

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: FREQUENCY AND IMPLICATIONS IN A SUB-SAHARAN AFRICAN VILLAGE

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Abstract

Glucose-6-Phosphate Dehydrogenase (G6PD) enzyme deficiency is the most prevalent X-linked recessive hereditary red blood cell abnormality, affecting over 500 million people globally (Luzzatto et al., 2016). This deficiency is critical as G6PD catalyzes the first step in the pentose phosphate pathway (PPP), the sole source of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) in red blood cells (RBCs). NADPH, in turn, is essential for converting oxidized glutathione (GSSG) to its reduced form (GSG), which protects cells from oxidative stress by neutralizing reactive oxygen species (ROS) (Gomez-Manzo et al., 2017). Interestingly, G6PD deficiency is believed to confer a protective advantage against malaria, suggesting that natural selection has played a role in maintaining its gene frequencies in malaria-endemic regions. This study explores the frequency of G6PD deficiency in a holoendemic village in Sub-Saharan Africa, examining its implications for public health and potential benefits in malaria-prone areas.

INTRODUCTION

Glucose-6 phosphate dehydrogenase enzyme deficiency is the most common x- linked recessive hereditary red blood cell abnormality, which affects over 500 million people globally (Luzzatto et al., 2016). It is the critical enzyme in the first step of the pentose phosphate pathway (PPP), which is the only source of generating the co-enzyme, Nicotinamide Adenine Dinucleotide Phosphate (NADPH) in red blood cells (RBCs). The NADPH passes the electron to oxidized glutathione (GSSG) in the oxidant pathway, producing reduced glutathione (GSG). The GSG generation helps to protect cells from oxidative stress by removing the reactive oxygen species (ROS) (Gomez-Manzo et al., 2017). G6PD enzyme deficiency is believed to protect against malaria, and it is thought that natural selection has been responsible for elevating and maintaining their genes frequencies in malarial regions of the world. Therefore, it can be no surprise that malaria parasites should have had a profound impact on recent human evolution. This is the proposition of the “malaria hypothesis”, which posits that specific human genetic polymorphisms, especially those affecting red blood cells (RBCs), have been selected for high frequencies because they have protection against the effects of malarial infections. Indeed, many identified human genetic

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polymorphisms meet some or all of the expectations of the malaria hypothesis that can be attributed to selection under any other single agent (Cavalli-Sforza et al., 1994).

Glucose-6-phosphate dehydrogenase (G6PD) is of broadly global distribution with sex-linked gene mutation, giving rise to G6PD deficiencies (Luzzatto et al., 2016). The gene encoding G6PD is highly polymorphic and is located at the q28 locus on the x-chromosome and is 18kb long with 13 exons. There exist numerous polymorphisms as a result of single point mutations, deletions, and insertions, leading to various variants. World Health Organization classified G6PD genetic variants, based on the level of enzyme activity, into five classes, from the most severe (class 1) to the mildest (class 4): Class I, severe deficiency of the enzyme with chronic non-spherocytic haemolytic anaemia; Class II severe deficiency with enzyme activity <10% of normal; Class III moderate deficiency with enzyme activity 10-60% of normal; Class IV very mild to none deficiency with enzyme activity 60-100% of normal and Class V increased enzyme activity; with the first three being deficiency states (WHO, 2019). The most common variant seen African population is G6PD-A. It is of mild to moderate deficiency that has 10-15% of normal activity in the hemizygous. The G6PD Mediterranean variant is more severe, with less than 1% normal activity. G6PD mutations frequently cause clinical manifestation because of decreased enzyme activity and stability (Cappellini and Fiorelli, 2008). The deficiency was recognized initially as a clinical condition known as *favism*, so-called because of the haemolytic crisis that can be caused in those affected by the consumption of oxidant foods such as fava beans. Most G6PD deficiency patients show no symptoms until exposure to external triggers could lead to moderate and severe symptoms, such as acute or chronic haemolytic anaemia, favism, hyperbilirubinaemia, and neonatal jaundice (Frank, 2005). Today, G6PD deficiency is a recognized hazard in malarious regions because of the associated risk of oxidant stress from taking antimalarial drugs such as Primaquine (Recht et al., 2018). The hereditary G6PD enzyme deficiency distribution varies in males and females and among ethnic groups in different geographic regions, ranging from 0% in native Americans to 20% or more in African and Asian and as high 98.6% in Brazil (Luzzatto et al., 2016; Howes et al., 2013).

