Effects of ABO/Rh blood groups, G-6-P-D enzyme activity and haemoglobin genotypes on malaria parasitaemia and parasite density.

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SUMMARY

The main objective was to investigate the effects of ABO/Rh blood groups, haemoglobin genotype and G-6-P-D enzyme activity on malaria. The study was carried out in Buea, South West Province, Cameroon. Subjects consulting at health care facilities in Buea were randomly recruited into the study. A total of 121 febrile patients 1-60 years old comprised the study subjects. Thin and thick blood films were prepared for malaria parasite detection. G-6-P-D enzyme activity was assayed using the met-haemoglobin reduction test. Determination of haemoglobin genotypes was by a rapid screening method alongside electrophoresis. Malaria positive patients were treated. The highest malaria prevalence of 74.5% was in Group O individuals and the lowest of 58.6% in group B individuals. Mean parasite density (Log_{10}^{-1}/ul blood) in the various blood groups was not significantly different. Individuals with G-6-P-D deficiency had a significantly lower malaria prevalence (47.5%) when compared with active individuals. Mean parasite density in enzyme deficient and active individuals was 3.7(SD± 3.9) and 4.4(SD ±5.0) respectively and the difference was significant ($p<0.05$). Malaria prevalence was lower (57.5%) in HbS individuals when compared with HbAA (74.6%) and HbSS (60%) but parasite density was not significantly different. Our results suggest that individuals with blood group O who have the HbAA genotype and show G-6-P-D enzyme activity may be more susceptible to malaria. Information on the influence of these genetic factors on malaria would be useful in the better management of the disease in the study area.

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Introduction

Despite the high morbidity and mortality, certain individuals living in malaria endemic regions appear relatively protected compared to those who suffer frequent severe malaria attacks. Resistance to malaria infection is dependent on the development of an immune response by the host and to a varying extent, on certain innate characteristics possessing protective value against infection. These factors include sickle cell trait (HbAS) and sickle cell disease HbSS [1,2], ABO blood type [3,4] and the level of G-6-P-D enzyme activity [5]. The high frequency of haemoglobin S in malaria endemic areas has been attributed to the selection advantage it offers to carriers of the trait against malaria [6]. The mechanism of protection offered by G-6-P-D deficient cells is related to the decreased levels of reduced glutathione in these cells, which render them sensitive to oxidant damage hence inhibiting parasite growth [7]. There is only limited data on the association between malaria and red blood cell ABO antigens. A study conducted at Edo State University in Nigeria reported that in males, individuals with blood group O and B were the most and the least susceptible to malaria parasitaemia respectively

[3]. Contrary to these findings, a related study in another part of Nigeria reported no significant association between ABO blood groups and resistance to malaria in non-severe cases [8,9]. It is possible that the ABO blood type, haemoglobin genotype and the presence or absence of G-6-P-D enzyme activity may have a cumulative effect on the outcome of malaria parasitaemia and parasite density. The main objective of this study therefore was to investigate the individual and joint effects of these genetic factors on malaria in the study area.

Patients and Methods

Study area

The study was conducted in the South West Province of Cameroon from January to November 2002. The study period covered both the rainy and dry seasons, which coincided with the high and low transmission of malaria respectively.

Study population

Participants comprised 222 febrile patients between the ages of 1-60 years who were referred to the hospital laboratory for a malaria test. Ethical clearance was obtained from the ethical Review Committee of the Delegation of Health in the South West Province. Each participant or guardian signed an informed consent form. A questionnaire was administered to each patient and additional information was obtained on the date of onset of fever, drugs taken prior to consultation, previous history of fever, presence of nausea, vomiting, convulsion and other illnesses.

Sample collection

About 3ml of venous blood was collected from which thick and thin blood films were made on glass slides. The rest of the blood was put into tubes containing anticoagulant to be used for the detection of ABO/Rh blood groups, G-6-P-D enzyme activity and haemoglobin genotype.

