Journal of Medical Laboratory and Diagnosis Vol. 1(1) pp. 1-4, April 2010
Available online http://www.academicjournals.org/JMLD

Full Length Research Paper

Glucose-6-phosphate dehydrogenase deficiency in blood donors and jaundiced neonates in Osogbo, Nigeria

E. O. Akanni[1]*, B. S. A. Oseni[1], V. O. Agbona[1], B. A. Tijani[2], E. Tosan[3], E. E. Fakunle[4] and V. O. Mabayoje[4]

[1]Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, P. M. B 4400, (OS 230001), Osogbo, Osun State, Nigeria.
[2]Department of Haematology and Blood Transfusion, Ladoke Akintola University of Technology Teaching Hospital, P. M. B. 5000, (OS 230001) Osogbo, Osun State, Nigeria.
[3]Medical Laboratory Science Council of Nigeria, 8 Harvey Road, Yaba, Lagos, Lagos State, Nigeria.
[4]Department of Haematology and Blood Transfusion, Ladoke Akintola University of Technology College of Health Sciences, P. M. B. 4400, (OS 230001) Osogbo, Osun State, Nigeria.

Accepted 11 March, 2010

A study on the prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in blood donors and in jaundiced neonates was carried out. 286 subjects consisting of 200 blood donors and 86 jaundiced neonates were screened for G6PD. Presence of G6PD and bilirubin levels (total and conjugated) were determined in all the subjects. G6PD was determined using two standard methods; methaemoglobin reduction test and fluorescent spots test. Total and conjugated bilirubin levels were also determined in neonates using Jendrassik and Groff method. Out of the 200 blood donors tested for G6PD, 39 (19.5%) were G6PD deficient and 41 (47.7%) out of 86 jaundiced neonates were G6PD deficient. There is a close association between the two methods used for determining G6PD in blood donors and jaundiced neonates as there was no significance difference "P < 0.05" between the results obtained from the two methods. With G6PD deficiency prevalence rate of 19.5% (39) in the blood donors, and the attendant reduced life span of red blood cells, this study therefore reveals the necessity of including G6PD testing in the blood donors screening criteria in the study area. Glucose-6-phosphate deficiency is also revealed as a major cause of haemolytic episode in neonates in the area.

Key words: G6PD deficiency, prevalence, jaundiced neonates, blood donors.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common known inherited human enzyme disease, affecting 10% of the world population, which amounts to 200 - 400 million affected people worldwide. It is an X-linked disorder genetically transmitted by a sex-linked gene of intermediate dominance (Beutler, 1993) causing haemolytic anaemia and neonatal hyperbilirubinaemia (Viroj, 2005). Deficiency of G6PD enzyme in the red blood cells, under certain circumstances, may lead to an abnormal rupture of the cell wall with resultant hemolytic anaemia (Lukens et al., 1999). The likelihood of developing haemolysis and its severity is determined by the magnitude of the enzyme deficiency, which is consistent with the biochemical characteristics of each G6PD variants (WHO, 1967). G6PD deficiency is also known to be associated with neonatal jaundice, kernicterus and even death (Valaes, 1994).

Glucose-6-phosphate dehydrogenase is one of the red cell enzymes that catalyze the initial step in the hexose mono phosphate shunt, which oxidizes glucose-6-phos-phate to phosphogluconate and reduces nicotinamide

*Corresponding author. E-mail: olufemiakanni@yahoo.com.

adenine dinucleotide phosphate (NADP) to NADPH (Bertil, 2002).

G6PD is also described as a house keeping enzyme essential for basic cellular functions, including protection of the red cell proteins from oxidative damage. Oxidant damage of haemoglobin leads to precipitation of haemoglobin, which may be morphologically recognized as Heinz bodies. The enzymatic activity of G6PD generates NADPH that is utilized for glutathione reductions, which restores haemoglobin to the soluble form. Thus, maintaining a high ratio of reduced to oxidized glutathione, represents the major defense against oxidative damage of haemoglobin (Prchal et al., 2005).

While some forms of G6PD deficiency are symptomatic, some others results in neonatal jaundice, chronic non-spherocytic anaemia, acute episodic haemolytic anaemia (caused by oxidative stress such as ingestion of fava beans), and drug induced hemolysis induced by infections. A mild phenotype characterized by neonatal jaundice, favism and hemolytic anaemia has become common in the world (Notaro et al., 2000) arising in regions of past-malaria risk such as Africa, India, the Mediterranean and south East Asia (Vulliamy et al., 1992). Individuals who are G6PD deficient live a normal or near normal life until particular agents trigger or sensitize them, leading to oxidative damage resulting into hemolysis. Hemolysis is induced by sudden destruction of the older and more deficient erythrocytes. This occurs after exposure to some drugs of high redox potentials, mothballs, henna, favabeans, or following certain infections and metabolic abnormalities (Hsia et al., 1993).

