Advances in Biochemistry
2016; 4(6): 66-73
http://www.sciencepublishinggroup.com/j/ab
doi: 10.11648/j.ab.20160406.11
ISSN: 2329-0870 (Print); ISSN: 2329-0862 (Online)

Prevalence of Glucose-6-phosphate Dehydrogenase Deficiency Among Children Aged 0-5 Years Infected with *Plasmodium falciparum* in Katsina State, Nigeria

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To cite this article:

Bello Ibrahim, Abdulsalami Mohammed Sani, Bulus Timothy. Prevalence of Glucose-6-phosphate Dehydrogenase Deficiency Among Children Aged 0-5 Years Infected with *Plasmodium falciparum* in Katsina State, Nigeria. *Advances in Biochemistry.* Vol. 4, No. 6, 2016, pp. 66-73. doi: 10.11648/j.ab.20160406.11

Received: August 22, 2016; **Accepted**: September 8, 2016; **Published**: November 15, 2016

Abstract: About 96 million people having Glucose-6-phosphate dehydrogenase (G6PD) deficiency worldwide are known to reside in malaria endemic countries and this G6PD-deficiency has been shown to protect against malaria infection, a disease which affect mostly children less than 5 years of age. This study was prompted by the paucity of scientific information on G6PD deficiency for malaria-infected children in Nigeria and so it was designed to determine the prevalence of G6PD deficiency among children (aged 0-5 years) infected with *Plasmodium falciparum* in Katsina State, a North-western region of Nigeria. A total of 200 blood samples were collected from children with *Plasmodium falciparum* malaria attending the six selected hospitals located across the three senatorial zones of the state from March 2015 to May 2015. Children's inform consent was obtained, their socio-demographic information and clinical presentations were also taken with the aid of structured questionnaire. G6PD deficiency was detected qualitatively using G6PD screening test. Thirty two (16%) samples were G6PD deficient and were significantly associated ($p<0.05$) with malaria. Higher prevalence was observed among male children (62.5%) compared with their female counterpart (37.5%). Prevalence rates of 31.25%, 25.00%, 18.75% and 12.50% were seen in children of 1, 2, 3, 4 and 5 years old respectively. These conditions reach life-threatening scenarios for all G6PD deficiency patients with different genetic variants. Hence, individuals that are required to use antimalaria drugs should be screened very carefully for their tendency to have G6PD deficiency. For effective control and treatment, either a reliable test for detecting G6PD deficiency or an anti-malaria drug that can be safely given to G6PD deficiency patients is required. The need for training paediatricians on routine screening of children for G6PD deficiency in developing countries in order to avoid cases of drug-induced anaemia associated with malaria treatment need to be taken into consideration.

Keywords: Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD), *Plasmodium Falciparum*, Malaria, Children, Katsina State, Nigeria

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme found in the cytoplasm of all cells catalyzing the first reaction in the pentose phosphate pathway, providing reducing power to all cells in the form of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). NADPH enables cells to counterbalance oxidative stress that can be triggered by several oxidant agents, and to preserve the reduced form of glutathione.

Since red blood cells do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defence against oxidative damage is dependent on G6PD [1].

It has been estimated that Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects around 353 million people worldwide, with 96 million in the malaria endemic countries [2]. Indeed, G6PD deficiency prevalence in a given region has been proposed as an important marker of the selective pressure exerted by malaria [3].

G6PD deficient status has been associated with protection against *Plasmodium falciparum* malaria [4, 5] and is one of the most common human enzyme deficiencies in the world. It is particularly common in populations living in malaria-endemic areas. The highest frequencies are detected in Africa, Asia, the Mediterranean region, and in the middle-east; owing to recent migrations, however, the disorder is also found in North and South America and in northern European countries [6].

G6PD deficiency is a common X-linked recessive hereditary genetic defect caused by mutations in the G6PD gene, resulting in protein variants with different levels of enzyme activity, that are associated with a wide range of biochemical and clinical phenotypes. It affects the erythrocyte metabolism, featuring non-immune haemolytic anaemia in response to a number of factors [7]. Patients with G6PD deficiency develops haemolytic anaemia during acute malaria infection and when treated with certain therapeutic agents such as anti-malarials, antipyretics and antibiotics which have oxidant properties. Increased oxidative stress in G6PD deficient cells is well documented [8] and the erythrocyte exposure to oxidative stress causes haemoglobin denaturation, ultimately resulting in haemolysis. Other clinical conditions include neonatal jaundice, which may result in neurological complications and death [9].

