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STUDIES ON ABO BLOOD GROUPS, HAEMOGLOBINOPATHIES AND G6PD GENOTYPES, AND *PLASMODIUM FALCIPARUM* INFECTION IN KPONE-ON-SEA, GHANA

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Erythrocyte variants such as the ABO blood groups, haemoglobinopathies and G6PD genotype are known to be associated with naturally acquired immunity against malaria. Despite some evidence of their protection, other epidemiological studies have provided evidence to the contrary, therefore their associations with malaria at Kpone-On-Sea, a coastal fishing village with high malaria incidence, was investigated. The design was cross-sectional, 592 individuals were randomly selected from whom 0.5ml of blood was collected and human DNA extracted using DNeasy Kit (Qiagen, USA). Blood groups and haemoglobinopathies were determined by standard agglutination method and cellulose acetate haemoglobin electrophoresis respectively. G6PD genotypes were determined by a PCR-based method using primers 5'-CCTGTTCCCTCTGCCACA-3' and 5'-GGGGGTCTCAAGAAGTAC-3', followed by restriction of the amplified product with Hsp 92II enzyme. Parasitaemia was determined using microscopy. Among the study participants, 60.5% were females and 39.5% males. The distribution of the blood groups O, A, B and AB were 44.76%, 20.61%, 31.25% and 3.38% respectively. The prevalence of HbAA, HbAC, HbAF, HbAS, HbSC and HbSS were 71.28%, 8.11%, 1.18%, 16.89%, 1.35% and 1.01% respectively. Of all study participants, 50.68%, 35.81%, 8.11%, 1.69% and 3.72% were G6PD homozygous normal, hemizygous normal, heterozygous deficient, homozygous deficient and hemizygous deficient respectively. Only 72 individuals among the total study participants were parasitaemic. The geometric mean parasite density was 829.7 parasites/µl of blood (95%CI, 574.0-1199.40). Blood group O was not associated with reduced parasitaemia (t = -0.546, P = 0.587). HbAS was not associated with reduced parasitaemia (t = -1.262, P = 0.212). HbAC was not associated with reduced parasitaemia (t = 0.189, P = 0.851). The heterozygous G6PD deficiency was also not associated with reduced parasitaemia (t = 0.437, P = 0.664). Sample collection occurred in a period following a long dry season, resulting in low parasite prevalence rates being recorded, therefore the need for more studies to further explore the associations of these RBC variants and parasitaemia in the area. A more sensitive diagnostic technique such as PCR should be used in future studies to determine parasitaemia. There may be a clinal trend in the distribution of HbS and HbC in the country so the need for nationwide screening.

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ANTIBODIES THAT INHIBIT BINDING OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES TO CSA ARE ACQUIRED DURING PREGNANCY AND CORRELATE WITH ANTI-VSA AND ANTI-VAR2CSA

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Pregnant women are infected by *Plasmodium falciparum* presenting with unique adhesion properties that allow them to specifically bind Chondroitin sulphate A proteoglycan in the placenta. Acquisition of protective immunity over successive pregnancies is attributed to antibodies that block the adhesion of infected erythrocytes to CSA. In this study we analysed plasma samples of women of various parity enrolled from their first trimester of pregnancy till delivery in the ongoing STOPPAM project in Benin. The plasma level of anti-VSA antibodies and adhesion inhibitory activity were measured on two parasite lines selected for CSA binding on Bewo cells (FCR3 and HB3). Specific antibodies to var2csa were measured on recombinant proteins of the DBL5 domain and the full-length extracellular part of the VAR2CSA. The majority of primigravidae had low levels or no anti-adhesion antibodies at enrollment compared to multigravidae. Women who experienced a detected parasitemia during the follow up significantly increased their levels of anti-VSA, anti-var2csa as well as their plasma inhibitory activity. However a difference in the kinetics of antibody production was observed between primigravidae and multigravidae following an infection. Women infected with HIV displayed an antibody acquisition pattern similar to that of HIV negative primigravidae. Overall, a significant correlation was found between the plasma level of anti-VSA and anti-var2csa IgG and the plasma anti-adhesion activity. The results from this study suggest that the anti-adhesion antibodies play a significant role in the protective immunity acquired against pregnancy malaria and should be considered a priority in strategies aiming at developing a vaccine against this pathology.

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DEVELOPMENT OF ANTIBODY RESPONSES AND RESTRICTED GLOBAL DIVERSITY OF *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN-1 IN MALARIA ENDEMIC REGIONS

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Clinical cases due to *Plasmodium falciparum* malaria in areas of high stable transmission reduce with age partially due to acquired humoral immunity to parasite proteins exposed on the surface of infected erythrocytes. To better understand the development of immunity to *P. falciparum*, we measured antibodies among a cohort of Kenyan children and adults to surface antigens expressed by the trophozoite stages of *P. falciparum* using five *P. falciparum* parasite isolates from different geographic origins. The isolates were selected for adhesion to ICAM-1, thought to be an important receptor for endothelial adhesion. Furthermore, we quantified the importance of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) as a target of acquired antibodies by using transgenic parasites with altered expression of PfEMP1. IgG was measured by flow cytometry. Most adults had IgG antibodies that reacted with the surface of infected erythrocytes, and all isolates were well recognized by serum antibodies. In contrast there was very low to no antibody reactivity in children aged below three years. PfEMP1 appeared to be the dominant target of antibodies among adults and children. Results suggest that there is restricted global diversity or common antigenic determinants in PfEMP-1 antigens and antibody reactivity increases with age and/or exposure. Further studies on PfEMP-1 are required to define the common epitopes for development as correlates of immunity and potential blood stage vaccines.