Clinical manifestation of G6PD deficiency results in abnormal breakdown of the red cell of the newborn since the neonates do not have ability to produce more glucoronyl tranferase, an enzyme needed for the conjugation of bilirubin release from the breakdown of the cell (Luzzatto, 1995). It leads to accumulation of the unconjugated bilirubin there by causing jaundice which is characterized by yellow pigment seen on the skin and eye (Ernest, 1996). Donor red blood cells from G6PD deficient donors therefore have shortened life span and will not benefit the recipients.

In 1998, Douglass defined blood donors as screened individuals that donate blood specifically to benefit a particular person (recipient) with the purpose of ensuring an adequate supply of blood when needed for accident victims, people needing surgery, people suffering from certain diseases and for medical research (Douglass, 1998).

The donor's blood are not routinely screened for G6PD deficiency in blood banks, because most common variants that cause acquired hemolytic anaemia pose little health hazards (Amoozegar, 2005). Regarding the potential hazards of severe hemolysis in neonates and 80% of donors are males; the aim of the study therefore is to determine the prevalence of G6PD deficiency in blood donors and in jaundiced neonates in Osogbo.

MATERIALS AND METHODS

Study area

Osogbo is the capital of Osun State situated in the tropical rainforest belt of south/western part of Nigeria with about 500 km from Abuja, the country capital. The estimated population based on 2006 census is about 450,000. This work was carried out in the Blood Bank and Hematology laboratory of LAUTECH Teaching Hospital Osogbo.

Samples collection

A total of 286 samples were collected for the study, 200 samples were collected from prospective and suitable donors and 86 samples from jaundiced neonates. The samples were collected and analyzed between May and August, 2009.

Exclusion criteria

Unsuitable candidates for blood donation and non-jaundiced neonates were excluded from the study.

All samples were screened for G6PD using methemoglobin reduction test and fluorescent spot test methods. Besides, serum total and conjugated bilirubin levels were determined in neonates. The various analyses were performed using the methods as described by the authors as below:

Methaemoglobin reduction test: Clean test tubes were arranged and labeled test, normal, and deficient. Into each of the tubes labeled test 0.05 ml of sodium nitrite and 0.05 ml of methylene blue reagents were dispensed. To the tubes labeled Deficient 0.05 ml sodium nitrite only was dispensed and to those labeled normal were no reagent dispensed. 1.0 ml of the blood sample was then dispensed into all the tubes and mixed after which they were corked with cotton wool and incubated at 37°C for 3 h. At the end of the incubation, 3 clean test tube were arranged and labeled as before (test, normal, deficient), 10 mls of distilled water was dispense into each of the tubes and 0.1 ml of the respective incubated sample was transferred into each of the tubes accordingly and a colour comparation of the three was done (Cheesbrough, 2002).

Fluorescent spot test: Exactly 0.01 ml of blood was added to 0.2 ml of substrate and 0.01 ml of the control (Deficient, intermediate and normal) was also added to the substrate separately. A drop of the mixture was transferred to its respective column on an absorbent paper at zero minutes and the remaining content was incubated at 37°C. Also at 5 and 10 min a drop of the respective sample was again transferred to the absorbent paper and allowed to dry after which the spots were viewed using ultraviolet light (Beutler and Mitchell, 1968).

Bilirubin estimation: Clean test tubes were arranged and well labeled for total and conjugated bilirubin estimation with a tube in each group labeled as blank and test. To the tubes labeled blank for total bilirubin, 400 µl of the blank solution, 200 µl of Diazo blank and 3.4 ml of Benzoate urea were dispensed and to the tubes labeled blank for conjugated bilirubin, 400 µl of blank solution, 200 µl of diazo blank and 3.4 ml of distilled water were dispensed and the tubes labeled tests for total bilirubin contained 400 µl of plasma sample, 200 µl of Diazo reagent and 3.4 ml of Benzoate urea. Also to the test tubes labeled test for conjugated bilirubin contained

Table 1. Prevalence of G6PD deficiency in blood donors and jaundiced neonates.

	Blood donors		Jaundiced neonates	
	Frequency	**Percent (%)**	**Frequency**	**Percent (%)**
Normal	161	80.5	45	52.3
Deficient	39	19.5	41	47.7
Total	200	100.0	86	100.0

Table 2. The distribution of the total and conjugated bilirubin levels.

