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MOLECULAR EPIDEMIOLOGY AND ACTIVITY OF ERYTHROCYTE G6PD VARIANTS IN A HOMOGENEOUS NIGERIAN POPULATION

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ABSTRACT

Objective: **To determine accurately the relative frequencies and enzyme activities of the polymorphic variants of G6PD in a homogeneous population in Nigeria.**
Setting: **Abanla village in the outskirt of Ibadan city and the University College Hospital, Ibadan Nigeria.**
Subject: **Seven hundred and twenty one subjects who belong to the Yoruba tribe of Southwestern Nigeria.**
Method: **Two mls of blood was withdrawn from each subject. G6PD activity was quantified by spectrophotometry. DNA was extracted for genotyping of G6PD by PCR.**
Results: **G6PD deficiency was 23.9% and 4.6% in males and females respectively. The gene frequencies of the different G6PD variants (Gd) were in accordance with expected Hardy-Weinberg equilibrium. Only GdA-1 type was found in subjects with deficient variants. G6PD activity decreased significantly with age among non-deficient individuals. The range of enzyme activities was wide and overlapping among the different G6PD variants.**
Conclusion: **G6PD deficiency was very high in the population. The gene frequencies were similar to previous findings. Molecular methods of typing G6PD allowed for direct and accurate genotyping of the enzyme in both males and females without having to combine several methods.**

INTRODUCTION

Glucose-6- phosphate dehydrogenase (G6PD) is a ubiquitous enzyme which has been found in every organism and tissues in which it has been sought(1). It is a membrane bound enzyme which helps to maintain the integrity of the cell. G6PD deficiency is the most common enzymopathy in humans. Red cell G6PD deficiency constitutes a considerable health problem in developing countries where it could cause neonatal jaundice, chronic haemolytic anaemia and acute haemolysis.

G6PD deficient erythrocytes have decreased enzyme activity as a result of accelerated breakdown, formation of molecules with decreased catalytic activity or production of enzyme molecules with reduced stability(1,2). These abnormalities result in a heterogeneous group of G6PD deficiency ranging from mild enzyme deficiency to almost complete lack of enzyme activity.

In Africa South of the Sahara, three different G6PD variants with polymorphic gene frequencies are well known. G6PD B is the commonest with normal enzyme activity and it is not associated with haemolysis. G6PD A is next in frequency, but has slightly reduced enzyme activity and also not associated with haemolysis. The third variant is G6PD A . This variant results in mild enzyme deficiency which can be associated with haemolysis especially after ingestion of certain chemicals like camphol and menthol or drugs like the antimalarial, primaquine. Newborns may develop hyperbilirubinaemia(3).

G6PD deficiency is inherited as an X- linked disorder(4). There is therefore lack of father to son transmission(5). A full expression of the trait therefore occurs only in hemizygous males and homozygous females in whom the X-chromosomes present carry the mutant gene. In the African populations, three different G6PD A^- genotypes are recognised in males (GdA, GdB and GdA$^-$) whilst in females six genotypes are recognised (GdB/GdB, GdA/GdB, GdA/GdA, GdB/GdA$^-$, GdA$^-$/GdA$^-$ and GdA$^-$/GdA$^-$).

Some work has been done in the past on the population genetics of G6PD in this area using different methods and in particular the qualitative fluorescent spot test(6). Until the advent of PCR methods, it has been difficult typing accurately the different variants without having to combine several methods, such as spot test, enzyme assay, enzyme electrophoresis and cytochemical elution test. We have used molecular techniques and enzyme activity measurements to characterise and determine gene frequencies of the however, it was realised that, there is a paucity of data on the relative frequencies of the polymorphic G6PD variants in the population.

MATERIALS AND METHODS

Seven hundred and twenty one subjects (children 0.6-12 years, adults 15-56 years) who gave informed consent were recruited into a study on genetic traits and malaria. They comprised 373 males and 348 females from Abanla village health centre and

the University College Hospital in Ibadan, Nigeria. All the subjects were from the predominant Yoruba tribe of south western Nigeria. The study received the approval of the joint ethical committee of University of Ibadan/University College Hospital, Nigeria. Two millilitres of blood was collected from each subject into citrate anticoagulated bottles and stored at 4°C until analysed.