Detection of malaria parasites

Thin and thick blood films were stained with Field's stains, and the slides were examined by microscopy for malaria parasites. Absolute parasite counts were obtained from thick smears by counting the number of parasites among 200 leucocytes and multiplying the count by the patients total leucocyte count divided by 200. Thick smears were recorded as negative only after 100 or more high-powered microscope fields had been scanned.

Determination of blood group

The ABO/Rh blood group was determined by placing a drop of each blood sample on three distinct areas on a slide and mixing each separate drop with commercially prepared antiserum-A, B or D and observing for agglutination.

G-6-P-D enzyme activity assay

The met-haemoglobin reduction test to detect G-6-P-D deficiency was used [10]. Essentially, 2 ml of test or reference (normal and deficient controls) sample was added to a tube containing 0.2ml of a mixture of sodium nitrite (180mmol/l), dextrose (28mmol/l) and methylene blue (0.4mmol/l). The tubes were thoroughly mixed and samples incubated at 37°C for 3 hours. After incubation 0.1ml was removed from each tube and put into 10 ml of water. The contents were gently mixed and the colour intensity measured.

Determination of haemoglobin genotypes

A rapid screening method was first done which consisted of placing a drop of sodium metabisulfite on a slide to which was added a drop of blood sample. The wet film was immediately covered with a cover slip and sealed with petroleum jelly/paraffin wax mixture. Each film was immediately observed under a microscope to determine the degree of sickling [11]. Results were confirmed with the traditional electrophoresis method.

Statistical methods

Comparison of malaria parasitaemia in the different groups was performed using the χ^2 tests. Mean values were compared using analysis of variance (ANOVA). The statistical significance level was $p<0.05$

Results

Patient population

A total of 222 patients were studied with 109 males and 113 females. Their ages ranged

between.1-60 years. Fifty of these were below 5 years of age. The clinical signs and symptoms commonly presented were fever, headache, diarrhoea and joint pain.

Relationship between ABO/Rh blood group and malaria

There was no significant difference (χ^2 =4.8; p>0.05) in the distribution of these blood groups in males and females. Similarly, no difference was observed in the prevalence of malaria in males and females of the various blood groups (χ^2 =0.07; p.0.05). However, a significant difference was found to exist between malaria prevalence in individuals of blood group O when compared with that of the other blood groups (Table 1). No significant difference occurred in mean parasite density in the different blood groups. The prevalence of the Rhesus factor was 65.8% and malaria prevalence in this group was 62.3%. However, no association was found between the Rhesus factor and parasite density (χ^2 =3.6; p>0.05).

Table 1. Malaria parasitaemia and parasite density in the different blood groups

Blood group	Positive for malaria*	Log_{10}^{-1} mean(SD ±)parasite density$^+$
A (n=64)	40(62.5%)	4.1 (±5.1)
B (n=29)	17(58.6%)	3.7 (±4.3)
AB(n=19)	12(63.2%)	3.9 (±4.5)
O (n=110)	82(74.5%)	4.2 (±4.9)

*χ^2 = 15.5; p<0.05
$^+$F = 0.01; p>0.05

Table 2. Association of the level of G-6-P-D enzyme activity with malaria parasitaemia and parasite density.

Level of G-6-P-D enzyme activity	Positive for malaria*	Log_{10}^{-1} mean (SD ±) parasite density$^+$
Active (n=111)	92(82.9%)	4.4 (±5.0)
Deficient (n=40)	19(47.5%)	3.7 (±3.9)
Heterozygote (n=69)	39(56.5%)	3.5 (±3.9)

*χ^2 = 23; p<0.05
$^+$F = 3.1; p>0.05

Table 3. Effect of haemoglobin genotype on intensity of malaria parasitaemia.

