Socio-Demographic Factors Associated with Glucose-6-Phosphate Dehydrogenase Deficiency among Children Diagnosed with *Plasmodium falciparum* Malaria

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Abstract **- Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency has been shown to protect against malaria infection which affect 241 million people worldwide with an estimate of 627 000 malaria deaths in 2020 of which 95% in the African region and 80% were children under 5 years of age. In view of this, a study to determine the basic socio-demographic factors associated with G6PD deficiency among children diagnosed with *Plasmodium falciparum* malaria in three senatorial district of Katsina state, northern Nigeria where there is paucity of demographic information was set out. A total of 200 patients diagnosed with Plasmodium falciparum malaria were included in the study. Their socio-demographic information and clinical presentations were also noted with the aid of scheduled questionnaire. All patients were analysed for G6PD enzyme deficiency using G6PD qualitative test. Out of 200 children sampled, 32 were G6PD deficient. Of the 32 deficient, 20 (62.5%) were males, 19 (59.4%) were from urban areas, 18 (56.3%) developed haemolytic anaemia between the age of 1-2years, 26 (81.3%) developed jaundice after birth which persist between one to two weeks, 7 (21.9%) had family history, 6 (18.8%) were consanguineously related, while only 5 (15.6%) were aware of the G6PD deficiency, thus, followed some diet and avoids some drugs. Factors that achieved statistical significance for severe haemolysis included younger age (*P*<05), male gender (*P*<05). Our study showed that there are significant co-relations between G6PD deficiency and factors such as sex, ethnic group, consanguinity, previous haemolytic crisis, jaundice outside of neonatal period, and anaemia. Among clinical signs, fever was significantly associated with the studied G6PD deficiency (p ≤ 0.0001). Prevalence of G6PD deficiency is considerably high in Katsina state. Therefore, the introduction of systematic neonatal screening is required.**

KEY WORDS: Children, G6PD deficiency, Katsina, *Plasmodium falciparum* malaria, Senatorial district, Socio-demographic factors

I.INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme in the pentose phosphate pathway and the main intracellular source of reduced nicotidamine adenine nucleotide phosphate (NADPH), involved in diverse physiological processes such as antioxidant defense, (for instance in the erythrocyte) endothelial growth modulation, erythropoyesis, vascularization and phagocitosis [1]. Although several enzymes can recycle the cofactors, G6PD has been identified as the only NADPH-producing enzyme that is activated during oxidative stress [2]. G6PD deficiency is X-linked and predisposes to hemolysis and to a lesser extent to methemoglobinemia in those persons in use of a substance with oxidative properties [3, 4].

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common hereditary hemolytic disorders in human, affecting around 400 million people worldwide [5]. Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency has been shown to protect against malaria infection which affect 241 million people worldwide with an estimate of 627 000 malaria deaths in 2020 of which 95% in the African region and 80% were children under 5 years of age. Malaria is the 3rd leading cause of death for

children under five years worldwide, after pneumonisa and diarrheal disease [6].

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked, hereditary genetic disorder with a wide range of clinical and biochemical phenotypes [7] and main clinical manifestations of acute or chronic haemolytic anaemia and neonatal jaundice [8] G6PD deficiency is more prevalent throughout tropical and subtropical regions, Africa, the Mediterranean region and Middle East [9].

World Health Organization recommends screening for all male newborns in countries with prevalence higher than 3–5% [10, 11] of which Nigeria fall with a prevalence rate of 4% to 26%. The aim of this study was to investigate whether socio-demographic factors have an impact on G6PD deficient children in Katsina state, northern Nigeria.

II. MATERIAL AND METHODS

A. Study Population

The study population was consisted of children aged ≤5 years admitted or presented with *Plasmodium falciparum* malaria cases to the selected hospitals. Two key hospitals were selected from each of the three senatorial districts of Katsina state.

The hospitals selected for the study are; General Hospital Funtua and General Hospital Malumfashi (South), General Hospital Dutsin-ma and General Hospital Katsina (Central), General Hospital Baure and General Hospital Daura (North).

B. Ethical Approval

The ethical approval for this study was obtained from Katsina State Ministry of Health Ethical Research Committee that grants ethical clearance for research that involves human subjects (MOH/ ADM/ SUB/ 1152/1/276).

