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Glucose-6-phosphate dehydrogenase (G6PD) deficiency and adverse reactions to antimalarial drugs in Lagos state, Nigeria

Akwaowo Orok[1]*, Adetayo Fagbenro- Beyioku[1], Godswill Iboma[1], Adedayo Mayowa[1] Hilary Okoh[2], Olusola Ajibaye[2], Bamidele Iwalokun[2], Katherine Egbuna[2], Chimere Agomo[2], Yetunde Olukosi[2], Olugbenga Aina[2], Samuel Akindele[2], Vera Enya[2], Jumoke Akinyemi[2], Philip Agomo[2]

1 Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos.
2 Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research, Yaba, Lagos.

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*Corresponding author:

Email : aborok2001@yahoo.com
Tel : +2348023773771

ABSTRACT

Individuals lacking in Glucose-6-phosphate dehydrogenase (G6PD) enzyme are selective to the use of drugs as deficiency can predispose to oxidation and subsequent hemolysis of their red blood cell. This study was undertaken to evaluate the effect of G6PD enzyme deficiency and correlation with adverse drug reaction to anti-malarial drugs. G6PD determination was done using Randox diagnostic kits while malaria diagnosis was done using standard microscopy technique. A total of 100 participants, comprising 44 males and 56 females (designated as group A & B) were recruited for the study. Of these numbers, 28 were G6PD deficient and 72 had normal G6PD activity. G6PD deficiency were similar in males 12 (27.3%) and in females 16(28.6%) (P=0.885). There was no statistical difference in G6PD activity with malaria parasite density (MPD) estimation (P=0.585) despite the fact that those with low G6PD activity also had low MPD than those with normal G6PD activity (415.57 ± 297.07 and 697.86 ± 1516.42 respectively). Also, among the 12 individuals who reported to having adverse drug reaction to some anti-malarials, the outcome was not statistically significant (P=0.659 and P=0.528). In conclusion, there was no relationship between G6PD activity and adverse drug reaction.

INTRODUCTION

Over the years, the quest for effective malaria control have witnessed several methods and strategies towards its eradication, elimination and or minimization especially in sub-Saharan Africa where deaths arising from malaria holds sway and the disease also accounting for about 63% in hospital attendance [1]. Among these numerous methods is the use of anti-malarial drugs (both herbal and orthodox) for curative and prophylactic purposes which have sustained the populations living in endemic areas from time immemorial. However, there are certain populations that are selective to drug use of which improper or wrong administration could result in fatal clinical consequence. One of such are persons lacking in Glucose-6-phosphate dehydrogenase (G6PD) enzyme, well known for its critical role in metabolic energy generation and in the control of oxidative stress a deficiency can predispose to oxidation and subsequent hemolysis of the red blood cells depending on the type [2].

Glucose-6-Phosphate dehydrogenase enzyme deficiency is an inherited condition in which the activity of red blood cell G6PD is markly diminished. The gene determining the structure of G6PD is carried on the X chromosomes and, therefore the defect is inherited in a sex-linked fashion which is fully expressed in the effected males. G6PD deficiency is associated with four main clinical syndromes: acute intravascular hemolysis following exposure to redox chemicals including drugs, neonatal jaundice, favism and chronic non-spherocytic hemolytic diseases. On administration of certain substances, it could precipitate hemolysis with resultant haemoglobulinuria which in most cases is erroneously seen as the worsening of the disease state most especially in places like ours where self medications, poverty and ignorance are common place. These enzymes are endemic in populations of countries of Africa, the Mediterranean basin and the far East, and their descendants in Europe, the Americas and Australia [3-6]. It has been estimated that there are more than 100 million affected individual worldwide.

Functionally G6PD is a cytoplasmic *"house keeping"* enzyme that catalyses the first and rate limiting step of the hexose

monophosphate pathway for pentose synthesis [7-9]. This pathway is an important source of Nicotinamide-adenine dinucleotide phosphate (NADPH) that is required for maintaining adequate intracellular levels of reduced forms of glutathione [9]. It functions in all cells especially in those that lack the mitochondria and nucleus like the red blood cell. It provides the reducing capacity for the red blood cells to reduce harmful substances like hydrogen peroxide (H_2O_2). The generation of reduced glutathione is a key product in the control of oxidative damage in erythrocytes [10-11].

By preserving and regenerating reduced forms of glutathione as well as promote the stability of catalase, NADPH plays a major role in a cell's ability to withstand oxidant stress [12].

MATERIALS AND METHOD

The study was cross-sectional in nature and was conducted in three medical facilities in Lagos. Participants who consented to the study were grouped by sex: Males (Group A) and Females (Group B). 2ml venous blood collected in acid citrate dextrose (ACD) bottles was used for G6PD assay using Randox kits. G6PD activity was measured in RBC over a 5mins interval at 37^{0}C. G6PD normal controls were run concurrently. Results were standardized with respect to total protein content and reported in the international units per gram of hemoglobin (U/gHb). Absorbance were read at 365nm using a UV spectrophotometer. Malaria parasite density (MPD) estimation was conducted according to WHO standard procedure. Interviewer administered semi-structured questionnaires to capture information on reactions to medications were used.

Inclusion criteria for the study includes:

Blood samples slides positive for P. falciparum, with MPD not <200 parasites/µL and willingness to participate in the study at no cost to the investigator. Exclusion criteria were patients with hemoglobin (Hb) < 12.0g/dl for males and < 11.0g/dl for females. Results were communicated to participants and appropriate counseling made where necessary.

