Impact of Haemoglobin Variants AS and AC on Asymptomatic Falciparum Malaria among Adults in Iwo, Southwestern Nigeria

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ABSTRACT: The aim of this study was to examine the impact of haemoglobin variants HbAS and HbAC on asymptomatic malaria compared to normal HbAA among adults living in a malaria hyperendemic area. Seemingly healthy individuals, 2,237 (≥16 years) without clinical symptoms in Iwo, Southwestern Nigeria were screened for this study after informed consent was obtained. A sample of 5 mL of blood was withdrawn from each participant for examination of malaria parasite and haemoglobin genotype. Thick and thin Giemsa stained blood smear were prepared for malaria parasite identification and quantification. Haemoglobin genotype was determined by cellulose acetate electrophoresis. The results showed that compared to HbAA, malaria infection and parasite densities were significantly lower in Hb AS ($\chi^2 = 26.66$, $p < 0.001$; $t = 5.05$, $p < 0.001$) and HbAC ($\chi^2 = 6.51$, $p = 0.01$; $t = 3.70$, $p = 0.002$). However, there was no significant difference between HbAS and HbAC individuals with respect to malaria infection and parasite density ($\chi^2 = 0.21$, $p = 0.64$; $t = 0.22$, $p = 0.83$). These findings suggested that among adults living in Iwo, Southwestern Nigeria, HbAS or HbAC offered better protection against asymptomatic falciparum malaria infection than HbAA while HbAS and HbAC offered similar protection.
Key Words: asymptomatic falciparum malaria; haemoglobin electrophoresis

INTRODUCTION

Many studies have shown that compared to haemoglobin (Hb) AA, HbAS is associated with resistance to severe falciparum malaria (Agarwal et al., 2000; Modiano et al., 2001; Williams et al., 2005; Danquah et al., 2010; Kreuels et al., 2010) and mild falciparum malaria (Williams et al.,2005; Kreuels et al., 2010) but there is no concensus on its protective effect against asymptomatic falciparum malaria (Williams et al.,2005; Kreuels et al., 2010). Similarly, there are reports of strong association between resistance to severe malaria and HbAC compared to HbAA (Agarwal et al., 2000; Modiano et al., 2001; Danquah et al., 2010; Kreuels et al., 2010). However, the studies available on association between mild malaria or uncomplicated malaria and HbAC show lack of protection (Agarwal et al., 2000; Danquah et al., 2010; Kreuels et al., 2010). While malaria protection from haemoglobin S comes at a cost as individuals with HbSS often develop potentially lethal sickle-cell anaemia (Weatherall and Clegg, 2001), HbCC only results in mild clinical phenotype and reports have shown that it protects against malaria (Modiano et al., 2001).

Moreso, studies on association between HbS heterozygosity (AS) and parasite density have shown contrasting results when compared with parasite density in AA individuals (Marsh et al., 1989; Cot et al., 1993; Blampain-Azzibrouck et al., 1999; Kreuels et al., 2010) while similar studies between AC and AA individuals have reported no difference (Danquah et al., 2010; Kreuels et al., 2010). In Nigeria, there is dearth of information on the association between asymptomatic malaria and haemoglobin genotypes among apparently healthy adults. The aim of this study was to determine the impact of haemoglobin variants on asymptomatic malaria among apparently healthy adults in Iwo community, Southwestern Nigeria.

MATERIALS AND METHODS

Subjects

The study was carried out in Iwo, a semi-urban community in Southwestern Nigeria. It is situated between Latitudes 7˚37´30˝ and 7˚38´30˝N and Longitudes 4˚10´30˝ and 4˚12´00˝S**.**

A total of 2237 individuals with no clinical signs and symptoms of ill health as of the time of investigation were screened for the study after clinical examination and informed consent was obtained. Ethical approval for this study was obtained from the Joint Ethical committee of Ladoke Akintola University of Technology, Ogbomoso and Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria.

A sample of 5 mL of venous blood was collected from each participant into ethylenediaminetetraacetic acid (EDTA) bottle for laboratory investigations. Thick and thin blood films stained with 3% Giemsa were examined for identification of malaria parasite. Lysate of each sample was prepared by lysing 2 volumes of washed packed cells in 1 volume of carbontetrachloride. The haemolysate of each sample was loaded on the cellulose acetate paper along with control samples. The 250-350 V was applied for 20 minutes or until visible and clear separation was obtained (Dacie and Lewis, 2001).

Statistical Analysis

The statistical package for Social Sciences (SPSS version 14) was used for statistical analysis. Differences between percentages and proportions were tested by chi-square test. Sample means were compared by ANOVA and Student's t test. A p-value of < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Results

The distributions of haemoglobin genotype, the number of those infected with *P. falciparum* and the mean values of parasite density among the study population are given in Table 1. Of the 2,237 subjects examined, 1299 (58.1%) were of genotype AA; 526 (43.9%) of which had malaria, 725 (32.4%) were of genotype AS; 232 (32.0%) of which had malaria, 170 (8.3%) were of genotype AC; 57 (33.5%) of which had malaria, 18 (0.9%) were of genotype SS; 6 (33.3%) of which had malaria; 13 (0.6%) were of genotype SC; 4 (30.8%) of which had malaria and 12 (0.5%) were of genotype CC; 3 (25.0%) of which had malaria. There was a significant association between haemoglobin genotype and *P. falciparum* infection ($\chi^2 = 29.78$; df = 5; $p < 0.001$). Falciparum infection was significantly higher in individuals with AA genotype than those with: (i) AS genotype ($\chi^2 = 25.5$; $p < 0.001$) and (ii) AC genotype ($\chi^2 = 6.51$; $p = 0.01$). There was no significant difference between the AS individuals and AC individuals who had malaria infection ($\chi^2 = 0.21$; $p = 0.65$).