Hemoglobin genotype	Positive for malaria*	Log_{10}^{-1} mean(SD ±)parasite density^{+}
AA (n=134)	100(74.6%)	4.4 (±5.0)
AS (n=73)	42(57.5%)	3.5 (±3.9)
SS (n=15)	9(60%)	2.5 (±2.7)

*$\chi^2 = 6.8$; $p<0.05$
$^{+}F = 1.1$; $p<0.05$

Association of malaria and G-6-P-D enzyme activity

Of the 220 individuals whose blood samples were analysed for enzyme activity, the prevalence of G-6-P-D active, deficient and heterozygote patients was 50.5%, 18.2% and 31.4% respectively. A significantly higher malaria prevalence and mean parasite density was observed for individuals with G-6-P-D enzyme activity (Table 2).

Effect of haemoglobin genotype on malaria

A Prevalence of 60.4%, 32.9% and 6.6% was obtained for HbAA, HbAS and HbSS genotypes respectively. No significant difference was found in the distribution of these genotypes in males and females (χ^2 =3,7;p>0.05). While malaria prevalence was higher in HbAA individuals, mean parasite densities in the three groups were not different (**Table 3).**

Discussion

Investigations on the effect of the human genetic background and of parasite genotype on malaria morbidity and infection have become recognized as an important area of malaria research. In the study described herein, we investigated the prevalence of G-6-P-D enzyme activity, haemoglobin genotype and ABO/Rh blood group in the study area and the influence of these on malaria parasitaemia and parasite density. The results obtained revealed that 64.8% of the population was blood group O and these individuals also had the highest prevalence (74.5%) of malaria. Group B individuals on the other hand had the lowest malaria prevalence (58.6%). The malaria observed in the study was classified as uncomplicated malaria. The ABO/Rh blood type generally did not appear to influence parasite density, as mean parasite density was not significantly different between members of the various blood groups. Our results are similar to those reported in a Nigerian population [3]. Earlier studies conducted in Nigerian children reported a lack of association between ABO blood group and non-severe falciparum malaria [8,9]. In similar studies carried out in Gambia a protective role for blood group O was demonstrated against severe malaria. Varying degrees of susceptibility of ABO blood groups to malaria parasites have been reported [12].

An overall prevalence of 18.2% was obtained for G-6-P-D deficiency. It was observed that G-6-P-D deficient patients had significantly lower malaria prevalence and mean parasite density when compared with G-6-P-D active individuals and heterozygotes. These findings support the view that the deficient enzyme offers some relative advantage against malaria to its carriers [5]. The most common African G-6-P-D deficiency variant, G-6-P-D A^{-} has been linked to an appreciable reduction in the risk of severe malaria for G-6-P-D female heterozygotes and male hemizygotes.

This study did not demonstrate that the protective effect of haemoglobin S on malaria is HbS content related as proposed by other studies [1,2] in which HbSS individuals were found to be more protected than HbAS individuals. A higher proportion of HbSS individuals were infected with parasites in our study compared with HbAS. This

genotype, however, did not exert significant impact on malaria parasitaemia since mean parasite densities were not significantly different in individuals of the three haemoglobin genotypes. It has been suggested, however, that the protective role is against severe disease and not infection rate. We included a faster and cheaper screening method to detect sickling in whole blood by microscopy, which was comparable with the results obtained using the standard electrophoresis method. It has been suggested that the sodium metabisulfite method should not, however, be used on cord and infant blood [11]. Mixed infection rates were not influenced by the different genetic factors under study. *Plasmodium falciparum* was the predominant species occurring only in 7 cases as mixed infection with *P. malariae*. Interestingly, we found that 9 out of 16 patients who had parasite densities >10,000 parasites/μl were blood group O, HbAA and showed G-6-P-D enzyme activity. This finding highly suggests that the presence of these genetic factors may influence the intensity of malaria parasitaemia. A larger sample size is required to validate this finding. It is clear from the present study that there is need for more investigations to be carried out in several epidemiological settings with varied levels of malaria endemicity to better evaluate the effects of the above genetic factors on malaria severity.

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