G6PD activity: Red cell G6PD activity was measured quantitatively within 24 hours of sample collection by using the standard WHO spectrophotometric method(8) as adapted by May *et al*(9). Briefly, 50 ul of the sample was haemolysed with 450 ul of 2% saponin and then incubated at room temperature for 10 min. The haemolysate was separated by centrifugation for 20min at 5000rpm. 20ul of the supernatant was added to the reaction mixture which was freshly prepared (0.68 mM G6P, 0.68 mM NADP, 10mM Mg Cl, 36 mM triethanolamine). G6PD activity was then measured at 340nm. The haemoglobin (Hb) level was determined using a coulter cell counter (Clinicon HC 555). Results were expressed as IU/g haemoglobin after a temperature factor was introduced.

G6PD genotype by PCR: Genomic DNA was purified from whole blood using the DNA Easy kit of Invitrogen, as earlier reported(10). Outer PCR was carried out by amplifying the region spanning the third to fifth exon of the gene using the primers 12.0 (5' GGTGGAGGATGATGTATG 3') and 15.0, (5 GCTCCCACCACTCTATGA3') with the following thermocycling programme:- Initial denaturation at 94°C, for 5min, 30 cycles of (94°C 40sec, 54°C 55sec, 72°C 55sec), and final extension at 72°C for 5 min. The resulting PCR product was then used as a template for a nested PCR using, the same thermocycling programme but with the primers 12 nl, (5' GTCCCCAGCCACTTCTAAC3') and 15 nl, (5 AGGGCAACGGCAAGCCTTAC 3').

The PCR products were analysed on 1.5% agarose gel electrophoresis stained with ethidium bromide for visual detection by UV transillumination. Amplified DNA was blotted and UV cross - linked onto nylon membranes. Four sequence specific oligonucleotide probes (SSOP) were designed to cover position 202 (exon 3, 202 .1: Gd^B, Gd^A; 202.2 Gd^{A-}) and 376 (exon 5, 376.1 Gd^B; 376.2 Gd^A and Gd^{A-}). In order to identify the Gd^{A-} variants, samples reactive with probes 202.1 and 376.2 were hybridized with additional SSOP covering other A- mutations at positions 542, 680 and 968(9,11).

RESULTS

Table 1 shows the distribution of G6PD status with sex. It showed a G6PD deficiency of 23.9% in males and 4.6% in females. 29.3% of the females had heterozygous deficiency. The gene frequencies of the various G6PD variants (Gd) in males and females are shown in Table 2. The frequencies were in accordance with Hardy-Weinberg Principle. Among males, percentage G6PD deficiency (GdA^-) was 24%, GdB and GdA were 59% and 17% respectively. In females, the frequency of homozygous deficiency (GdA^-/GdA^-) was 4.6% whilst those with normal variants GdB/GdB, GdB/GdA and GdA/GdA were 33.9%, 27.9% and 4.3% respectively. Frequency of intermediate G6PD deficiency, GdB/GdA^- and GdA/GdA^- were 25.3% and 4.0% respectively. Only one type of G6PD deficient variant (GdA^{-1}) was recognised in the study. The gene frequency of the variants were similar among all age groups (data not shown).

Table 1

G6PD status in males and females

G6PD status	Males N	Males %	Females N	Females %	Total
Normal	284	76.1	230	66.1	514
Heterozygous deficient	–	–	102	29.3	102
Deficient	89	23.9	16	4.6	105
Total	373	100	348	100	721

Table 2

Gene frequency of G6PD variants in males and females

G6PD genotype	Gene frequency	(%)
Male		
Gd^B	219	(58.7)
Gd^A	65	(17.4)
Gd^{A-}	89	(23.9)
Females		
Gd^B/Gd^B	118	(33.9)
Gd^B/Gd^A	97	(27.9)
Gd^B/Gd^{A-}	88	(25.3)
Gd^A/Gd^A	15	(4.3)
Gd^A/Gd^{A-}	14	(4.0)
GdA-/GdA-	16	(4.6)