	N	**Minimum**	**Maximum**	**Mean ± SD (µmol/L)**
Age in days	86	1	12	4.81 ± 2.65
Total bilirubin	86	70	447	228.90 ± 101.20
Conjugated bilirubin	86	4	170	28.52 ± 27.94

400 µl of plasma, 200 µl of diazo reagent and 3.4 ml of distilled water. The content of the tubes were mixed and then incubated in the dark for 10 min after which the absorbance was read at 545 nm (Jendrassik and Grof method, 1978).

***Statistical analysis*:** Data are expressed as mean ± SD, presented pictographically and also using chi-square (X^2)

RESULTS

There was no difference in the results obtained using the two methods. Out of the 200 blood donors, 19.5% were G6PD deficient while 80.5% were normal. Out of the 86 jaundiced neonates 47.7% were G6PD deficient while 52.3% were normal (Table 1).

Regarding the prevalence of G6PD deficiency, there was a significant difference between the jaundiced neonates and blood donors; since the x^2 calculated, 24 are greater than the X^2 tabulated 3.84.

The total and conjugated bilirubin levels were also estimated to confirm jaundice (hyperbilirubinaemia) in the neonates. The results were shown in Table 2.

81.5 % of the blood donors were males and 18.5% were females whereas 75% of jaundiced neonates are males 25% were females. Among blood donors, 35 of the 163 males (21.5%) were G6PD deficient and 4 of the 37 females (10.8%) were G6PD deficient. Among jaundiced neonates, 33 of the 65 males (50.8%) were G6PD deficient while 32 of the 65 males (44.2%) were G6PD normal. The sex distribution of G6PD deficiency in blood donors and jaundice neonates is represented in Figure 1.

DISCUSSION

In this study, we found the prevalence of G6PD deficiency in blood donors as 19.5% in Osogbo Osun State. This is similar to the findings of the study conducted at Imam Sajjad's Hospital in Yasuj about the prevalence of G6PD deficiency in blood transfussion recipients. Out of the 261 blood bags screened for G6PD deficiency, 14.17% were G6PD deficient (Seyed et al., 2007). Sex distribution in this study showed that 89.7% of the deficient patients are males and 10.3% were females. The reason behind this fact is that the abnormal gene responsible for deficiency is located on the X-chromosome. Therefore, the illness associated with G6PD deficiency occurs more frequently in males than in females (Beutler, 1993). G6PD deficiency is inherited from females who carry one copy of the gene on one of their X chromosome to half of their sons. Sons who receive the gene are G6PD deficient and daughters who do not receive the gene are unaffected (Rector et al., 2006).

The higher rate of male donors than females may be a result of their high packed cell volumes (PCV) which is usually higher than that of their female counterparts. The normal range for the male is between 40 - 54% and that of the females is between 36 - 46%. Besides, higher hemoglobin values in males (normal range 13 - 18 g/dl) compared to females (12 - 15 g/dl) or other screening criteria of the donors usually exclude the females from blood donation. Those females excluded are the lactating mothers, pregnant women and menstruating women (Cheesbrough, 2002). Similarly, a study carried out in Shiraz, Southern Iran about the prevalence of G6PD deficiency among male donors established that 80% of the blood donors were male (Amoozegar et al., 2005).

In this study, we found the prevalence of G6PD deficiency in jaundiced neonates as 47.7% in Osogbo Osun State. In a similar study carried out in Al-Hofuf area in the Eastern Province of Saudi-Arabia on G6PD deficiency and neonatal jaundice. Out of the 211 jaundiced neonates involved in the study, 30.3% were G6PD deficient (Abbas et al., 1999). Kaplan et al. (1998) reported that 22.5% of the jaundiced infants had G6PD deficiency in a pilot kernicterus registry between 1984 and 1998, in which 80 jaundiced infants from 21 states in

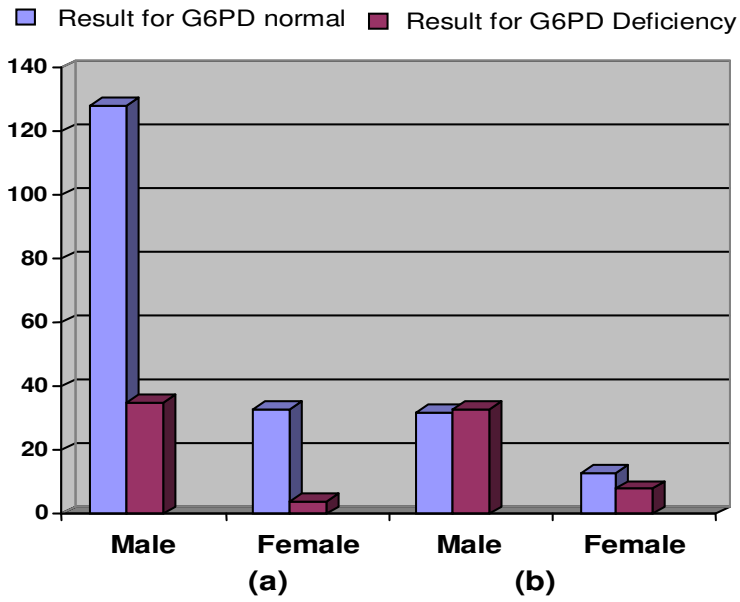

Figure 1. Histogram showing; (a) Sex distribution of G6PD deficiency in blood donors; (b) Sex distribution of G6PD deficiency in jaundiced neonates.