The prevalent rate varies according to the infection rate and depends on the study's locality. In heterozygous and hemizygous combinations, G6PD deficiency has now been associated with a level of protection of about 50% against severe *P. falciparum* malaria (Ruwende et al., 1995). Therefore, high rates of G6PD deficiency in many parts of the world can probably be accounted for as the result of selection by malaria infection. Recent evidence indicates these alleles for G6PD deficiency were selected in African populations between 4,000 and 12,000 years ago. This period coincides with some estimates for the time of emergence of *P. falciparum* as a significant human pathogen around 4,000 years ago (Rich et al., 1998; Volkman et al., 2001). However, the selection for G6PD deficiency may have preceded this event and overlapped with when *P. vivax* malaria could still have been a major selective force in Africa. The mechanisms underlying these clinical and genetic observations are not yet fully understood. A review of relevant literature showed that the model is based on the interaction between haemoglobin and membrane components, which may provide a molecular basis for the involvement of the immune response in genetic adaptation to malaria. G6PD, as a red cell enzyme, maintains red cells' stability through the reduction of the protein molecule (glutathione). The glutathione mops oxygen radicals that lyse RBCs, which the malaria parasite depends on for its metabolic activities. A deficiency of G6PD causes an increase in the blood level of oxygen radicals which is detrimental to the parasite at its various stage of development (Ginsburg and Golenser, 1999). This study is an essential since it undermines the current idea that red blood cell genetic defects give protection against falciparum malaria by reducing intra-erythrocytic growth and development of the parasite (Pathak et al., 2018). Given the putative stable transmission in these areas, we, therefore, sought to determine the frequency of this genetic polymorphism in the population of this area to confirm it as a protective factor for high-level asymptomatic malaria subjects encountered in our study.

MATERIALS AND METHODS

Abina community in Ikwo Local Government Area of Ebonyi State, Nigeria, was chosen for the study. Though it is part of a broader study of insecticide-treated nets funded by Tetfund, (2016 National University Research Grant) under the auspice of Ebonyi State University. Ebonyi state is located in the rainforest belt of southeastern Nigeria. Precisely, it lies between latitude 05 40 and 06 45 E, North of the equator and longitude 07 30 N and 08 30 N. It is bounded in the North by Benue state, in the South by Abia state, in the East by Cross River state and the West by Enugu state. The state has a land area of 5,932 square kilometres and is divided into 13 local government areas. The population for the year 2000 is estimated at 2.1 million. This is projected from the 1991 National population census figure with a growth rate of 3%.

The state has a high temperature and humidity, and is drained by the Cross River, which rises from the Cameroon Mountains and empties into the Atlantic Ocean. The soil is clayey and clayeyloamy, with pockets of water collections in several places during the rainy season (as swamps). The roads are hardly accessible, especially during the rainy season, which starts in March/April and ends in September/October. Many agricultural practices occur at the subsistence level due to the crude technology in use. The major crops grown are rice, yam, cassava and sweet potato.

Ethical consideration: Ethical approval was obtained from Ebonyi state university Research Ethic Committee, UREC Ref No: EBSU/UREC/TETFUND/2015/19.

Table 1. Baseline characteristics of 100 schoolchildren studied in a rural area of Eastern Nigeria.

Age (years)	Gender		Parasite density/UL	frequencies			
	Male	Female		Normal	Homo	Hetero	Hemi
≤ 1	15	10	500-2000	8	2	3	12
2-3	14	20	1000-6000	8	5	10	11
4	21	18	500-6000	12	4	8	15
6	2	0	0	0	0	0	2

Glucose-6-phosphate dehydrogenase enzyme polymorphic

Source: Author

Table 2. Infection rate and parasite density of the study population.

Study population	Number of subjects	Infection rate	Parasite density/UL
Normal	28	10	6000
Heterozygous	11	5	6000
Homozygous	21	1	1000
Hemizygous	40	00	1500

Source: Author

Laboratory and field methods

One hundred drug-naïve school pupils aged between 3 and 6 years, without signs and symptoms of any disease, were randomly selected and enrolled in the study. During the study, a finger-prick blood sample was drawn from each subject at noon. The blood was used to prepare thick films, stained with Giemsa (30 to 45 minutes, with 3% Giemsa stain, pH 7.2) and examined after being air-dried for the presence of malaria parasites. A random sample of 10% of the slides was examined a second time by another experienced independent microscopist. The study was approved by the Ethics Committee of Ebonyi State University, Abakaliki. Written informed consent was also

obtained from the Parents, Guardians, Parents Teachers Association and the Head Master of the school prior to the study.

G6PD enzyme activity was measured spectrophotometrically using Sigma Kit 203-A (Sigma-Aldrich, Inc., St Louis, MO, USA). Three mls of freshly withdrawn venous blood devoid of haemolysis were used for the assay. Principle of the method: Sodium nitrite oxidizes haemoglobin to methaemoglobin, and the addition of a redox dye, methylene blue, pentose phosphate pathway is activated. This results in the reconversion of methaemoglobin to oxyhaemoglobin in cells with normal G6PD. The intensity of the colour developed depends on the status of the G6PD of the cell. For quantitation of parasite density, thick blood films made on grease-free clean slides were stained with 3% Giemsa stain buffered at 7.2pH for 30 min, dried and read by two independent microscopists using x100 objective. Parasite density was calculated as the number of parasites counted per 200 white blood cells (WBC) on a thick film, assuming a mean WBC count of 8000/uL. Plasmodium falciparum gametocyte count was done per 1000 leucocytes.

