# Human Glucose-6-phosphate Dehydrogenase Deficiency and Haemoglobin Genotypes in Owerri

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***Abstract***

***One hundred and fifty three (153) subjects (70 males and 83 females), aged between 10 – 60 years were investigated for erythrocyte glucose-6-phosphate dehydrogenase deficiency by methaemoglobin reduction method. Haemoglobin genotype in each of the 153 subject was determined using cellulose acetate paper electrophoresis. A methaemoglobin elution (cytochemical) test was used to ascertain heterozygous G6PD status. Glucose–6–phosphate dehydrogenase deficiency was found in 37 (24.2%) of the 153 subjects and in 33 (22.7%) of the randomly selected 145 subjects. With the female subjects, 12 (14.45%) were homozygous while 9 (10.8%) were heterozygous for G6PD deficiency. With the male, 16 (22.8%) were found to be homozygous for G6PD deficiency. There is a significant relationship (P≤0.05) between Glucose-6-phosphate dehygrogenase deficiency and haemoglobin genotypes. The enzyme (G6PD dehydrogenase) appears to be inherited through a partially dominant gene, which is sex-linked and has variable expression.***

**Keywords**: Human erythrocyte G-6-P-D- Deficiency, Haemoglobin genotypes, Sex

## Introduction

Glucose–6–phosphate dehydrogenase (G6PD) catalyses the conversion of β-D-glucose –6-phosphate to 6-phosphoglucono-1-5–lactone, which is an oxidation of the aldopyranose glucose-6-phosphate to a cyclic ester, called a lactone. Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme of the pentose phosphate pathway that is responsible for the generation of NADPH, required in many detoxifying reactions. There are evidences that G6PD expression is induced by a variety of chemical agents acting at different steps in the biochemical pathway controlling the intracellular redox status. (Pannerrselvam and Govindaswamy, 2002). It has been recently suggested that the primary physiological role of G6PD in mammalian cells is the defence against oxidative injuries Paudolfi et al, 1995, Martini and Ursini, 1996, Ugochukwu and Babady, 2002).

It is well known that G6PD is expressed in all cell types although at varying levels (Pandolfi et al, 1995). Although G6PD is present in leucocytes and platelets, it is the erythrocytes G6PD that is of clinical interest (Mordumuller et al. 1999). A deficiency of G6PD in red cells leads to acute anaemia.G6PD deficiency is an extremely widespread enzymopathy that affects over 11% of Black Americans and over 400 million people worldwide. Prevalence rate of 14.9% has been reported in Enugu and 21.6% in Ibadan (Luzzato and Allan 1968, Shu and Iluono 1996). G6PD deficiency is genetically inherited by a sex-linked gene which is of intermediate dominance (Beutler 1966). A normal male carries XY chromosomes while a normal female carries XX chromosome. A G6PD deficient gene is denoted as X. Consequently a G6PD deficient male carries XY genes (hemizygous). In females, deficient gene is denoted as XX gene (homozygous) and XX gene (heterozygous) (Luzzato and Roper, 1995). Full expression of G6PD deficiency occurs in hemizygous male and homozygous female. Heterozygous females manifest intermediate expression of G6PD deficiency. HbAA is the normal genotype; abnormal genotypes include HbAS, HbSS, HbSC, HbCC and HbEE. These and their clinical effects constitute the haemoglobinopathies. The relationship between G6PD deficiency states with these heamoglobinopathies is of great interest.

## Materials and Methods

**Subjects:** One hundred and fifty three (153) persons (70 male and 83 females) with an average age of 45 (range 10-60) years participated in this study. The 153 subjects comprises of 145 persons randomly selected and 8 persons drawn from the clinic and wards of Holy Rosary Hospital Emekuku and General Hospital Owerri respectively.

The Haemoglobin AA group were 91 subjects of age range 18-60 years and consisted of 37 males and 54 female with PCV of between 34% - 42% and have no history of recent haemolytic episode. The haemoglobin AS group was 38 subjects of age 15-58 years and consisted of 15 males and 23 females, with PCV of between 35-50% with no recent history of haemolytic episode. The Haemoglobin SS group were 24 subjects of age 10-40 years and consisted of 18 males and 6 females. Those considered from the haemoglobin SS group were only those having a PCV levels of 20% and above.