Mean parasite densities varied significantly with haemoglobin genotypes ($F = 5.87$; $p < 0.001$). The mean parasite density was significantly higher in AA individuals than in: (i) AS individuals ($t = 5.05$; $p < 0.001$) and (ii) AC individuals ($t = 3.70$; $p = 0.002$). The mean parasite density of AS individuals was not significantly different from that of AC individuals ($t = 0.22$; $p = 0.83$). The numbers of SS, SC and CC individuals infected were too small to be compared statistically.

Table 1: Distribution of Haemoglobin Genotype and *Plasmodium falciparum* Infected Subjects among the Study Population in Iwo, Nigeria

Haemoglobin genotype	No Examined (%)	*Plasmodium falciparum* No	Mean ± S.D x10^3/µL
AA	1299 (58.1)	526 (43.9)	3.23±2.89
AS	725 (32.4)	232 (32.0)	2.21±2.00
AC	170 (7.6)	57 (33.5)	2.17±1.71
SS	18 (0.8)	6 (33.3)	1.87±1.65
SC	13 (0.6)	4 (30.8)	1.81±1.50
CC	12 (0.5)	3(25.0)	0.32±0.16
TOTAL	2237 (100.0)	828 (37.0)	

DISCUSSION

This study examined the impact of haemoglobin variants AS and AC on asymptomatic malaria infection among adults in Iwo, a malaria holoendemic community in Southwestern Nigeria. Our data showed that asymptomatic malaria infection and parasite density were significantly lower in AS individuals or AC individuals compared to AA individuals but there were no statistically significant differences in infection and parasite density between AS individuals and AC individuals.

Our finding showed that both HbAS and HbAC offered protection against asymptomatic malaria in terms of the number of individuals infected and the mean values of parasite density. The sickled cell trait had been associated with protection against severe falciparum malaria (Agarwal et al., 2000; Modiano et al., 2001; Williams et al., 2005; Danquah et al., 2010; Kreuels et al., 2010) mild malaria and asymptomatic malaria (Danquah et al., 2010) though some studies had suggested that its greatest impact seemed to protect against either death or severe disease while having less effect on infection per se (Hill et al., 1991; Cooke and Hill, 2001).

Although early researches to find a link between haemoglobin C and malaria resistance were inconclusive, recent studies had shown its protective effect against severe malaria (Agarwal et al., 2000; Modiano et al., 2001; Kreuels et al., 2010; Mockenhaupt et al., 2004; May et al., 2007). Studies on the effect of HbC on uncomplicated and asymptomatic malaria which had reported lack of protection had been largely based on children Danquah et al., 2010; Kreuels et al., 2010). Our study observed that HbC among adults in a malaria endemic area offered protection against asymptomatic malaria.

In this study, no statistically significant differences in malaria infection and parasite density were observed between AS individuals and AC individuals. There are conflicting reports on whether or not haemoglobin C offers better protection against malaria compared to haemoglobin S. Modiano *et al*. (2001) reported that HbC reduced risk of clinical malaria was greater than that of HbS and that HbC could replace HbS in central West Africa. Also, Agarwal *et al*. (2001) observed that the protective effect of HbAC was greater than that of HbAS. However, Danquah *et al*. (2010) and Kreuels *et al*. (2010) reported that the protective effect of HbAS was greater than that of HbAC. Our result showed that adults who were HbAS and HbAC offered similar protection against asymptomatic malaria.

Several mechanisms had been suggested for protective effects of HbS and HbC. According to Weatherall and Glegg (2001), parasitaemia was lower in AS because AS cells sickled in the circulation and were removed by the spleen before the parasite could develop into schizonts. Weatherall and Clegg (2001) and Cooke and Hill (2001) suggested both impaired entry into and growth of parasites in red cells. Similarly, Rihet *et al*. (2004) observed that parasite multiplication was inhibited in individuals with HbC. According to Oslon and Nagel (1986) these abnormal cells probably constituted a barrier for the parasite because of their inability to lyse and release merozoites at the appropriate stage. Some authors had suggested that the protective effect of HbC might act in synergy with specific acquired immunity as suggested for the protective effect of HbS (Abu et al., 1992; Ntoumi et al., 2002). Reduced and impaired cytoadherence had been observed in both HbS and HbC carriers suggesting similar protection mechanism for these two haemoglobin variants (Verra et al., 2007).

Unlike HbSS whose lethality offsets its protective effect, the HbCC presents with lack of clinical disability or haematological changes. Owing to the small number of cases involved, SS and CC individuals' results were not compared in this study. However, available studies had shown that HbCC offered greater protection against malaria than HbAC, AS together with SS (Agarwal et al., 2000; Modiano et al., 2001).

Our findings suggested that asymptomatic malaria infection among the study population was significantly associated with haemoglobin type. When compared to the normal haemoglobin (HbAA), HbAS or HbAC significantly protected against asymptomatic malaria infection and their protection was comparable.

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