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Antibody responses to *P. falciparum* Apical Membrane Antigen 1(AMA-1) in relation to haemoglobin S (HbS), HbC, G6PD and ABO blood groups among Fulani and Masaleit living in Western Sudan

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ABSTRACT

Fulani and Masaleit are two sympatric ethnic groups in western Sudan who are characterised by marked differences in susceptibility to *Plasmodium falciparum* malaria. It has been demonstrated that Glucose-6-phosphate dehydrogenase (G6PD) deficiency and Sickle cell trait HbAS carriers are protected from the most severe forms of malaria. This study aimed to investigate a set of specific IgG subclasses against *P. falciparum* Apical Membrane Antigen 1 (AMA-1 3D7), haemoglobin variants and (G6PD) in association with malaria susceptibility among Fulani ethnic group compared to sympatric ethnic group living in Western Sudan. A total of 124 children aged 5–9 years from each tribe living in an area of hyper-endemic *P. falciparum* unstable malaria transmission were recruited and genotyped for the haemoglobin (Hb) genes, (G6PD) and (ABO) blood groups. Furthermore, the level of plasma IgG antibody subclasses against *P. falciparum* antigen (AMA-1) were measured using enzyme linked immunosorbent assays (ELISA). Higher levels of anti-malarial IgG1, IgG2 and IgG3 but not IgG4 antibody were found in Fulani when compared to Masaleit. Individuals carrying the HbCC phenotype were significantly associated with higher levels of IgG1 and IgG2. Furthermore, individuals having the HbAS phenotype were associated with higher levels of specific IgG2 and IgG4 antibodies. In addition, patients with G6PD A/A genotype were associated with higher levels of specific IgG2 antibody compared with those carrying the A/G and G/G genotypes. The results indicate that the Fulani ethnic group show lower frequency of HbAS, HbSS and HbAC compared to the Masaleit ethnic group. The inter-ethnic analysis shows no statistically significant difference in G6PD genotypes (P value = 0.791). However, the intra-ethnic analysis indicates that both ethnic groups have less A/A genotypes and (A) allele frequency of G6PD compared to G/G genotypes, while the HbSA genotype was associated with higher levels of IgG2 (AMA-1) and IgG4 antibodies. In addition, patients carrying the G6PD A/A genotype were associated with higher levels of specific IgG2 antibody compared with those carrying the A/G and G/G genotypes. The present results revealed that the Fulani ethnic group has statistically significantly lower frequency of abnormal haemoglobin resistant to malaria infection compared to the Masaleit ethnic group.

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1. Introduction

Malaria is a prominent health issue in Africa where it contributes to the death of about 429,000 children annually (WHO, 2016b). In areas where Malaria transmission is stable, children mostly have uncomplicated infections (Greenwood et al., 1987; Hill et al., 1991). Whereas in the areas of unstable transmission, severe malaria is likely to affect all age groups (Giha et al., 2005). Although the mechanism of this protective immunity is unclear, it might be related to host genetic factors involving abnormal haemoglobin. In addition, transferred IgG from immune adults may result in clearance of blood- stage parasite and malaria symptoms (McGregor, 1964).The mortality associated with malaria is believed to be evolutionarily selective for haemoglobin (Hb) S in disease endemic areas such as Africa. This hypothesis is supported by epidemiological studies showing that heterozygosity for adults (HbA), sickle ce; (HbS) and sickle-cell trait protects children from developing severe malaria (Taylor et al., 2012). In addition, inconsistent findings from multiple previous studies further suggests that HbAS protects against uncomplicated *Plasmodium falciparum* malaria (Allen et al., 1992; Lell et al., 1999). However, several mechanisms were proposed to explain how HbAS confers malaria protection, including: (i) limited parasite invasion and/or growth in HbAS erythrocytes, especially when oxygen tension is low; (ii) augmented phagocytosis of infected HbAS erythrocytes by macrophages; and (iii) impaired adhesion of infected HbAS erythrocytes to host vascular endothelial and other cells (Lopez et al., 2010).

Recently, modification of carbon monoxide levels by HbS in mice demonstrated protective effects against malaria (Ferreira et al., 2011). In addition, several studies revealed that IgG may play a significant role in clearance of the parasite from the blood in children infected with *P. falciparum,* which also indicates the protective role of the acquired immunity (Cohen et al., 1961a; Gong et al., 2012; Williams et al., 2005a). However, the protective effect of IgG and the influence of Hb needs further clarification. Furthermore, several studies have shown that haemoglobin C trait (HbAC), offers protection against both uncomplicated and severe malaria (Modiano et al., 2001c); in particular against cerebral malaria (May et al., 2007). On the other hand, different contradicting studies suggested that ((HbAC) has no protective effects against both uncomplicated (Crompton et al., 2008; Kreuels et al., 2010) and severe malaria (Gilles et al., 1967; Guinet et al., 1997).