**Bloods sample collection:** 5 mls of blood was withdrawn from the veins of each subject. 1ml was added to 0.02ml of 0.1g/ml ethylenediaminetetra-acetic acid (EDTA), which served as anticoagulant. The sample was kept for haemoglobin genotype and PCV determinations. 4 ml of the venous blood was added into 0.6ml of acid citrate dextrose (ACD) for G6PD detection and cytochemical test Analysis was carried out on samples within 12 hours of collection.

**Detection of glucose-6-phosphate dehydrogenase**

***Methaemoglobin reduction test:*** 1ml of blood each from ACD was added to test tubes A (test), B(positive control), and C(negative control). 100µl glucose solution was added to A and C. 50µl of sodium nitrite was added to A and B while 50µl of methylene blue solution was added to A and C. the tubes were incubated in water bath at 37 °C. After 1 hour contents of the tubes were mixed by gentle tapping, then aerated by blowing gently through a micropipette and tubes re-incubated. After 2 hours, the aeration was repeated and tubes incubated for the third hour. Tubes were compared. If A (test) stays brown as in B, subject is G6PD deficient. If A changes to red as in C, subject is not G6PD deficient. If A has colour between B and C, result was termed 'indeterminate' and investigated further using the cytochemical test.

***Cytochemical test:*** Indeterminate result from the methaemoglobin reduction method of brewer et al (1962) was subjected to methaemoglobin elution test method of Gall et al (1965). Into the tube with indeterminate result, 20µl of potassium cyanide was added and mixed gently. Blood films were made on clean dry glass slides and dried quickly in air. The slide were immersed in the elution fluid and agitated for 1 minute. Then immersed in methanol for 30 seconds and also washed in water for 30 seconds. The films were then stained for 2 minutes with haematoxylin, rinsed in tap water, and counter stained with eosin for 2 minutes. The slide were rinsed in water and allowed to air dry. The films were examined with oil immersion for stained cells and ghost cells. The percentage of ghost cell was calculated thus: (number of ghost cells / total number of cells counted) x 100. Values greater than 5% indicates heterozygous G6PD state while values less than 5% indicates normal G6PD activity.

**Haemoglobin electrophoresis:** Haemoglobin variants were investigated using Cellulose Acetate membrane Electrophoresis at Alkaline pH (8.6).One volume of washed packed cell was lysed in four volumes drabkin solution (lysing reagent). The compartment of the electrophoretic tank was filled with Tris EDTA Borate buffer solution and the wicks soaked and positioned. The cellulose acetate paper was gently soaked for 5minute in a separate dish with Tris buffer, blotted lightly in between two absorbent papers. About 10ul of lysate was placed on the sample well. Using the applicator stick, test samples and known controls were applied to the membrane about 3cm from one end of the paper. Cellulose acetate paper was placed cross the bridge of the tank so that the surface was in contact with buffer. Power was connected and haemoglobin allowed separating at 220v until visible separation was obtained. Results were read directly alongside with the controls.

**Packed cell volume (PCV) estimation:** Capillary tubes of 75mm length with internal diameter of 1mm were filled with anticoagulant blood, sealed with Cristaseal at one end and centrifuged at 12,000g for 5minutes. The height of the red cell column was determined with the microhaematocrite reader.

**Results and Discussion**

The relationship between G6PD deficiency and haemoglobinopathies has received considerable scientific attention on the premise that a possible genetic link or inheritance pattern may exist between them. Scientific opinion on this subject is still discordant. We present our findings on the relationship between G6PD deficiency and haemoglobin genotypes in Owerri.

G6PD deficiency was found in 33 of the 145 subjects randomly selected representing a, 22.7% prevalence in Owerri. This result is very close to reports from Ibadan of a 21.6%. Prevalence rate of 14.9% have been reported in Enugu (Shu and Iluonu 1996). The distribution of G6PD deficiency according to heamoglobin genotype (Table 1) gives a positive correlation between G6PD deficiency and Haemoglobin Genotype in Owerri individuals.

