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Co-inheritance of Glucose-6-phosphate dehydrogenase Deficiency and Sickle Cell Traits in Sokoto Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Author IJ designed the study, produced the initial draft. Author NG reviewed the manuscript and interpreted the data. Authors AZ and SYI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Transfusion from glucose-6-phosphate dehydrogenase (G-6-PD) deficient and Sickle cell traits (HbAS) blood constitute a major health burden and social challenge, especially to jaundiced neonate and sickle cell disease patient. Currently, routine screening of these two abnormal genes on blood donors in our locality is yet to be introduced. However, this study aimed at bridging the gap.
Methodology: This study screened 1000 volunteers for G-6-PD deficiency using methaemoglobin reduction method. Haemoglobin phenotypes of the deficient subjects were determined by alkaline cellulose acetate electrophoresis.
Results: Out of 1000 volunteers; 36.7% were G-6-PD deficient [128 (36.2%) were males; 248 (62.5%) were females]. Haemoglobin phenotypes (HbAA, HbAS, HbSS, HbAC, HbSC and HbCC) of these deficient subjects were; 71.30%, 23.90%, 0.50%, 3.70%, 0.30% and 0.30% respectively.

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No incidence of HbSS, HbSC, and HbCC in G-6-PD deficient male was recorded.
Conclusion: Co-inheritance with G-6-PD deficiency and HbAS is high. This finding has importance in blood transfusion setting, as routine screening of these inherited disorders prior to blood donation may help in reducing the potential risk of haemolytic complications and also prevent failure of white blood cell filtration among high risk persons.

Keywords: G-6-PD deficiency; haemoglobin variants; jaundiced neonates; haemolytic complication; blood transfusion.

1. INTRODUCTION

Glucose -6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49) catalyses the first committed step in the pentose phosphate pathway, generating NADPH, which protects the mature erythrocyte cell against oxidative damage by maintaining a high level of reduced glutathione (GSH) and assisting the action of catalase [1]. G-6-PD deficiency is the most common human enzymopathy, affecting about 400 million people worldwide [2], and almost unknown to the public [3]. It is transmitted from parent to child [4]. Both homozygous (affected females) and hemizygous (affected males) people with G-6-PD deficiency show episodes of acute haemolytic anemia and, more rarely, non-spherocytic chronic haemolytic anaemia [5,6]. The use of G-6-PD deficient blood has been studied for simple therapeutic intervention such as exchange blood transfusions (EBT). It has been proposed that several biochemical changes and depletion in the antioxidant defence system occur on storage of G-6-PD deficient blood [7]. It was observed in a study that preterm infants who received G-6-PD deficient blood developed haemolysis requiring further intervention by exchange transfusions [8]. Lesser than expected drop in post-EBT total serum bilirubin, prolongation of the duration of phototherapy, the need for repeat EBT and the occurrence of massive intravascular haemolysis have been reported in infants in whom G-6-PD deficient donor blood were used for EBT practice [9]. These complications were reported in patients with normal G-6-PD activity receiving G-6-PD deficient blood. In areas with high prevalence of G-6-PD deficiency, more neonates with jaundice that might require EBT are likely to be G-6-PD deficient; Hence the incidence and severity of these complications are expected to be higher [10].

Nigeria, by virtue of her population, is the most sickle cell disease (SCD) endemic country in the world, with over 40 million people (24% of its population) being a carrier of the Haemoglobin “S” [11]. It is caused by an autosomal structural single-point mutation, and characterized by poor solubility haemoglobin in the deoxygenated state followed by polymerization leading to RBC shape distortion, rigidity and extravascular haemolysis [12]. Sickle cell traits (HbAS) have been considered as a benign condition that impose a life-threatening complications as reported in several *in vitro* studies in which HbAS red cells showed abnormality of their filterability [13]. RBCs collected from HbAS donors frequently occlude WBCs' reduction filters caused by haemoglobin polymerization [14]. Other parameter including temperature, platelets, osmolarity, type of anticoagulant, time of storage and oxygen saturation of the blood unit might further affect the adequacy of WBC filtration [14,15]. Failure of adequate WBC reduction has been shown to increase the incidence of febrile non-haemolytic transfusion reaction, transmission of leukocyte-associated viruses and HLA alloimmunization [15]. Storage of HbAS whole blood in large-capacity oxygen-permeable bags increases oxygen tension and allows more effective WBC reduction by filtration [14] The acute congestive crisis-like syndrome associated with HbAS red cell exchange transfusion is rarely seen, mainly due to the modifying effect of a concurrent normal blood transfusion [16].