C. Sample Size

The sample size for this study was determined using the formula of [12] at 95% confidence level and a reported 15% prevalence of Glucose-6-phosphate dehydrogenase deficiency in Nigeria [13].

$$N = \frac{Z^2pq}{d^2}$$

Where;

N = Sample size

Z = Statistics for a level of 95% confidence interval = 1.96

P = Prevalence rate of Glucose-6-phosphate dehydrogenase deficiency from previous studies = 15%

d = Level of significance (allowable error) = 5%

q = 1- p (i.e. 1-15%)

Thus,

$$N = \frac{(1.96)^2 \times 0.15 \times (1 - 0.15)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.15\ 0.85}{0.0025}$$

$$= 195.9216$$

The calculated sample size is 195.9216; hence a total of 200 samples were used for the study.

D. Collection and Transport of Samples

Venous blood samples (2.0mls) were withdrawn from each child of the study population at the selected hospital by the laboratory technician and ethical guidelines was followed. All of the variables including age, sex, presence of anaemia, cause of anaemia, presence of jaundice, duration of jaundice, family history of G6PD deficiency and parent knowledge about G6PD deficiency were recorded based on a scheduled questionnaire. 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 screening.

E. Analysis of Samples

i. Detection of Plasmodium falciparum parasite using Rapid Test Device

The test device contains monoclonal malaria antibody coated on the membrane.

i.i Test Principle

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.

i.ii Test Procedure

The test device, specimen and buffer were allowed to equilibrate to room temperature (37^oC) prior to testing.

1. 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.
2. The test device was then placed on a clean and flat surface. The specimen was then transferred to a pipette. Then 10µl of whole blood was transferred to the specimen well of the test device and 3 drops of

The following reagents are provided with the kit:

1. Substrate Vials
2. Phosphate Buffer pH 8.5
3. Mineral oil

ii.ii Reagent preparation

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.

ii.ii.i *Preparation* of Red Blood Cell Hemolysate

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.

III. RESULTS

According to the biochemical screening of the 200 children admitted or presented to the selected hospitals diagnosed with *Plasmodium falciparum* malaria in this study, 35 children (17.5%) were found to be G6PD deficient while the remaining 165 children (82.5%) are normal. Therefore, we observed

phosphate buffer was added and the timer was started.

3. The result was read within 15 minutes only to allow for coloured line(s) to appear.

ii. 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.

ii.i Test Principle

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.

ii.ii.ii Assay Procedure

1. 1 ml of the hemolysate was added to the reconstituted substrate vial and mixed gently by swirling.
2. 1 ml of mineral oil was added immediately.
3. The plug and the cap were replaced tightly.
4. The mixture was then incubated undisturbed at 37 ^0C for 60 minutes.

ii.ii.iii Interpretation of Results

ii.ii.iii.i Decolourising time

Normal subject: Up to 60 minutes

G6PD deficient subject: 2-24 hours (In heterozygous male and homozygous female)

a prevalence of 17.5% (35/200) among the 200 *Plasmodium falciparum* positive children studied which is statistically significant.

The number of G6PD deficient subjects based on age encountered in this study showed no significant differences (χ^2= 3.0547, d.f = 4, P=0.549) among the age group. However, children within the age-group of 0-12 months have the highest deficiency, 14 (22.6%)

while those between the age of 49-60 months shows the least deficiency 03 (12.0%).

In this study, it is found that among the 35 deficient children out of the 200 children screened, male children shows high prevalence of G6PD deficiency 23(65.70%) compared to the female children 12(34.30%). The differences observed was statistically not significant (χ^2= 0.6799, d.f = 1, P=0.409).

In this study, the prevalence of G6PD deficiency did not differ between the sampling area, (χ^2= 0.0127, d.f = 2, P=0.9937). The prevalence of G6PD deficiency was 34.30%, 34.30% and 31.40% for Katsina Central, Katsina North and Katsina South respectively.