More than 176 mutations and 500 different variants have been described to date for the G6PD gene; however most are single nucleotide changes, leading to amino acid substitutions [10, 11]. The world health organization grouped the G6PD variants into five classes based on their enzyme activity and clinical manifestations, with class I demonstrating the severely deficient cases that are associated with chronic non-spherocytic haemolytic anaemia [12].

Malaria is a febrile illness caused by sporozoa of the genus *Plasmodium,* four species of which infect humans: *P. falciparum, P. vivax, P. ovale,* and *P. malariae.* The malaria parasites undergo a developmental cycle in the female anopheles mosquito, which is the vector. They are transmitted to the human host following a bite by the mosquito, rapidly enter the liver where they undergo a developmental phase of varying duration among the four species (pre-erythrocytic phase), and then enter the red blood cell (intra-erythrocytic phase) where they continue their multiplicative cycle. The asexual erythrocytic parasite is mostly characterized by fever, chills and sweats, anaemia, enlargement of the liver and the spleen [13].

Despite the clinical and epidemiological significance of the interaction between G6PD deficiency and malaria, the extent of its occurrence and consequences has not been properly measured. Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counseling, and abstinence from disease-precipitating drugs like anti-malaria.

Previous reports in Nigeria showed that the prevalence of G6PD deficiency ranged from 4% to 26%. However, a study by [14] in Sokoto, Nigeria among 118 children visiting the Emergency Paediatric unit of Usman Danfodiyo University Teaching Hospital for paediatric related care indicated G6PD deficiency of 14.4%. Also a study by [15] in Oshogbo, Nigeria among 200 blood donors and 86 jaundiced neonates indicated G6PD deficiencies of 19.5% and 47.7%, respectively. A study by [16] among males resident of Jos, Nigeria showed prevalence of 20% G6PD deficiency. In Asia, the deficiency prevalence ranges from 6.0% to 15.8% [17, 18]. In India [19] it is 10.5%, and in the Middle East [20, 21] the prevalence varies from 3% to 29%. In Brazil, a few studies have found prevalence between 1.7% and 6.0%[22, 23, 24].

To the best of our knowledge this is the first prevalence data on the G6PD deficiency among children in Katsina Locality in Nigeria.