In view of these, this work was undertaken to provide information about co-inheritance with G-6-PD deficiency and Haemoglobin variants. Due to the economic burden associated with transfusing G-6-PD deficient and/ or HbAS blood to high risk persons. It may be necessary that routine G-6-PD and haemoglobin variants screening be adopted in blood banking system as part of management policy to avert haemolytic complications.

2. MATERIALS AND METHODS

This was a prospective study carried out between the months of February and April 2015 within the metropolitan city of Sokoto state, Nigeria. Ethical approval for the research design was obtained from the Usmanu Danfodiyo

University Teaching Hospital Ethical committee, UDUTH/HREC/2014/No. 238 and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

The relevance of the study was explained to all the subjects to ensure their voluntary participation. For those children less than 18 years of age, consent was given by their parents or guardians.

Venous blood samples (6 ml) were collected and divided into two parts from each subjects. Four (4) ml was put into lithium heparin container for G-6-PD screening. The remaining two (2) ml was dispensed into EDTA container for haemoglobin phenotypes determination. All blood samples so collected were analyzed within 48 hours of collection in batches. The methaemoglobin reduction method described by Chessbrough [17] was used for G-6-PD screening. Three clean test tubes were arranged and labeled test, normal, and deficient. Into each of the tubes labeled test, 0.05 ml of sodium nitrite-glucose solution and 0.05 ml of methylene blue reagents was dispensed. To the tubes labeled deficient 0.05 ml sodium nitrite-glucose solution only was dispensed and to those labeled normal no reagent was dispensed. One (1) ml of the blood sample was then dispensed into all the tubes and was rocked gently to mix. All the tubes were corked with cotton wool and incubated at 37℃ for 3 hours. At the end of the incubation, three clean centrifuge tubes were arranged and labeled as before (test, normal, and deficient). Ten (10) ml of distilled water was then dispensed into each of the tubes. 0.1 ml of the respective incubated sample was transferred into each of the tubes accordingly and a colour comparison of the three tubes (test, deficient and normal) was done after 5 minutes. Normal blood samples showed a clear red colour like that of the normal reference tube. Glucose-6-phosphate dehydrogenase deficient blood samples give full expression of brown colour, like the positive (deficient) reference tube. In contrast, samples of female heterozygote give colour variations between red and brown in accordance with the degree of expression of the trait.

The standard method described by Evans [18] was used for haemoglobin electrophoresis. About 1 ml of the blood sample was washed three times using normal saline (0.85% NaCl) to remove plasma proteins. The washed cells were re-suspended with equal volume of normal saline. The red cell suspension was mixed with equal volume of distilled water to lyse the blood cell. The resulting lysate was used for haemoglobin phenotype determination [19] A small quantity of haemolysate of venous blood from each of the subjects was placed on a cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris-EDTA-borate buffer at pH 8.6 [18]. Electrophoretic separation was then allowed to operate for 15-20 minutes at an electromotive force (e.m.f) of 160 V. The results were read immediately against known haemoglobin (AA, AS, AC, SC and SS) which serves as controls. The data obtained were subjected to descriptive statistics using SPSS Software package Version 20 and results presented as percentages.

3. RESULTS

Out of the 1000 volunteer subjects; 376 (37.6%) persons were found to be G-6-PD deficient. Furthermore, sex distribution of G-6-PD deficiency indicated that 128 (36.2%) were males and 248 (62.5%) were females (Table 1). Table 2 displays the haemoglobin phenotypes AA, AS, SS, AC, SC and CC among G-6-PD deficient persons in the study population and their prevalence were 71.3%, 23.9%, 0.5%, 3.7%, 0.3% and 0.3% respectively. There was no record for G-6-PD deficiency co-inherited with HbSS, HbSC and HbCC among males.