Data analysis:

The data generated in this study were analysed using SPSS 11.0 statistical software. Comparison of continuous variables was done with ANOVA, while comparison of proportions was done using Chi-square test. Test for significance was set at P value less than 0.05 at 95% confidence limit.

RESULTS AND DISCUSSION

In Nigeria, preponderance of private medicine vendor (PMV) trading outlets are common place which also serve as a catalyst for indiscriminate self administration of drugs especially as it pertains to malaria which is a household name among numerous tropical diseases. G6PD is not a routine test even in Ante-natal-clinical setting, yet sulfadoxine-pyrimethamine (SP) is adopted as a model for Intermittent Preventive Treatment (IPT) of malaria in pregnancy. There is also paucity of data on the contribution of G6PD to adverse drug reactions and malaria pathogenesis.

Our study was designed to test for G6PD deficiency and possible link to adverse drug reaction. Previous studies have confirmed the prevalence of G6PD A- in the context of malaria protection in Nigeria [13-16].

A total of 100 participants, comprising 44 males (group A) and 56 females [group B] were recruited for the study. Of these, 28 were G6PD enzyme deficient and 72 had normal G6PD enzyme activity. The mean G6PD activity for group A was 11.7485±5.05 U/gHb and 11.4403±4.55 U/gHb for group B with no apparent difference between the two as shown in Table 1. Also, G6PD deficiency was similar in males 12 (27.3%) and in females 16(28.6%); (P=0.885) as shown in Table II.

Table 1: Summary of the mean values for Age, Haemoglobin, MPD and G6pd activity

GROUP	SERIES	AGE	Hb (g/dl)	MPD (uL)	G6PD (U/gHb)
A	Mean	34.73±10.8	13.30±1.0	718.15±1518.3	11.7485±5.0
	N	44	44	44	44
	Range	12 – 62	11-15	45-11970	5.97-20.60
B	Mean	33.06+16.5	12.81+1.0	409.88+632.2	11.4403+4.5
	N	56	56	56	56
	Range	7-75	11-15	72-3900	6.02-20.10
TOTAL	Mean	34.16	13.13+1.0	613.34+1291.6	11.6437+4.8
	N	100	100	100	100
	Range	7-75	11-15	45-11970	5.97-20.60

Table 2: G6PD activity distribution with sex

G6PD activity	FEMALES	MALES	TOTAL
Low	16 (28.6%)	12 (27.3%)	28 (28.0%)
Normal	40 (69.6%)	32 (72.7%)	72 (72.0%)
TOTAL	56 (56.0%)	44 (44.0%)	100

$X^2 = 0.834$ df =2 P = 0.885

Table 3: G6PD activity and mean parasite density

S/N	G6PD Activity	MPD	N
1	Low	415.57 ± 297.07	28
2	Normal	697.86 ± 1516.42	72

F= 0.539 P=0.585

Table 4: GThe relationship between G6PD activity and Adverse drug reaction (ADR) among G6PD deficient individuals; n=28

GROUP	G6PD activity	No ADR	ADR to CQ	ADR to SP	TOTAL
A	Low	n=12	n=2	n=5	n=17
B	Low	n=4	n=1	n=4	n= 9
TOTAL		16	3 ADR to CQ	9 ADR to SP	28
			TOTAL number with ADR= 12		

GROUP A: P=0.659 GROUP B: P=0.528

KEY: NR (No reaction); CQ (Chloroquine); SP (Sulphadoxine-Pyrimethamine)

*Total case of G6PD deficient individuals; n=28

*Reported cases of adverse drug reaction in the group; n=12

There was no statistical difference in G6PD activity with malaria parasite density (P=0.585) despite the fact that those with low G6PD activity had lower MPD than those with normal G6PD activity (415.57 ± 297.07U/gHb and 697.86 ± 1516.42/gHb respectively) as shown in Table III. Similar results have been reported elsewhere [17-19]. Others have reported a positive effect of G6PD deficiency on MPD reduction [20-22].

Over the years, Studies of G6PD deficiency and malaria reduction or protections have been hypothetic and contentious. Different results were found in studies of the Mediterranean variant, where no differences in malaria invasion and growth of G6PD deficient and non-deficient cells were found [23]. Similarly, conflicting results have been reported about whether hemizygous G6PD deficient males have protection against malaria [24-26]. According to [9], the gene that codes for G6PD activity is on the X- Chromosomes and hence one of the two G6PD alleles present in females is subject to inactivation. Variable X-Chromosomes inactivation means that expression of G6PD deficiency differs markedly among female heterozygotes as their red blood cell populations are variable mosaics of deficient and normal cells [27].

Finally, our study also showed that, among the G6PD deficient individuals (n=28), some of them reported adverse drug reactions (n=12). However, it was not statistically significant (P= 0.659 and P=0.528 in group A and B respectively) as in Table IV.

Adverse Drug reactions (ADRs) in G6PD deficient subjects is an age-long issue and dates back to 1926, when hemolytic anemia arising from the administration of anti-malaria, primaquine, was reported and 24years later (1950), G6PD deficiency was considered to be culpable [28]. Most common biological features of ADRs in G6PD deficient patients include: rapid fall in hemoglobin, Heinz bodies' accumulation, increased Fe^{2+}, protein and dark brown urination. In most cases, these conditions are self-limiting but can be fatal as well. The use of Sulphonamide for the treatment of malaria (in combination with quinine sulfate and pyrimethamine), has been shown to produce shortening of the life span of RBC [29].

CONCLUSION

There was no association between G6PD activity and adverse drug reaction in our present study. A more expanded study to include 1000 pregnant women on IPT and correlation with pregnancy outcome is presently on-going.

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