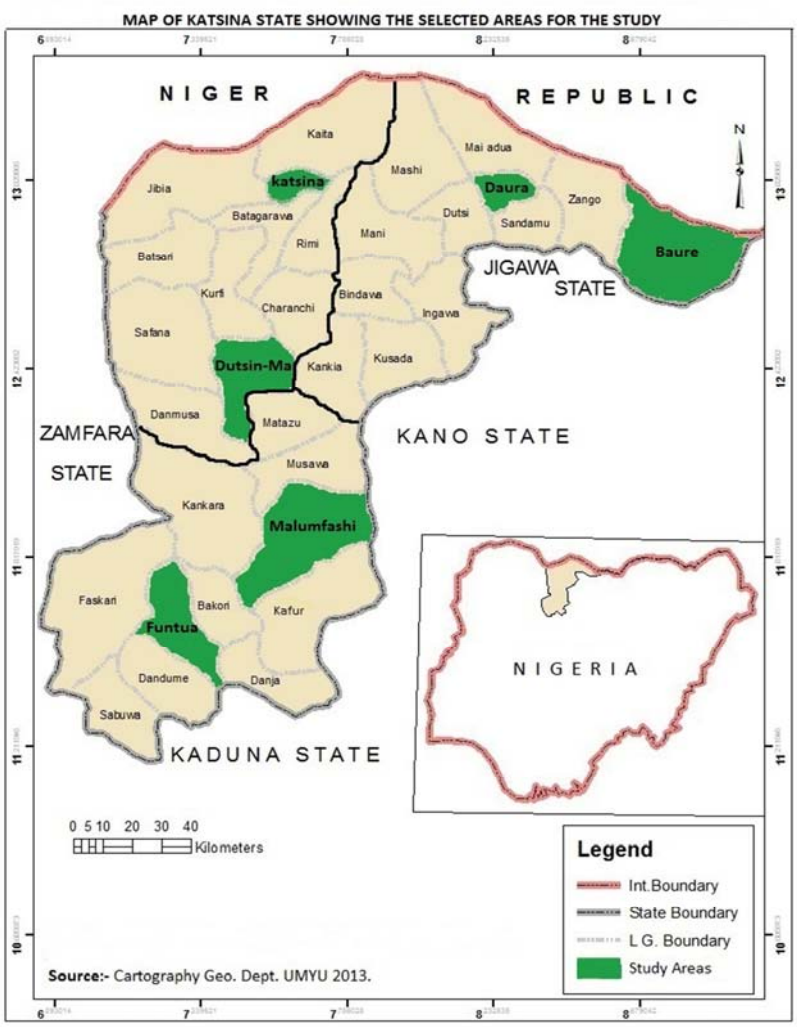
2. Materials and Methods

2.1. Study Design

This study was designed to determine the prevalence of G6PD deficiency among 200 children which comprises of 120 (60%) males and 80 (40%) females, aged 0-5 years that are admitted or presented with *Plasmodium falciparum* malaria in the selected hospitals from March 2015 to May 2015. Written informed consent was obtained from parents/guardians after counseling. Ethical clearance was obtained from the Health Research Ethical Committee (HREC), Ministry of Health Katsina State, Nigeria.

2.2. Study Area

The study was carried out in Katsina State, located at the extreme northern margin of Nigeria and covers a total area of about 23,938 square kilometres with a total population of 5,801,584 people, going by 2006 census. It lies between latitudes 11°08'N and 13°22'N and longitudes 6°52'E and 9°20'E. The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. For the purpose of the study, the thirty four (34) local governments were divided into three (3) senatorial zones according to their geographical locations, namely; Funtua zone (South), Katsina zone (Central), and Daura zone (North) as show infigure 1 below [25].



Source: Cartography unit, Geography Department, Umaru Musa Yar'adua University, Katsina (2013).

Figure 1. *Map of Katsina state showing the selected areas for the study.*

2.3. Sample Collection and Methods

After screening children for malaria parasite using *Plasmodium falciparum* Rapid Test Device, venous blood samples (2.0mls) were withdrawn from each child of the study population positive for malaria at the selected hospital by the laboratory technician. The samples were collected in EDTA (Ethylene Diamine Tetra-acetic Acid) tubes and transported immediately in ice-cooler box to the Laboratory Department of General Hospital Dutsin-ma for G6PD deficiency screening using Biorapid G6PD qualitative in vitro test (Biorapid Diagnostics Nig. Ltd.).

2.4. Screening of Samples

2.4.1. Detection of Plasmodium Falciparum Parasite Using Rapid Test Device

The test device contains monoclonal malaria antibody coated on the membrane. The malaria *Plasmodium falciparum* Rapid Test Device (whole blood) is a quantitative, membrane immunoassay for the detection of *Plasmodium falciparum* antigen in whole blood. The membrane is pre-coated with *Plasmodium falciparum* antibody. During testing, the whole blood specimen reacts with the dye conjugate, which had been pre-coated in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with *Plasmodium falciparum* antibody on the membrane on the test line. If the specimen contains *Plasmodium falciparum* antigen, a coloured line in the test region indicates that the specimen contain *Plasmodium falciparum* antigen. To serve as a procedural control a coloured line will always appear in the control region indicating that proper volume of specimen had been added and membrane wicking had occurred.

The test device, specimen and buffer were allowed to equilibrate to room temperature (37°C) prior to testing. The test device was removed from the poil puch and used immediately, as best results are obtained if the assay is performed within one hour from the time it was removed. The test device was then placed on a clean and flat surface.

The specimen was then transferred to a pipette. Exactly 10µl of whole blood was then transferred to the specimen well of the test device and 3 drops of phosphate buffer was added and the timed. The result was read within 15 minutes, a time sufficient only for the appearance of coloured line(s) [26].

