as a control.

The results of the polymorphism analysis in mild and severe malaria are depicted in Table 2. For *GSTM1* there was a higher prevalence of *GSTM1*-null genotype in the severe group (40%) than in the mild group (26.2%), although not statistically significant ($P = 0.08$). The observed distributions in mild malaria are comparable to the reported distribution of *GSTM1*-null in African population (22–39%).[19–23] In Caucasian population, however, the *GSTM1*-null frequency is higher (around 50%) than in the African population.[21,23,24] In our previous study with 138 children from Cameroon, we found a statistically significant difference for the *GSTM1*-null frequency: 32% and 58% in uncomplicated malaria and severe malaria, respectively.[12]

TABLE 2

Genotypic and allelic distribution of glutathione S-transferases (GSTs) gene polymorphisms among mild vs. severe falciparum malaria groups in Tanzanian children

	Mild malaria	Severe malaria	*P* value
GSTM1-null, % (n/*N*)	26.2 (28/107)	40.0 (20/50)	0.08
GSTT1-null, % (n/*N*)	47.7 (51/107)	54.0 (27/50)	0.46
GSTP1			
Wild type, % (n/*N*)	39.3 (42/107)	16.0 (8/50)	
Heterozygous mutant, % (n/*N*)	50.5 (54/107)	58.0 (29/50)	
Homozygous mutant, % (n/*N*)	10.3 (11/107)	26.0 (13/50)	0.004*
GSTM1 and *P1* combined			
One or both enzyme wild type, % (n/*N*)	83.2 (89/107)	64.0 (32/50)	
Both enzymes mutant (*P1*-hetero/homozygous), % (n/*N*)	16.8 (18/107)	36.0 (18/50)	0.007

* *P* value of mutants (combined homo- and heterozygotes) compared with wild type.

The prevalence of homozygous *GSTP1* I105V in the severe malaria group (26.0%) was significantly higher than in the mild group (10.3%). This indicates for the first time an association of the *GSTP1* I105V genotype with severe malaria. There is evidence that the *GSTP1* I105V polymorphism may have a substrate-dependent effect on the enzyme activity.[18,25,26] The general distribution of the homozygous *GSTP1* I105V genotype in the mild malaria group is comparable to previous studies in a Brazilian population of African descent (8.3%) and Caucasians (11.3%).[21,24] In our previous study with Cameroonian children,[12] we found in uncomplicated malaria (21%) and severe malaria (26%) frequencies that are comparable to what we now observe in severe malaria (26.0%).

When a combined analysis of *GSTM1* and *GSTP1* was performed, the presence of wild-type condition on one or both of the two genes was 64.0% and 83.2% for the severe and mild malaria groups, respectively, and for the presence of mutations on both enzymes (hetero- or homozygous mutant) was 36.0% and 16.8% for the severe and mild malaria groups, respectively ($P = 0.007$).

The prevalence of *GSTT1*-null was comparable in both groups (48% and 54%). In the Cameroonian study we also observed no differences; although the frequencies were lower (21% and 29%).

This study has shown association of *GSTP1* I105V, and a trend but not significant association of *GSTM1*, with severe malaria anemia. *GSTP1* and *GSTM1* are expressed in all blood cells with higher expression in lymphoid than erythroid cell types, whereas *GSTT1* and *GSTA* are expressed in higher levels in erythrocytes than in lymphoid cells.[27] In this study, we did not investigate on *GSTA* and we did not detect an association of *GSTT1* with malaria, which is consistent with our previous observation.[12] It is not clear how GST polymorphisms can affect the malaria infection outcome. The GSH is important for parasite growth and *in vitro* studies have documented detoxification of heme via a GSH-utilizing pathway, that can be inhibited by chloroquine and amodiaquine.[28,29] Furthermore, drugs known to reduce cellular GSH were shown to potentiate the action of chloroquine in drug-resistant rodent malaria.[30]

Glutathione S-transferase (GST) polymorphisms can change the enzyme activity, which can lead to reduced detoxification of the host cell or increased availability of host GSH that might be used by the parasite. In both cases the malaria pathology could be accelerated. It is also likely that the impact of GSTs is not direct on erythrocytes but on other cells that are involved in the immune response mechanisms and that severe malarial anemia as an outcome can partly be attributed to such responses. Therefore, further studies including *in vitro* cellular studies to assess malarial outcomes for specific GST polymorphism genotypes are important.

In conclusion, *GSTP1* and possibly *GSTM1* may have protective effects against severe falciparum malaria in children. The contribution of specific GST polymorphisms to severe disease may differ between populations or geographic areas. These findings do not undermine the importance of oxidative stress in malaria clearance, but rather provide a broader perspective on the impact of oxidative stress on both the host and parasite cells.

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