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The Relationship Between G6PD Variations And Malaria In Calabar, Nigeria

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ABSTRACT

Introduction: This study was carried out to ascertain the burden of malaria based on the G6PD status of the participants. A total of 225 inhabitants were recruited for this study.

Materials and Methods: Participants were tested for malaria infection using microscopic and serological techniques.

Results: The prevalence of malaria infection in the study was 27.1%. The polymerase chain reaction (PCR) technique detected G6PD and its variations. The prevalence rate of G6PD deficiency in the entire study population was 15.1%, and the difference between malaria-positive subjects who were G6PD deficient and those with normal G6PD was significant. The study indicated a male sex bias in the prevalence of G6PD deficiency in the population studied.

Conclusion: This study recorded a significant association between G6PD variation, a human genetic factor, and malaria disease.

Keywords: Malaria, G6PD, Microscopic, Serological, PCR.

INTRODUCTION

Malaria, a potentially fatal, vector-borne disease caused by the protozoan parasites of the genus *Plasmodium* (1), is transmitted to humans through the bite of an infected female *Anopheles* mosquito. The predominant species responsible for over 95% of malaria cases in Nigeria is *P. falciparum*. This is also the most dangerous malaria parasite species (2). Calabar is one of the coastal cities in Nigeria and is considered a hyper-endemic malaria city due to its typical rainforest vegetation, which has been sustained by heavy rainfalls almost all year round (3). In malaria-endemic climes like Calabar, the clinical outcome of malaria and its advancement to pathological complications depends on many factors involving the specific and dynamic combination of host and parasite properties (4).

In malaria-endemic countries, humans acquire protection against malaria due to naturally occurring mechanisms involving inheritable modifications of genes. *Plasmodium falciparum* malaria infection is a well-recognized relevant force of evolution, which has helped to shape the human genome and can select genes that contribute to resistance (5). A couple of host genes have been implicated in conferring specific protection against the progression and severity of the malaria disease. Glucose-6-phosphate dehydrogenase is a well-characterized human genetic defect that confers a certain degree of resistance to malaria, especially its severe forms (6).

Glucose-6-phosphate dehydrogenase is an X-linked enzyme that helps red blood cells (RBC) work properly and ensure survival. A lack of this enzyme, which is hereditary, causes RBCs to break down faster than they are made (7). This enzyme deficiency causes partial protection against malaria by producing oxygen radicals in the red cell, causing oxidative stress and eventual death of the parasite. The geographical prevalence of this disorder correlates with malaria distribution through natural selection, which accounts for its persistence and high frequency (8). The first line of natural defense against malaria is mainly exerted by G6PD deficiency, alongside abnormal haemoglobins.

The polymorphism of this enzyme deficiency is high, with more than 160 genetic variants identified globally. Sub-Saharan Africa's 376G/202A haplotype is the most prevalent (9).

This study assesses the prevalence of G6PD variations and their impact on malaria. This information will be useful in understanding the acquisition of malaria immunity and provide baseline data of value in malaria vaccine trials.

MATERIALS AND METHODS

Study Area

This study was conducted at the University of Calabar Teaching Hospital (UCTH) in Calabar, Cross River State, Nigeria, between July 2022 and May 2023. Calabar is located in the mouth of Cross River, and its latitude and longitude coordinates are 4.982873 and 8.334503, respectively. This area lies in the SouthSouth geographical zone within the coastal rainforest region of Nigeria. The majority of the population speak Efik and Ejagham dialects. The vegetation is typical of the tropical rainforest, which makes malaria transmission stable throughout the year but more intense during the rainy season, lasting from April to October (3).

Ethical Approval

This study was approved by the Health Research Ethics Committee of the University of Calabar Teaching Hospital, Calabar-Nigeria. Informed consent was obtained from the participants.

Determination of Sample Size

A total of 225 participants were selected for the study. Only subjects who reside permanently in the study area were recruited.

Collection of Samples

Each study participant was subjected to capillary blood collection for blood film preparation and malaria serological tests. In addition, 2ml of venous blood was collected by venipuncture from each individual into an ethylenediaminetetraacetic acid container for molecular analyses.