2.4.2. G6PD Enzyme Detection Using Test Kits

In this work, the activity of G6PD enzyme was measured qualitatively using commercially available G6PD screening test (Biorapid Diagnostics Nig. Ltd.) according to manufacturer's instructions using fresh blood samples as enzyme activity reduces on refrigeration. Briefly, Glucose-6-phosphate dehydrogenase present in red blood cell hemolysate acts on glucose-6-phosphate and reduces $NADP^+$ which in the presence of Premium Motor Spirit (PMS) reduces the blue coloured 2, 6-dichlophenol indophenol into a colourless form leaving behind the original cherry red colour of the hemolysate. The rate of decolourisation is proportional to the enzyme activity.

All the reagents were brought to room temperature. The substrate vials were taped gently on the flat surface to dislodge all the substrate powder. Using clean pipette, each substrate vial was reconstituted with 0.5ml of buffer reagent and gently swirl to dissolve and then allowed it to stand for 5 minutes. In 50µl of distilled water, 1 ml of well mixed EDTA whole blood sample was added and mixed well, then allowed to stand for 5 minutes at room temperature. One ml of the hemolysate was added to the reconstituted substrate vial and mixed gently by swirling and 1 ml of mineral oil was added immediately. The plug and the cap were replaced tightly. The mixture was then incubated undisturbed at 37°C for 60 minutes.

Decolourising time for normal subject is up to 60 minutes while for G6PD deficient subjects (In heterozygous male and homozygous female), approximately 2-24 hours as shown in figure 2 below.

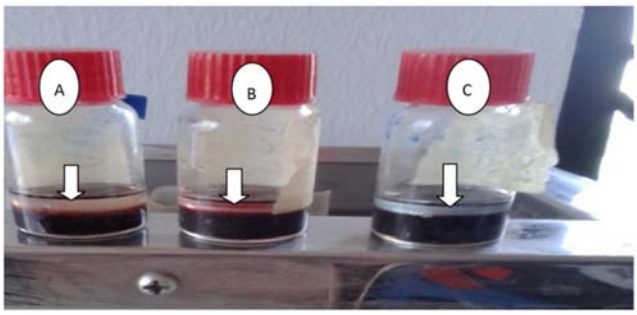


Figure 2. *Biochemical manifestation of Glucose-6-phosphate Dehydrogenase in normal (A and B) and G6PD deficient blood (C) samples treated with 2, 6-dichlophenol indophenol.*

[Arrows in the first two bottles show the original cherry red colour of the hemolysate of normal blood observed after reduction of blue coloured 2, 6-dichlophenol indophenol; Arrow in the third sample bottle is indicative of the absence Glucose-6-phosphate Dehydrogenase activity]

2.5. Statistical Analysis

The data generated from this study were analysed by descriptive statistics (mean, standard deviation, percentage), Pearson correlation by 2 tailed-tests of significance, and comparing means by paired samples t-test using IBM Statistical Package for Social Sciences (SPSS) grad pack 20 software. The statistical level of significance was set at $p<0.05$.

3. Results

A total of 200 children (120 males and 80 females, i.e. 3:2 male to female ratio) that are *Plasmodium falciparum* malaria positive were screened; of these, 32 (16%) were shown to have G6PD deficiency.

3.1. Prevalence of G6PD Deficiency Among Children with Plasmodium Falciparum in the Study Area

Figure 3 shows the prevalence of G6PD deficiency among the children studied with *Plasmodium falciparum* malaria. According to the biochemical screening of the 200 children admitted or presented to the selected hospitals with *Plasmodium falciparum* malaria in this study, 32 children (16%) were found to be G6PD deficient while the remaining 168 children (84%) are normal. Therefore, we observed a prevalence of 16% (32/200) among the 200 *Plasmodium falciparum* positive children studied which is statistically significant.