RESULTS

Glucose-6 phosphate dehydrogenase deficiency was observed in 72 (72%) of 100 primary school children randomly selected for the study. Table 1 shows the characteristics of the study subjects. All subjects presented no signs and symptoms of any illness or infirmity during the study.

Hemizygote glucose -6-phosphate dehydrogenase polymorphism was most prevalent (Table 2). The study population with normal glucose-6 phosphate dehydrogenase enzyme had higher parasite density (parasite density $\geq 6000/\mu\text{L}$) than subjects with enzyme deficiency activity (Figure 3). A higher parasite density of 6000/ μL was found in children aged between 3 and 4 years. The percentage prevalence of malaria parasite infection was higher in subjects with normal glucose-6 phosphate dehydrogenase enzyme activity (Figure 2) than in subjects with a heterogeneity of G-6PD (Figure 1).

DISCUSSION

The present study investigated the frequencies of G6PD as a protective polymorphism to malaria-infected subjects in our broader study subjects in a West African rural village with high malaria parasite transmission. Screening individuals for G6PD status before instituting antimalarial agents is uncommon in Nigeria, which could put a patient at risk of haemolysis. G6PD deficient populations are relatively high in sub-Saharan Africa, with a prevalent rate dependent on study area. An unpublished study showed that the malaria prevalence rate in this area was 60%, which is over and above WHO roll back malaria initiative and the pledge by African Heads of state by 2005. Out of 100 subjects randomly selected from the broader population of our study, 72% had G6PD of different heterogeneity. This agrees with the earlier statement that G6PD enzyme deficiency distribution correlates with the malaria-endemicity of that area (Howes et al., 2012). This high prevalence rate is not intriguing as a study conducted in Brazil showed a much higher rate of 98.6% (Earnest and Stephen, 2007). This study shows that the prevalence rate of G6PD polymorphism depends on the particular study area and not on the geographical contraption. The high prevalence of hemizygous, 40 (55.565%) observed in the present study corroborated previous clinical studies where higher G6PD enzyme deficiency is usually seen in hemizygous males (Guindo et al., 2007). We observed a correlation between G6PD enzyme activity and parasite density, which could explain the subjects' resistance to a high level of parasite density in the area. The present study also showed a difference in parasite density between the G6PD deficient and G6PD normal subjects. The high prevalence rate of G6PD deficiency (72%) found in the hinterland of sub-Saharan Africa, where mosquito bites is very high with subsequent high malaria infection rate, calls for attention. Therefore, an in-depth and broader study is needed to obviate the medical consequences of this red blood cell enzyme abnormality.

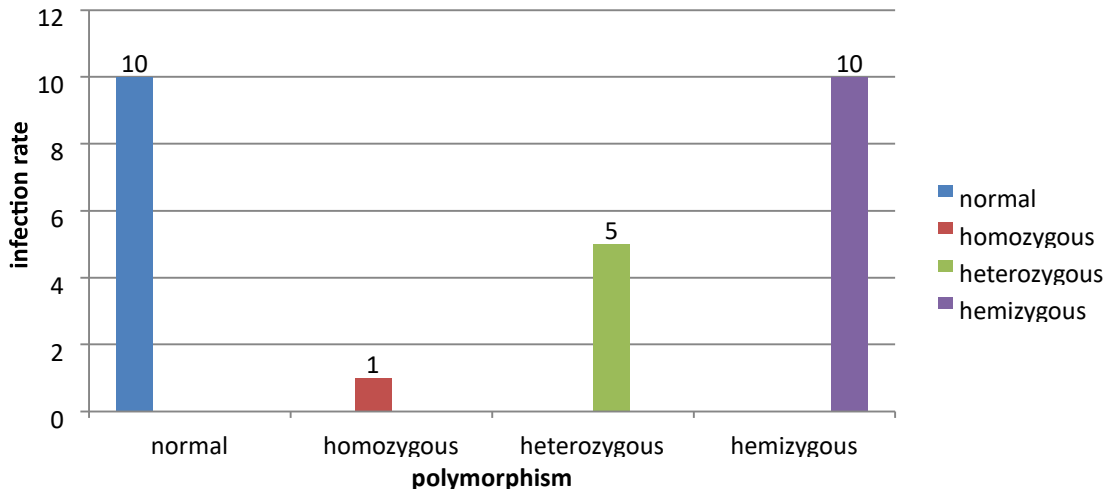


Figure 1. Prevalence of malaria parasite infection according to G6PD activity. Source: Author

