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Research Article

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

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Abstract: Several studies have shown that haemoglobin (Hb) variants AS and AC are associated with resistance to severe falciparum malaria infection. However, reports on association between these haemoglobin variants and asymptomatic malaria infection are conflicting. The objectives of this study were to examine the associations between asymptomatic falciparum malaria infection and haemoglobin variants AS and AC. 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 A sample of 5 mL of blood was withdrawn from each participant for examination of malaria parasite and haemoglobin genotype.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. There was no significant relationship between haemoglobin variants and sex (χ^2 = 1.46, df = 5, p = 0.918). Compared to HbAA, malaria infection and parasite densities were significantly lower in Hb AS (χ^2 = 26.66, p < 0.001; t = 5.05, p < 0.001) and Hb AC (χ^2 = 6.51, p = 0.01; t = 3.70, p = 0.002). There was no significant difference between AS and AC individuals with respect to malaria infection and parasite density (χ^2 = 0.21, p = 0.64; t = 0.22, p = 0.83). These findings suggest 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.
Keywords: Adults, Asymptomatic falciparum malaria, Haemoglobin variants, Iwo.

INTRODUCTION

Many studies have shown that, compared to haemoglobin (Hb) AA, HbAS is associated with resistance to severe falciparum malaria [1-5] and mild falciparum malaria [3, 5] but there is no concensus on its protective effect against asymptomatic falciparum malaria [3, 5]. Similarly, there are reports of strong association between resistance to severe malaria and HbAC compared to HbAA [1, 2, 4, 5]. However, the studies available on association between mild malaria or uncomplicated malaria and HbAC show lack of protection [1, 4, 5]. While malaria protection from haemoglobin S comes at a cost as individuals with HbSS often develop potentially lethal sickle-cell anaemia [6], HbCC only results in mild clinical phenotype and reports have shown that it protects against malaria [2]. Also, studies on association between HbS heterozygosity (AS) and parasite density have shown contrasting results when compared with parasite density in AA individuals [5, 7-9] while similar studies between AC and AA individuals have reported no difference[4, 5].

In Nigeria, there is dearth of information on the impact of haemoglobin variants on malaria infection among apparently healthy adults. The aim of this study was to determine the impact of haemoglobins S and C on asymptomatic malaria among adults in a malaria endemic community in Southwestern Nigeria.

MATERIALS AND METHODS

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 2,237 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 [10].

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

Of the 2,237 subjects examined, 1107 (49.5%) were males and 1130 (50.5%) were females. The distributions of haemoglobin variants of the participants with respect to sex are given in Table 1. Of the 1107 males, 641 (57.9%) had genotype AA, 361 (32.6%) AS, 82 (7.4%) AC, 8 (0.7%) SS, 8 (0.7%) SC and 7 (0.6%) had genotype CC while of the 1130 females, 658 (58.2%), 364 (32.2%), 88 (7.8%), 10 (0.9%), 5 (0.4%) and 5 (0.4%) had genotype AA, AS, AC, SS, SC and CC respectively. There was no significant relationship between haemoglobin variants and sex ($\chi^2 = 1.458$, df = 5, $p = 0.918$).

The distributions of haemoglobin variants, the number of those infected with *P. falciparum* and the mean values of parasite density among the study population are given in Table 2. 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 HbAA individuals than in: (i) HbAS individuals ($\chi^2 = 25.5$; df = 1; $p < 0.001$) and (ii) HbAC individuals ($\chi^2 = 6.51$; df =1; $p = 0.01$). There was no significant difference between the AS individuals and AC individuals who had malaria infection ($\chi^2 = 0.21$; df =1; $p = 0.65$).

Similarly, 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 ($t = 3.70$; $p = 0.001$). 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 Variants with Respect to Sex among the Study Population in Iwo, Nigeria

Haemoglobin genotype	No Examined (%)	Male (%)	Female (%)
AA	1299 (58.1)	641 (57.9)	658 (58.2)
AS	725 (32.4)	361 (32.6)	364 (32.2)
AC	170 (7.6)	82 (7.4)	88 (7.8)
SS	18 (0.8)	8 (0.7)	10 (0.9)
SC	13 (0.6)	8 (0.7)	5 (0.4)
CC	12 (0.5)	7 (0.6)	5 (0.4)
Total	2237 (100.0)	1107 (49.5)	1130 (50.5)

Table 2: Distribution of Haemoglobin Variants 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)	2.84±2.23

DISCUSSION

This study examined the association between asymptomatic malaria infection and haemoglobin variants 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.

Also, 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 [1-5], mild malaria and asymptomatic malaria [4] 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 [11, 12].

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 [1, 2, 5, 13, 14]. Studies on the effect of HbC on uncomplicated and asymptomatic malaria which had reported lack of protection had been largely based on children [4, 5]. This 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. Some studies reported that HbC reduced risk of clinical malaria was greater than that of HbS [1, 2] and that HbC could replace HbS in central West Africa [2]. However, others reported that the protective effect of HbS was greater than that of HbC [4, 5]. The present 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. In the case of AS cells, some studies had suggested both impaired entry into and growth of parasites in red cells [6, 12]. Lower parasitaemia in AS individuals was said to be due to the sickling of AS cells in the circulation and their subsequent removal by the spleen before the parasite could develop into schizonts [6]. Similarly, it had been observed that parasite multiplication was inhibited in individuals with HbC and that these abnormal cells constituted a barrier for the parasite because of their inability to lyse and release merozoites at the appropriate stage [15, 16]. 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 [17, 18]. Reduced and impaired cytoadherence had been observed in both HbS and HbC carriers suggesting similar protection mechanism for these two haemoglobin variants [19].

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 [1, 2].

CONCLUSION

The present findings suggested that asymptomatic malaria infection among the study population was significantly associated with haemoglobin type. When compared to HbAA individuals, HbAS and HbAC individuals were significantly more protected against asymptomatic malaria infection and their protection was comparable.

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