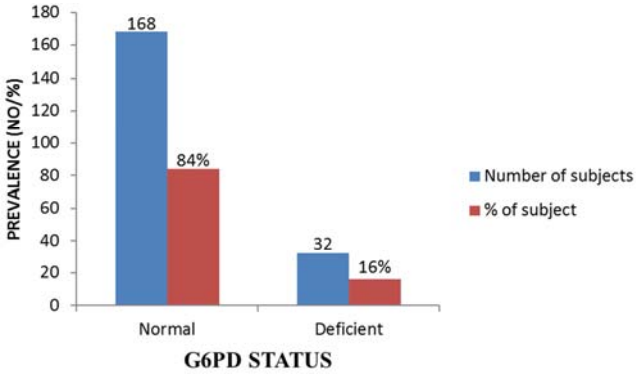


Figure 3. *Prevalence of G6PD deficiency among children with P. Falciparum in the study area.*

3.2. Prevalence of G6PD Deficiency Among Age Group

Figure 4 shows the distribution of G6PD deficiency among the age of the deficient subjects. The number of G6PD deficient subjects based on age encountered in this study showed that there are significant differences among the age group with children at 1 year old having the highest prevalence. The second highest are children at 2 years old followed by those at years 3 but those at 4 and 5 years shows the same prevalence.

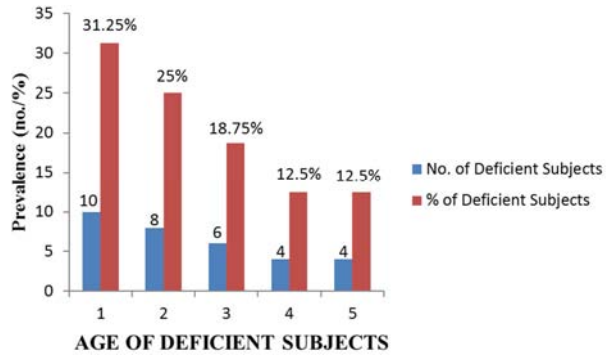


Figure 4. *Prevalence of G6PD Deficiency among Age Group.*

3.3. Prevalence of G6PD Deficiency Among Children Based on Sex

Figure 5 shows the distribution of G6PD deficiency based on sex. In this study, it is found that among the 32 deficient children out of the 200 children screened, male children shows high prevalence of G6PD deficiency (62.5%) (20/32) compared to the female children (37.5%) (12/32).

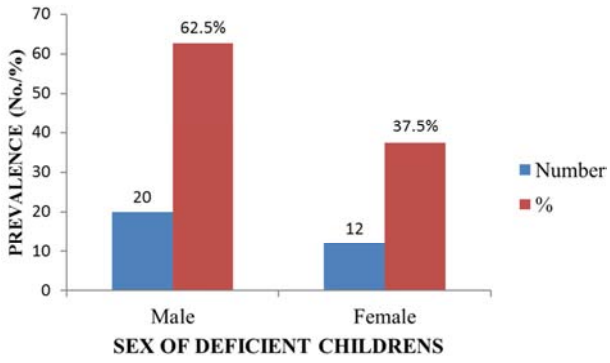


Figure 5. *Prevalence of G6PD Deficiency among Children Based on Sex.*

3.4. Prevalence of G6PD Deficiency Among the Three Senatorial Zones

Figure 6 shows the prevalence of G6PD deficiency among the three senatorial zones of Katsina state. In this study, it has been found that the percentage of deficient subjects in the three senatorial zones of Katsina state were nearly similar. The percentages are 16.2, 16.2 and 15.6 for Katsina, Daura and Funtua senatorial zones respectively.

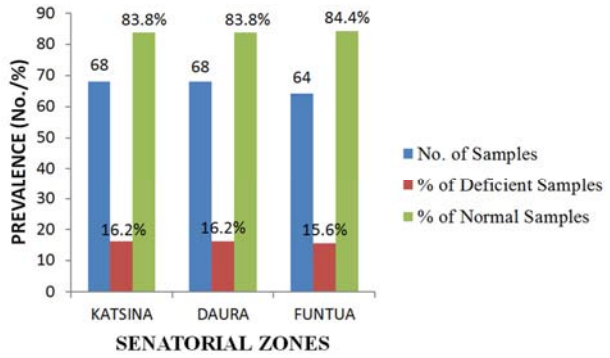


Figure 6. *Prevalence of G6PD Deficiency among the three Senatorial Zones.*

4. Discussion

This study sought to determine the prevalence of G6PD deficiency among children (aged 0-5 years) infected with *Plasmodium falciparum* in Katsina State, a North-western region of Nigeria. High prevalences of G6PD deficiency in many malaria endemic countries account for considerable difficulty in malaria eradication efforts [27]. Testing individuals for G6PD status in field conditions is currently unrealistic due to the costs involved and logistic aspects, and therefore, most countries opt not to administer primaquine in order to avoid drug related haemolysisthough African A-variant may take it at reduced dosage under close monitoring. This is because Primaquine is the only effective antimalarial drug that provides inhibition of persistent liver stages of *P. falciparum, P. vivax,* and *P. ovalae* parasites that lead to relapses of malaria [28]. This highlights the need for comprehensive estimates of G6PD deficiency in malaria endemic regions and its clinical consequences.