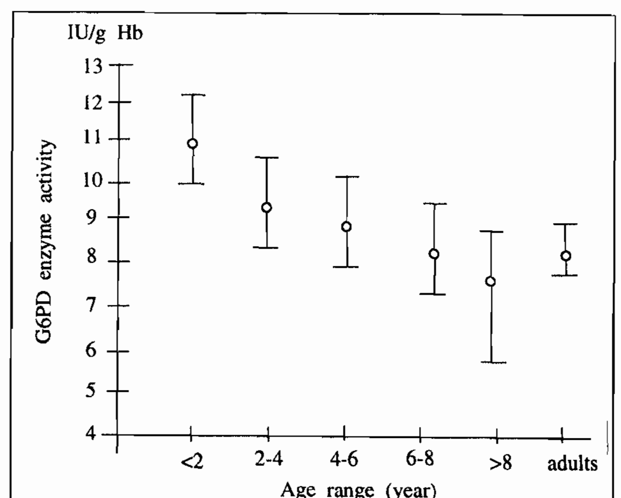
Table 3

G6PD genotype and mean enzyme activity

GP6D genotype	Activity IU/g Hb (mean ± SD)	Range
Male		
Gd^B	9.5 ± 3.7	2.2 – 39.6
Gd^A	9.4 ± 4.6	1.2 – 34.2
Gd^{A-}	1.9 ± 1.7	0.1 – 8.9
Female		
Gd^B/Gd^B	8.6 ± 2.6	2.5 – 18.2
Gd^A/Gd^B	8.6 ± 2.7	1.3 – 17.0
Gd^A/Gd^A	8.0 ± 3.1	3.8 – 13.9
Gd^B/Gd^{A-}	6.7 ± 2.5	1.1 – 13.5
Gd^A/Gd^{A-}	5.5 ± 2.6	1.2 – 10.6
Gd^{A-}/Gd^{A-}	1.7 ± 1.1	0.5 – 4.1

Figure 1

Mean G6PD enzyme activity and age among non-deficient subjects



Result of G6PD activity and genotype in males and females is shown in Table 3. It indicated the highest activity for those with Gd^B and Gd^A variants in hemizygous males as well as in females who were homozygous or heterozygous for the two variants. This is followed by heterozygotes with G6PD deficient gene (Gd^B/Gd^{A-}, Gd^A/Gd^{A-}). Hemizygous deficient males and homozygous deficient females, (Gd^{A-} and Gd^{A-}/Gd^{A-} respectively) had the least G6PD activity (20% that of Gd^B).

The range of enzyme activity was very wide and overlapping among the different G6PD variants. Enzyme activity was observed to decrease significantly ($p<0\ 05$) with age among non-deficient individuals (Figure 1) Consequently, enzyme activity was higher in children than in adults among non-deficient individuals.

DISCUSSION

We have determined the gene frequencies of the various polymorphic variants of G6PD and their activities in a homogeneous Nigerian population. Our result showed gene frequencies which conformed with expected Hardy-Weinberg equilibrium and was in agreement with existing data(1,12). The result indicated a high frequency of G6PD deficiency in the population studied (24% in males and 5% in females). The only type of GdA^- variant found in the population was GdA^{-1} relating to the mutation at position 202. In males, GdB has the highest enzyme activity followed by GdA with as high as 98% activity and GdA^{-1} with only 20% of the activity of GdB. The trend was the same in females except that the activity of GdA/GdA was 93% of GdB/GdB. Enzyme activity of the heterozygous normal GdB/GdA is similar to that of GdB/GdB whilst heterozygous deficient variants GdB/GdA^{-1} and GdA/GdA^{-1} have intermediate activity of 78% and 64% respectively. The enzyme activities of the different variants had a wide overlapping range. This is believed to be due to the relatively different population of young and old erythrocytes in the individuals. Young red cells are known to have higher enzyme activities than older ones(1,9). Generally, enzyme activity was slightly reduced in females than in males with the same G6PD type (Table 3) though not statistically significant.

All the subjects including those with hemizygous or homozygous GdA^{-1} were clinically normal as there was no haemolysis. This is because variants that are known to cause severe enzyme deficiency have far reduced enzyme activity of usually less than 10%(1). It is therefore not surprising that G6PD deficient individuals in the studied population were clinically normal as their enzyme activities far exceed the limit required to protect the cells under normal conditions. It has been reported earlier that the number of active enzyme molecules in deficient cells is sufficient to maintain normal red cell metabolism even though the half life of GdA^- is 13 days and that of GdB is 62 days(1,13).

It is concluded that there is a very high frequency of the deficient Gd^{A-} variant in the population. This is thought to be due to the selective advantage afforded the mutant gene by malaria in endemic areas where it is believed to confer protection against lethal effect of *P. falciparum*. However, the advantage appears to favour heterozygous females more than homozygous females and hemizygous males(1,14). Until now the complexity of typing female heterozygotes involving combining several methods led many studies to use only males for G6PD analyses. The use of molecular methods for typing G6PD will allow for accurate genotyping of both males and females. This will be highly beneficial in field and epidemiological studies.

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