Collectively, these epidemiological results suggest that HbAS and HbAC may exert their antimalarial effects by different mechanisms. However, the impact of HbAC on the incidence of uncomplicated and severe *P. falciparum* malaria is not well understood compared with that of HbAS (Taylor et al., 2012).Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common X-linked inherited enzyme defect; which commonly predisposes to red blood cell haemolysis, and it is believed to protect against malaria (Guindo et al., 2007). The A allele is associated with a reduction in (G6PD) and is a diagnostic Single Nucleotide Polymorphism (SNP) of the type A- (G6PD) deficiency genotype. Type A- is typically characterised by the presence of the (rs1050828A) allele and is predominantly found in individuals with African ancestry. The (rs1050828A) allele is also associated with malarial resistance (Clark et al., 2009; Kariuki et al., 2013). The A allele is recognised as the most common (G6PD) deficiency allele in sub-Saharan Africa (Clark et al., 2009). As the (rs1050828) is located on the X chromosome, males are either homozygous for the A or G allele [rs1050828 (G; G)] (Clark et al., 2009; Kariuki et al., 2013). The (rs1050828G) allele is located within the (G6PD) gene and is associated with normal levels of glucose-6-phosphate dehydrogenase enzyme production [22,23].

The apical membrane antigen-1 (AMA-1), is located on malaria merozoite membrane (Peterson et al., 1989) and has been suggested to play a role in the invasion of erythrocytes and hepatocytes (Florens et al., 2002). Specific antibodies to AMA-1 inhibit erythrocyte invasion *in vitro* (Hodder et al., 2001) and provide protection *in vivo* (Polley et al., 2004). The inhibitory action of anti-AMA-1 antibodies shows strain specificity in allelic exchange animal studies (Dutta et al., 2007; Healer et al., 2004). In this study, we investigated a set of specific IgG subclasses against *P. falciparum* Apical membrane antigen 1 (AMA-1 3D7), haemoglobin variants and G6PD in association with malaria susceptibility among Fulani ethnic group compared to Sympatric ethnic group living in Western Sudan.

2. Materials and methods

2.1. Study area and demography

This study was conducted at the National Health Insurance Funds Hospital (NHIFH) in AL-Obied city (North Kordofan, Western Sudan) during September 2013 to February 2015. North Kordofan is a region that falls between latitude (12°43′–13°42′N) and longitude (30°14′–31°55′E). It is typically characterised by a dry and hot tropical continental climate, with a relatively short raining season. The area is hyper-endemic and characterised by seasonal and unstable malarial transmission. Al-Obied (North Kordofan) combines Afro-Arab, Arabs and non-Arab ethnic groups (Nasr et al., 2012). The major inhabitant groups are Arabs which include (Kababish, Kawahla, Hamr, and Hawawir) tribes (Nasr et al., 2012). The inhabitant tribes are mainly Arabs, which includes Dar Hamid, Danagla, Gawamaa and Bedaireia (Nasr et al., 2012). A few tribes are non-Arabs; which are mainly of Fulani and Masaleit origin from West Africa (Nasr et al., 2012). North Kordofan is an agricultural area (Most crops grown are millet, sorghum, ground nuts and sesame), and pastoral activities (cattle and goats) characterise the way of living in the region.

2.2. Study design and sampling

This study is a matched cross-sectional hospital based study that was carried out over two successive malarial transmission seasons (2013–2015) in the outpatient clinic at NHIFH, where patients were presenting to the hospital for consultation with fever (axillary temperature ≥ 37, 5 °C) or history of fever in the preceding 48 h before inclusion in this study (suspected of malaria infection received treatment). After clinical examination by the physician; informed consent was obtained from parents or guardians of children then 5 ml of whole blood were collected in EDTA tubes from each child.

In this study, a total of 124 Fulanis (62 children with malaria free controls and 62 patients with uncomplicated malaria symptoms) and 124 Masaleits (62 children with malaria free controls and 62 patients with uncomplicated malaria symptoms) children were enrolled with an overall median age of 7 years (range 5–9 years). The controls were malaria free (MFC) children (included individuals with negative blood smears for *P. falciparum* and those who did not show any clinical symptoms of malaria attending to the dental clinic and dermatology clinic) were individually matched to cases on age, sex, and ethnicity, and were recruited within 3 weeks of their matched case being identified. The recruited children fulfilled the World Health Organization (WHO) criteria for uncomplicated malaria and as such were admitted to hospital (WHO, 2016b).

Full details of the study design, malaria definition, malaria diagnosis, characterisation of patient enrolment and clinical findings were adopted from previous reports (Nasr et al., 2012; Nasr et al., 2007). Children with other detectable infections or other causes for the clinical presentation were excluded from the study. On admission and after obtaining parental informed consent; children were weighed, physically examined and a venous blood sample (5 ml) was drawn for measurement of parasitaemia, blood glucose level, serum creatinine, haemoglobin concentration, haematocrit, complete blood cell count, genotyping of haemoglobin, (G6PD), (ABO) blood grouping and humoral response to malaria antigens. Patients were treated according to the WHO guidelines (WHO, 2016b) with a complete regimen of drugs that