Table 1. Sex distribution of G-6-PD deficient subjects in Sokoto

Sex	Deficient subjects		Screened subjects
	Number	%	
Male	128	36.2	603
Female	248	62.5	397
Total	376	37.6	1000

4. DISCUSSION

This is the first report of its kind concerning the co-inheritance with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and some haemoglobin variants in Sokoto. The prevalence of G-6-PD deficiency in Sokoto was found to be high. Contrastingly, the prevalence rate is low in developed countries like Europe and America [20]. An explanatory to this may be due to high frequency of G-6-PD deficiency seen in malarial endemic region across the globe [21,22]. Sex distribution among deficient subjects was higher

Table 2. Distribution of G-6-PD deficiency among Haemoglobin variants in Sokoto metropolis

Deficient subjects	Haemoglobin variants					
	HbAA	HbAS	HbSS	HbAC	HbSC	HbCC
Male	81(63.3)	41(32.0)	nil	6(4.7)	nil	nil
Female	187(75.4)	49(19.8)	2(0.8)	8(3.2)	1(0.4)	1(0.4)
Total	268(71.3)	90(23.9)	2(0.5)	14(3.7)	1(0.3)	1(0.3)

Hb=haemoglobin, Figures in parentheses are percentages

in female than in male. This finding is corroborated by a study in Gabon [19] and contrasted many studies [23,24]. A previous study by Ella et al. [20] indicated that the frequency of G-6-PD deficiency in females of Nigeria ranged from 21.1 to 51.4% including those studied by DNA method. In Ilorin, equal occurrence in both sexes among neonates was reported by Obasa et al. [25]. It is worth nothing that, G-6-PD deficiency being a genetic disorder that affects x-chromosome is transmitted from mother to son and not from father to son. Males carry a single x-chromosome against their female counterpart that carries dual x-chromosome. Affected males are hemizygous. Females that code for the abnormal gene for G-6-PD on both of their x-chromosomes are homozygous female while heterozygote female had two red cell populations, normal cells and a deficient cell, since one of their x-chromosome carries the defective G-6-PD gene. Therefore, severe enzyme deficiency occurred in hemizygous males and homozygous females while heterozygous females have normal or moderately lower enzyme level [26,27]. This extremely high rate for female deficient volunteers may be due to different Screening procedures which are quite robust in the detection of the fully developed defect in hemizygous males and female homozygous, but they fall short in the ascertainment of female heterozygote. The methaemoglobin reduction assay employed for this study may have overestimated the frequency of heterozygous females due to subjective judgement of the colour in the test tube which depends absolutely on the haemoglobin concentrations in the red cells.

Although there have being scarcity of published data on the co-inheritance with G-6-PD deficiency and some haemoglobin variants in Nigeria. In the current study, co-inheritance of G-6-PD deficiency with HbAS is higher than those with HbAC, HbSS, HbSC and HbCC. This finding agrees with a study in Ekiti by Esan et al. [10] but disagree with that of Egesie et al. [13] who reported 5.4% in Jos and a study in Ghana found G-6-PD and HbAS co-inheritance of 7% compared to the 23.9% found in this study. These discrepancies may be attributed to the differences in geographical areas and subjects included for the studies. While this present study included large number of apparently healthy individuals in North-Western part of Nigeria, the study of Egesie et al. [13] and Patrick et al. [28] recruited small cohort subjects of blood donors in the North central of the country and in Ghana respectively.

Sex distribution shows No incidence of HbSS, HbSC and HbCC in G-6-PD deficient male, thus showing sex-specific prevalence of HbSS, HbSC and HbCC in G-6-PD deficient female.

Bearing in mind that malaria transmission is stable throughout the year within the study area. The implication will be that G-6-PD deficient people living with HbAS probably may have more protections against malaria infection. Conversely, individuals with HbSS and HbSC may be more prone to malaria infection than HbAC.

Due to the significant implication that G-6-PD deficient blood may have when transfused to a jaundiced neonates and sickle cell disease patients, it may be necessary to introduce routine screening test for these abnormal genes before transfusion.

5. CONCLUSION

Blood transfusion is a major therapeutic intervention to many sufferers from sickle cell disease and jaundiced neonate. Screening donors for G-6-PD deficiency and sickle cell traits is recommended; Perhaps some complications impose on high risk recipients averted.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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