the United States were included (Kaplan et al., 1998).

The 52.3% of the jaundiced neonates had normal G6PD levels. Jaundice may be as a result of ABO incompatibility, Rhesus incompatibility, sepsis, elliptocytosis or physiological in these patients. It is also possible that some could have been G6PD deficient with normal levels during haemolysis (Abbas et al., 1999). Out of the 86 jaundiced neonates 75.6% were males and 24.4% were females. 50.8% of the total males were G6PD deficient and 38.1% of the females were the G6PD deficient. A total of 47.7% were deficient, out of the 86 jaundiced neonates. Similar reasons are responsible as in the case of blood donors' sex distribution.

Conclusively, this study showed that there are more male blood donors than females and also more male jaundiced neonates than females. Majority of the males were G6PD deficient in both groups. Therefore, G6PD deficiency is responsible for high number of neonatal jaundice in the study population as reflected in this report. Regarding that 19.5% of blood donors are G6PD deficient in this study, we suggest the inclusion of G6PD screening in blood donors in this area. Also, this study showed that G6PD is a major cause of haemolytic episode in neonates in the area.

REFERENCES

Amoozegar HM, Mirshakeris, Paishva N (2005). Prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency among male donors in Shiraz, Southern Iran. Iran J. Med. Sci. 30(2): 94-96.

Bertil E, Glader (2002). A review on genetics and Pathophysiology of Glucose-6-Phosphate Dehydrogenate deficiency. Blood 15: 150.

Beutler E, Mitchel M (1968). Special Modification of the Fluorescent Screening method for Glucose-6-phosphate dehydrogenase deficiency Blood pp. 32: 816.

Cheesbrough M (2002). Blood Transfusion Practice: Blood donation and Storage of blood, District Laboratory Practice in tropical countries, low-price edition by Cambridge Universal Press pp. 352-353.

Hsia YE, Miyakawa F, Baltazar J (1993). Frequency of Glucose-6-Phosphate Dehydrogenase mutations in Chinese Filipinos and Laotians from Hawali 92: 470-476.

Jendrassik L, Grof P (1978). Modified Jendrasik-Grof method for bilirubin, Adapted to the Abbott biochromatic analyzer. Clinical chemistry by Am. Assoc. Clin. chem.. 24: 1841-1845.

Kaplan M, Hemmerman C (1998). Severe neonatal hyper-bilirubinemia: A potential complication of glucose-6-phosphate dehydrogenase deficiency Clin. Perinatal. 25: 575-590.

Lukens JN, Glader BE (1999). Hereditary hemolytic anaemias associated with abnormalities of erythrocyte glycolysis and nucleotide metabolism, wintrobe's clinical hematology 10th ed: pp. 1160-1175.

Luzzatto L (1995). Haemoglobins, G6PD and Parasites in red cells experiential, 51: 206-208.

Notaro ORA (2000). "Human mutations in glucose-6-Phosphate Dehydrogenase reflect evolutionary History" Fazeb J. 14(3): 485-494.

Prchal JT, Gregg XT (2005). Red cell enzymopathies. In: Hoffman R, Benz E, eds, Haematology: Basic Principles and Practice, 4th ed. Churchill living stone Philadelphia pp. 653-659.

Seyed HN, Amir AV (2007). The Prevalence of G6PD deficiency in blood transfusion recipent. Haematology 12: 85-88.

Valaes T (1994). Severe neonatal Jaundice associated with glucose-6-Phosphate Dehydrogenase Deficiency. Pathogenesis and global epidemiology. *Acta* Paediatr. Supply 394: 58-76.

Vulliamy TPM (1992). The molecular basis of G6PD deficiency. Trends Genet. 8(4): 138-143.

Viroj W (2005). Is the G6PD activity assay more cost effective than the methaemoglobin reduction test in screening for G6PD deficiency? Haema 8(1): 61-63.

WHO Scientific Group (1967). Standardization of Procedures for the Study of Glucose-6-Phosphate Dehydrogenate World Health Organization Rep ser 366: 1-53.