The prevalence rate 0.016, approximately 16% in this study is consistent with previous reports conducted in Nigeria and other parts of the world. It is known that red blood cells that are deficient in G6PD are resistant to *Plasmodium falciparum* invasion since the parasite requires the enzyme for its normal survival in the host cell. This deficiency offers a selective protection against *Plasmodium falciparum* malaria [3, 29].

In this study, a significant number of prevalence with G6PD deficiency was in the children within 1 year old (31.25%). The second highest are the children within 2 years old (25.00%) followed by those within 3 years old (18.75%) but those within 4 and 5 years old indicated the same prevalence (12.50%). This is statistically significant and is consistent with the work of [14] where a significant number of subjects with G6PD deficiency in their study were in the 2- to 3- and 4- to 5-year age-groups. In this study, a high prevalence of G6PD deficiency was observed in male children (62.5%) compared to female children (37.5%) and this variation is statistically significant. G6PD deficiency is an X-linked recessive hereditary disease characterized by abnormally low levels of G6PD. The deficiency is X-linked since the X chromosome carries the gene for G6PD enzyme; therefore this deficiency mostly affects males. G6PD deficiency is inherited from females who carry one copy of the causative gene on one of their X chromosomes. Males who inherit the causative gene from the mother have G6PD deficiency, while females who receive the gene are carriers (carrier females generally do not show any characteristic symptoms). The deficiency is rare in females because the mutation would have to occur in both copies of the gene to cause the disorder, whereas in males only one abnormal copy of the gene is required for manifestation of the disease. This is consistent with previous reports that indicated that the sex of the patient is important and that males are at greater risk based on severity compared to females [30].

In this study also, there was no statistical correlation between G6PD deficiency with either of the Senatorial zones (Central, North and South senatorial zones) in figure 6.

Therefore this shows that G6PD deficiency does not depend on the locality of the children within the state. Irrespective of the senatorial zone of origin a child may have the G6PD deficiency or not. The geographic distribution of G6PD deficiency suggests that some polymorphisms confer resistance to *Plasmodium falciparum* malaria [2]. This phenomenon has been investigated mainly for the African variant (G6PD A^-), showing that it also confers protection against lethal falciparum malaria [31].

The higher prevalence of G6PD deficiency in malaria endemic countries is an indication that malaria infection has exerted a strong selective pressure in many human populations [3, 18]. In *Plasmodium falciparum* infection it has been demonstrated that shorter half-life and rapid clearance of red blood cells of G6PD deficient individuals make them less susceptible to malaria attacks from these parasites [32]. Conducting such kind of research in this locality with larger samples size is of paramount important because our findings were limited to small population (200 samples).

Malaria and Glucose-6-Phosphate Dehydrogenase Deficiency

There is a strong relationship between malaria and G6PD deficiency diseases. Several epidemiological studies have shown that distribution of malaria was nearly the same with distribution of G6PD deficiency [33, 34, 35]. This situation reveals two important facts. One of them is that G6PD deficiency provides great protection from malaria, especially for *Plasmodium falciparum* infections. On the other hand, using antimalarial drugs can cause life threatening haemolytic anaemia in patients with G6PD deficiency. Hence, malaria patients should be screened for their tendency to G6PD deficiency before their treatment with antimalarial drugs.

As pointed out before, according to epidemiological studies, the prevalence of malaria deeply relates to glucose-6 phosphate dehydrogenase (G6PD) enzyme deficiency. It was demonstrated that 66 of 77 genetic variants that have reached polymorphic frequencies were seen in populations living in tropical and subtropical areas where malaria was endemic. On the other hand, this genetic diversity does not occur in populations living in non-endemic regions of the world for malaria, indicating that high polymorphism is the indicator of G6PD deficiency [36].