Detection of Malaria Infection

Microscopy and serological techniques (rapid diagnostic test) were used to screen for the presence of malaria parasites. Thick and thin blood films were prepared for each individual and stained using the Giemsa staining method as described by Onile & Tawo (10). Malaria parasites were counted against white blood cells (WBCs). A minimum of 200 WBCs were counted, and the number of malaria parasites counted per white cells were recorded. The parasite density was then converted to parasite per milliliter of blood according to the formula below:

$$\frac{\text{Number of parasites counted} \times \text{WBC standard (6000)}}{\text{Number of WBCs counted}}$$

Determination of G6PD Status

Molecular genotyping of G6PD variant mutations was done using venous blood. Extracted DNA was amplified and analyzed for the presence or absence of common genotyped G6PD variants in Africa (A374G and G202A) using PCR assay (9). PCR used a 20 µl reaction mixture containing 5 µl of genomic DNA, 10 µl of PCR Master Mix (Thermo Fisher), and 0.3 µM of each forward and reverse primer. Polymerase water was used in a separate reaction as a negative control. Amplifications were done through an initial denaturation at 95°C for 5 minutes, followed by 40 cycles at 94°C for 30 seconds, 56°C for 1 minute, and 65°C for 45 seconds, with a final 7 min extension at 65°C. Then, the PCR products were run by gel electrophoresis with 2% agarose gel tinged with SafeView DNA stain at 120 volts for one hour and sequenced on an ABI 3730 xl DNA analyzer following standard protocols (Genewiz Inc., La Jolla, CA). Sequences were analyzed using BLASTN. All sequences were aligned to the National Centre for Biotechnology Information (NCBI) reference sequence to check the specificity of the PCR products.

Statistical Analysis

Data generated in this study were analyzed using differences of Mean, percentages, Chi-square, descriptive statistics, t-test, ANOVA, and Pearson's correlation. Chi-square and Pearson's correlation were used to assess the association among variables, while t-test and ANOVA were used to determine differences between two and multiple means.

RESULTS

The study included 225 participants, 85 (37.8%) males and 140 (62.2%) females, with a mean age of 32.1 ± 6.90 years. Table 1 shows the distribution and intensity of malaria infection based on gender of the population studied.
Table 1: Prevalence and Intensity of Malaria Infection among Study Subjects by Gender

Gender	No. of Subjects Examined	No. (%) of Subjects Infected with Malaria	Mean Parasite Density (per µl of blood)
Males	85	21 (24.7)	400±108.9
Females	140	40 (28.6)	150±68.0
Total	225	61 (27.1)	
		p-value= 0.0816	0.0028

Table 2: Prevalence and Intensity of Malaria Infection in the Study Population by Age

Age Group (years)	Number of Subjects Examined	Number (%) Infected with Malaria	MPD (per µL of blood)	P
5-14	37	11 (29.7)	30± 14.7	0.0017
15-24	33	10 (30.3)	120± 51.5	
25-34	43	13 (30.2)	320± 150.0	
35-44	42	10 (23.8)	167± 70.1	
45-54	38	09 (23.7)	261± 105.0	
55-64	17	03 (17.7)	640± 207.0	
65-74	15	05 (31.3)	500± 188.1	
Total	225	61 (27.1)		

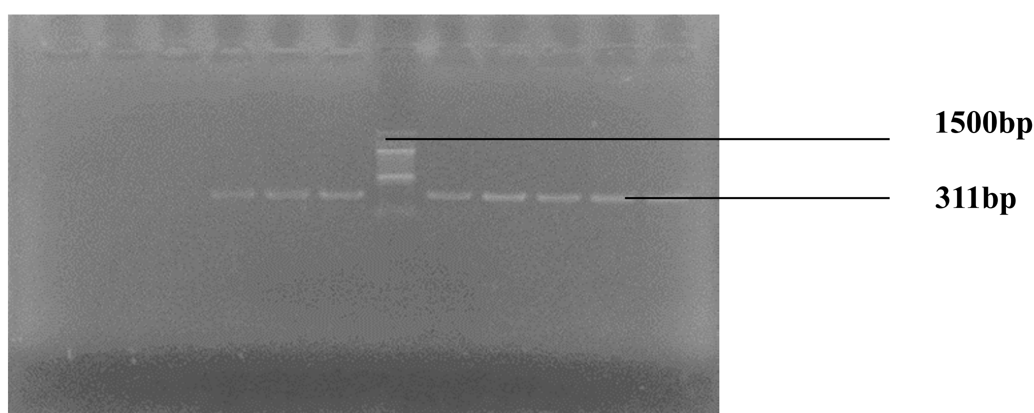

Plate 1: Representative photomicrograph of agarose gel electrophoresis showing glucose-6-phosphate dehydrogenase genes of some selected samples. Lanes 1-3 show no mutation, thus have normal glucose-6-phosphate dehydrogenase.