However, the cause of anaemia in the study children were mainly as a result of taking certain food 179 (89.50%) while very few shows anaemia as a result of taking drug 21 (10.50%) and none of the children were shown to have crisis due to infection 0 (0.00%). Greater number 160 (80.00%) develops jaundice during anaemia and mostly last within 1-2weeks while the remaining 40 (20.00%) do not. Family history and parent's knowledge about G6PD deficiency among the study children shows that majority 171 (85.50%) do not have both history and knowledge while very few 29 (14.50%) have it as presented in table 1.

IV. DISCUSSION

This is the first few study if any, to determine the basic socio-demographic factors associated with G6PD deficiency among children diagnosed with *Plasmodium falciparum* malaria in three senatorial district of Katsina state, northern Nigeria.

The study show high prevalence of G6PD deficiency was also observed in male children (11.5%) compared to female children (6.5%) and 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 [14-15].

The study also shows that there was no correlation statistically between G6PD deficiencies with either of the Senatorial zones i.e the sampling areas (Central, North and South senatorial zones). 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 geographical distribution of G6PD deficiency suggests that some polymorphisms confer resistance to *Plasmodium falciparum* malaria [16]. This phenomenon has been investigated mainly for the African variant (G6PD A-), showing that it also confers protection against lethal falciparum malaria [17]. 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 [18-19]. 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 [20].

The study also indicates that 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 [13] 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. However, this is different from a previous report [21] assessing the frequency of G6PD deficiency in Sardinian patients with nonarteritic anterior ischemic optic neuropathy, which indicated based on sex and G6PD deficiency interaction that sex does not have any modifier effect on G6PD

deficiency. Also, another report [22] among children in Malaysia indicated that sex was not a significant predictor associated with actual G6PD enzyme levels. However, the cause of anaemia in the study children were mainly as a result of taking certain food 179 (89.50%) while very few shows anaemia as a result of taking drug 21 (10.50%) and none of the children were shown to have crisis due to infection 0 (0.00%). Greater number 160 (80.00%) develops jaundice during anaemia and mostly last within 1-2weeks while the remaining 40 (20.00%) do not. Family history and parent's knowledge about G6PD deficiency among the study children shows that majority 171 (85.50%) do not have both history and knowledge while very few 29 (14.50%) have it as presented in table 1. This is consistent with [23] inherited deficiencies of G6PD can result in acute hemolytic anemia during times of increased ROS production which may be caused by stress or exposure to certain foods that contain high amounts of oxidative substances, for example, fava beans, or certain medications. In particular, anti-malarial agents have a strong association with inducing hemolytic anemia in patients with G6PD deficiency [24-25]

Table 1. General characteristics of the study population

Characteristics	Number	Percentage
Gender		
Male	119	59.5%
Female	81	40.5%
Age (Months)		
0-12	62	31.0%
13-24	49	24.5%
25-36	34	17.0%
37-48	30	15.0%
49-60	25	12.5%
Locality		
Urban	80	40.00%
Rural	120	60.00%
Presence of anemia		
Yes	179	89.50%
No	21	10.50%
Cause of anemia		
Food	179	89%
Drugs	21	10.50%
Infection	0	0.00%
Presence of Jaundice		
Yes	160	80.00%
No	40	20.00%
Duration of Jaundice		
Less than one week		
One week	29	14.5%
Two weeks	120	60.00%
Three weeks and above	51 0	25.5% 0.00%
Family History		
Yes	29	14.50%
No	171	85.50%
Parent Knowledge		
Yes	29	14.50%
No	171	85.50%

V. CONCLUSION

In conclusion, our study showed that there are significant co-relations between G6PD deficiency and factors such as sex, ethnic group, consanguinity, previous haemolytic crisis, jaundice outside of neonatal period, and anaemia. Among clinical signs, fever was significantly associated with the studied G6PD deficiency ($p \leq 0.0001$). Prevalence of G6PD deficiency is considerably high in Katsina state. Therefore, the introduction of systematic neonatal screening is required.

This study has been done only in six selected hospitals of the state and with limited sample size. Hence does not reflect the whole picture of G6PD prevalence in Katsina state. It is highly recommended that a comprehensive study that includes all hospitals and medical centres in the state should be performed to better determine the actual relationship between G6PD deficiency and socio-demographic factors among children (0- 5 years).

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Competing Interests

The authors declare that there is no conflict of interest.

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