**Table 1: Distribution of G6PD deficiency according to haemoglobin genotypes**

| Genotype n=153 | no | G6PD Deficiency | G6PD Deficiency (%) Among each genotype | G6PD Deficiency in whole Population |
|---|---|---|---|---|
| **HbAA** | 91 | 18 | 19.8 | 11.8% |
| **HbAS** | 38 | 10 | 26.3 | 6.5% |
| **HbSS** | 24 | 9 | 37.5 | 5.9% |

Comparing normal haemoglobin AA and abnormal haemoglobin AS and SS (Table 2), Haemoglobin AA individuals showed a lower G6PD deficiency (19.8%) while AS and SS individual showed a higher G6PD deficiency (30.6%).

**Table 2: Occurrence of G6PD deficiency in normal haemoglobin (HbAA) compared to abnormal Haemoglobin (HbAS and HbSS)**

| G6PD Deficiency Status | Male (n=70) | Female n=83 | % Prevalence |
|---|---|---|---|
| **Hemizygous (XY)** | 16 | - | 22.8 |
| **Homozygous XX** | - | 12 | 14.45 |
| **Heterozygous XX** | 1 | 9 | 10.8 |

Similar results have been obtained in Bahrain individuals whose abnormal haemoglobin genotypes showed a 47 % G6PD deficiency (Mohammad et al. 1998). This is similar to the situation in the Eastern Province of Saudi Arabia (Mohammad *et al* 1998). We hypothesize that the observation could be explained on the basis of historic endemicity of falciparium malaria in all these regions. G6PD expression is enhanced by oxidative stress induced by agents that either increase the intracellular concentration of oxygen or decrease the reduced glutathione (GSH) pool (Ursini *et al* 1997). It would then follow that G6PD expression will be higher in Haemoglobin genotypes HbSS in which there is a higher tendency for oxidative stress than the Haemoglobin HbAA. Invariably, G6PD deficiency should be enhanced in genotype in which there is reduced rate of oxidative stress e.g. HbAA. Therefore expression of G6PD deficiency would be

expected to be higher in HbAA than HbAS and HbSS: but the situation is a reverse. We speculate that although gene coding for the G6PD and that of haemoglobin are on different loci, the relationship existing between G6PD deficiency and haemoglobin genotypes could be explained on the basis of genetic polymorphism. The common occurrence of these genes in many populations led some researcher to the conclusion that acquisition of the G6PD deficiency gene and HbSS gene are related (Lewis and Harthrone 1996, Piomelli *et al* 1972) some workers posit that the two genes are independent (Lambotte *et al* 1968, Luzzato and Allain 1968, Bienzle *et al* 1975, Shu and Iluonu 1996) while other reports say that the two genes are even on different chromosomes and therefore the increased frequency of G6PD deficiency in abnormal Haemoglobin (Table 2) cannot be explained by the genetic mechanism (Pionelli 1993). The percentage distribution of G6PD deficiency according to sex (Table 3) showed that, the males have a significantly ($P \leq 0.05$) higher prevalence rate than females.

**Table 3: Distribution of G6PD deficiency according to sexes**

| Genotype | Males (n=70) | | Females (n=83) | |
|---|---|---|---|---|
| HbAA (n=91) | 37 | 40.7% | 54 | 59.3% |
| HbAS (n=38) | 15 | 39.5% | 23 | 60.5% |
| HbSS (n=24) | 18 | 75% | 6 | 25% |

Males showed 22.8% hemizygous while females were 14.45% homozygous and 10.8% heterozygous. This observation would be due to a higher occurrence of abnormal haemoglobin in male (47.1%) than in females (34.9% (Table 4).

**Table 4: Distribution of Heamoglobin Genotypes**

| Heamoglobin Genotype | G6PD Deficiency | % G6PD Deficiency |
|---|---|---|
| Normal Hb Genotype n = 91 | 18 | 19.8 |
| Abnormal Hb Genotype n = 62 | 19 | 30.6 |

The observation agrees with the fact that the enzyme inheritance is sex-linked (Piomelli 1993). There is a relationship between the expression of G6PD deficiency and Haemoglobin genotypes in individuals found in Owerri of Eastern Nigeria.

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