ABSTRACT

Plasmodium falciparum (*P. falciparum*) malaria is a major cause of childhood morbidity and mortality in Sub-Saharan Africa despite the integrated approaches put in place to control the disease. Most of the mortality in holoendemic transmission areas occurs due to severe *P. falciparum* disease complication of severe malarial anaemia (SMA), a condition which largely presents in children below five years. Molecular determinants have been implicated in the pathogenesis of SMA (Hb<6.0 g/dl with any density parasitemia). Toll-like receptors (TLRs) induce production of pro-inflammatory cytokines such as interferon gamma (IFN-γ) and possesses promoter polymorphisms that could contribute to the development of SMA by influencing the circulating levels of IFN-γ, a major cytokine that activates phagocytes in malaria infection.TLR 4 is a well-established receptor for the toxigenic glycosylphosphatidylinositol (GPI) of *P. falciparum*. However, the contribution of these receptor polymorphisms in the pathogenesis of SMA and circulating levels of IFN-γ remains largely unknown in peadriatic populations resident in *P. falciparum* holoendemic areas of western Kenya. Identifying genes that naturally condition susceptibility to SMA in children population is important in designing a long lasting malaria vaccine. The purpose of the current study was to assess the associations between TLR-4 polymorphisms and IFN-γ levels in children (aged 3-36 months) that presented with clinical symptoms of falciparum malaria in Siaya County Referral Hospital (SCRH), western Kenya. Specifically, it determined the associations between TLR-4 (-8984C/G and 299Asp/Gly) polymorphic variants and susceptibility to SMA and functional differences in IFN-γ production. In addition, the study determined the functional differences in IFN-γ levels between the clinical groups. In this case-control study, 414 children with SMA and their age- and sex-matched with non-SMA (Hb ≥6.0g/dl with any density parasitemia) controls were targeted. Parasite genomic DNA was extracted from stored blood spot samples and genotyped for TLR-4 (-8984C/G and 299Asp/Gly) polymorphisms using TaqMan real-time polymerase chain reaction technique. Circulating IFN-γ levels was quantified from 50µl of stored plasma using Human Cytokine 25-plex Antibody Bead Assay. Haematological and parasitological parameters were determined in all study participants prior to administration of medication. Multivariate logistic regression analysis (controlling for confounders), demonstrated that none of the genotypes TLR-4 -8984C/G (GG vs. GC, OR, 0.78, 95% CI, 0.55-1.67, *P*=0.89 and GG vs. CC, OR, 0.62, 95% CI, 0.20-1.89,*P*=0.740) and TLR-4 +299Asp/Gly (Asp/Asp vs. Gly/Asp, OR, 1.67, 95% CI, 0.24-11.62, *P* =0.60 and Asp/Asp vs. Gly/Gly, OR, 2.06, 95% CI, 0.330-12.99, *P*=0.44) or haplotypes TLR-4 -8984G and +299Gly(OR, 0.89; 95% CI, 0.46-1.47; *P*= 0.51), TLR-4 -8984G and +299Asp (OR, 0.79; 95% CI, 0.35-1.76; *P*= 0.56) and TLR-4 -8984C and +299Asp (OR, 0.84; 95% CI, 0.52-1.36; *P*=0.486) showed any significant associations with susceptibility to SMA. Further analyses revealed that there were no differences between individual genotypes and circulating IFN-γ levels. However, carriers of C/Asp (-8984C and +299Asp) haplotype showed significantly lower levels of circulating IFN-γ (*P*=0.041) relative to non-carriers. The circulating levels of IFN-γ were similar between the clinical groups (*P*=0.224).These results demonstrate thatTLR-4 (-8984C/G and 299Asp/Gly) variants are not associated with SMA in this population, but co-inheritance of functional variations in TLR-4 condition changes in circulating IFN-γ levels. The findings are important in further enhancing knowledge on the immune genes that may alter pathogenesis of malaria, thus making more informed decisions in designing rational novel interventions in the control against malaria. Therefore, the pathological role of lower IFN-γ should be investigated as it may act peadriatric as a basis of vaccine development against malaria.