When investigated in terms of cellular biology, it was observed that Plasmodium parasite that causes malaria use erythrocytes as host cells. Erythrocytes are also the most affected cells from G6PD deficiency. This situation also suggests the relationship between the two diseases [36]. In several studies, it was demonstrated that G6PD deficiency provides a protection against malaria infections. In one of the early studies, it was indicated that *P. falciparum* and *P. vivax* parasites preferred to invade younger erythrocytes, which possessed high levels of G6PD enzyme. Since enzyme levels are diminished in older erythrocytes, parasites do not prefer to invade these erythrocytes. These studies suggested the protective effect of G6PD deficiency from parasitemia [37, 38]. In the recent past, [3] also carried out a case-control study on more than 2,000 African children and exhibited that risk of contracting malaria in patients that have the African form of G6PD deficiency decreased at a rate of 46 to 58%. In this study, it was suggested that the selective advantage of resistance to malaria was counterbalanced with selective disadvantageous results of G6PD deficiency, and this stopped the rise of malaria frequencies in endemic regions [3]. In another study, [39] investigated 345 healthy adults for G6PD deficiency on Phuket Island, which had been determined to be a malaria-endemic region and found out that 10% of these individuals had G6PD deficiency. Interestingly, it was observed that none of the individuals had molecular evidence of malaria infection. According to this study, researchers postulated that G6PD deficiency provided an advantageous genetic trait against malaria [39].

The exact mechanism of this protection is still unknown. However there are two postulated explanations. According to the first suggestion, it was found that parasites that cause malaria can only survive in conditions with low oxygen levels [40]. This demonstrates that these parasites are very susceptible to oxidative stress. It is known that in the pentose phosphate pathway of erythrocytes, glucose-6 phosphate dehydrogenase (G6PD) enzyme has an important role in production of NADPH and GSH. This is the only mechanism for erythrocytes to survive. GSH that is produced by $NADP^+$ reduction reacts with H_2O_2 and reduce it to H_2O. This prevents the generation of oxidative stress within red blood cells. Since oxidative stress is the most important factor for the disruption of red blood cells, these cells are protected from this effect. However, in G6PD deficient erythrocytes, G6PD activity is significantly reduced. In G6PD A- variant, enzyme activity level reduces to 10 or 20% of normal levels, while enzyme activity completely disappears in G6PD variant [40].

Therefore, oxidative stress can be induced in erythrocytes whose G6PD enzymes are deficient. In this situation, GSH is not produced and H_2O_2 is not reduced to H_2O and leads to oxidative stress. Hence, it is thought that since malaria parasites are susceptible to oxidative stress, they do not live within the erythrocytes where their maturation occurs [41, 42]. Additionally, during oxidative stress, the loss of potassium from the cell and from the parasite can cause the death of the parasite [43].

According to the second suggestion, *Plasmodium* parasites oxidize NADPH and reduce the level of reduced glutathione (GSH) in erythrocytes. In the situation of G6PD deficiency, this effect becomes more severe and induces oxidative-induced damage within erythrocytes [40].

5. Conclusion

The study has shown a high prevalence of G6PD deficiency among children with *Plasmodium falciparum* malaria residing in Katsina state in North-western Nigeria. Findings indicated a high prevalence of G6PD deficiency

in male sex (60%) compared to female sex (40%) among the children studied. In most children (75%) G6PD deficiency cases occurred in early childhood (1 - 3 years). There is a need for the routine screening of children for G6PD deficiency in our environment to allow for evidence-based management of malaria in these children, to ensure the avoidance of food and substances that can potentially predispose them to oxidative stress. Establishing and conducting educational awareness programmes for G6PD deficiency especially among mothers may also play an important role. There is also a need to build capacity among paediatricians in our setting to ensure the effective management of children with G6PD deficiency.

Acknowledgments

The authors are grateful to Heath Research Ethical Committee (HREC), ministry of health Katsina state, Nigeria and staff of all the selected Hospitals (General Hospital Dutsin-ma, Katsina, Daura, Baure, Funtua and Malumfashi) in the state for their sacrifices and services rendered.

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