Table 3: Age Distribution of G6PD Deficiency among Subjects

Age Group (years)	Number of Subjects Examined	Number (%) of Subjects with Normal G6PD	Number (%) of Subjects with G6PD deficiency	P
5-14	37	24 (64.9)	13 (35.1)	0.077
15-24	33	28 (84.8)	05 (15.2)	
25-34	43	39 (90.7)	04 (9.3)	
35-44	42	40 (95.2)	02 (4.8)	
45-54	38	33 (86.8)	05 (13.2)	
55-64	17	15 (88.2)	02 (11.8)	
65-74	15	12 (80.0)	03 (20.0)	
Total	225	191 (84.9)	34 (15.1)	

Table 4: Gender Distribution of G6PD Deficient Individuals in the Entire Population

Gender	Screened Individuals	Number (%) of G6PD Deficient Individuals	χ^2	P
Males	85	23 (27.1)	10.8395	0.000994
Females	140	11 (7.9)		
Total	225	34 (15.1)		

Table 5: Distribution of G6PD variations, malaria parasitaemia and parasite density amongst malaria positive subjects

G6PD Variation	Number positive for malaria (n=61), n (%)	Mean parasite density	f-ratio
Normal G6PD	53 (86.9)	3600 ± 512	91143.61
Deficient G6PD	08 (13.1)	581 ± 208	
Total	225 (27.1)		

DISCUSSION

In this study, females (28.6%) had a higher malaria infection rate than males (24.7%). However, there was no significant association (p>0.05) between gender and the occurrence of malaria. This is similar to studies conducted by Malar *et al.* (11), Okiring *et al.* (12), and Nwele *et al.* (13). Though there is no scientific evidence that susceptibility to malaria is gender-based, the disease may not be gender-blind after all (14). Social and cultural factors (such as cooking outdoors in the early hours of the morning and late hours of the evening) are believed to aid malaria transmission, which makes females more vulnerable to malaria infection (15). Malaria infection intensity was higher among males (400±108.9) than female subjects (150±68.0) (Table 1), and this was statistically significant (p<0.05). The male bias in parasite count may be attributed to behavioural differences in alcohol and tobacco consumption, which increases males' attractiveness to mosquitoes (12). Older subjects (aged 65-74 years) had the highest malaria prevalence rate (31.3%) and intensity of malaria infection (500±188.1). There was a strong statistically significant association between age and malaria infection (p<0.05). Such

trends have been reported by Mawili-Mboumba *et al.* (16); Ogah *et al.* (17); Nwaneli *et al.* (18), and Okiring *et al.* (12). However, this is contrary to the report by Nwele *et al.*, (13), who reported a low malaria transmission in older age groups.

Of the 225 participants tested, 34 (15.1%) were G6PD-deficient. The prevalence of G6PD deficiency was concentrated predominantly among male participants (27.1%) compared to their female counterparts (7.9%). Male gender was significantly associated with G6PD deficiency among the participants studied. These values are similar to those reported by Okafor *et al.* (7) and Isaac et al. (19). G6PD deficiency is an X-linked recessive disorder where males usually manifest the abnormality and females are carriers (19). The highest prevalence of G6PD deficiency occurred among participants aged 5-14 years.

Amongst malaria-infected subjects, those with normal G6PD had a higher malaria prevalence (86.9%) and intensity (3600±512) than their G6PD deficient counterparts. There was a strong statistically significant association between G6PD variations and malaria infection. Increased oxidative stress of the erythrocytes due to G6PD deficiency impairs *P. falciparum* survival, and this has been attributed to genetic adaptation to malaria in malaria-endemic regions as the malaria parasite requires the enzyme for its normal survival in the host cell (20).

CONCLUSION

Glucose-6-phosphate dehydrogenase offers some protective effects against severe malaria. Individuals with this deficiency do not regularly get infected with malaria, and if they do, they may experience milder forms of malaria or lower parasite loads. However, individuals in malaria-endemic regions, especially those with a family of G6PD deficiency, should be screened to identify carriers and prevent complications (such as haemolytic crises) during malaria treatment.

This study further enhances our understanding of how G6PD can influence susceptibility to malaria. It also informs public health policies on screening and treatment protocols in malaria-endemic regions, improving patient care. Insights into the interplay between G6PD deficiency and malaria can aid in developing targeted interventions and preventive measures for affected populations. The relationship opens avenues for further research into genetic factors affecting disease outcomes, potentially leading to novel therapeutic approaches. Overall, this research enriches our understanding of malaria pathogenesis and the role of genetic factors in malaria disease dynamics.

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