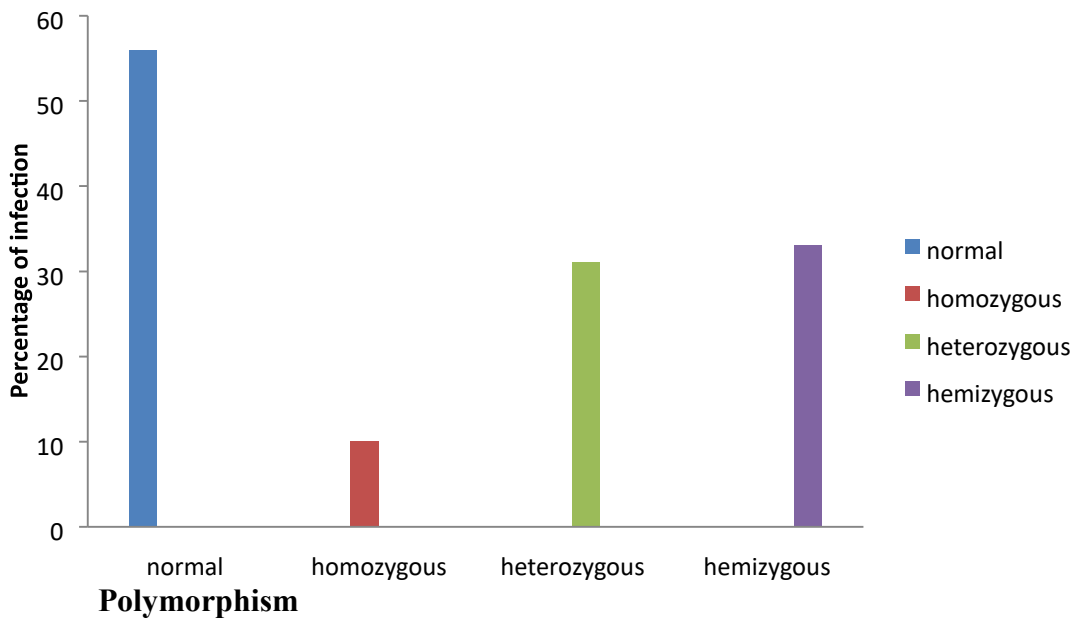


Figure 2. Percentage of subjects with G6PD polymorphism. Source: Author

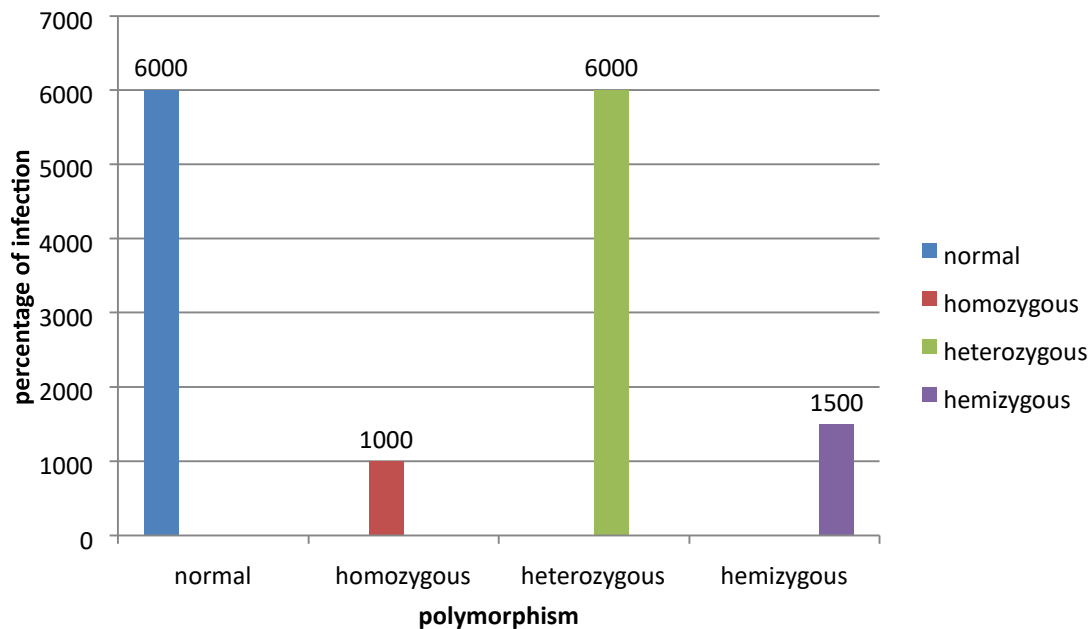


Figure 3. Parasite density of the study population.

Source: Author

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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