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, GSTTI, 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 GSTTI 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 (O2−), 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. 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.

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. 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. 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. Polymorphisms resulting into absence or reduced enzyme activity have been identified and linked with 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,
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, Bröckmoller and others, and Watson and others, respectively. The PCR conditions for GSTM1, GSTT1, and GSTP1 were followed as previously described. Samples that gave negative results for both enzymes (hetero- or homozygous mutant) was 36.0% and 16.8% 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. 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. 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. Furthermore, drugs known to reduce cellular GSH were shown to potentiate the action of chloroquine in drug-resistant rodent malaria.

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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GLUTATHIONE S-TRANSFERASE POLYMORPHISMS IN MALARIA

365

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