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Prevalence of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency among Neonates with Jaundice in a Tertiary Hospitalin Nigeria

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Abstract: G6PD deficiency is known to be associated with neonatal jaundice, kernicterus and even death. G6PD is the first enzyme of the pentose phosphate pathway and catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone, with the concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form (NADPH). Three hundred and twenty five neonatal blood samples were collected for the study between the ages of 1–10days of both sexes. About 4ml of blood sample was collected from newborn baby with jaundice, 2ml of blood sample was dispensed into di-potassium ethylenediaminetetracetic acid (K_2EDTA) bottles for packed cell volume and haemoglobin estimation using haematology analyzer (sysmex model KX-21N) also K_2EDTA blood sample was used for G_6PD status determination, remaining 2ml of blood sample was dispensed into heparin bottles for bilirubin estimation. Out of 325 newborn babies with neonatal jaundice, 96(29.5%) were G6PD deficient; 57 were male and 39 were female. Mean± SD of total bilirubin **(B_1)** andconjugated bilirubin**(B_2)** were significantly ($P<0.05$) higher in G6PD- deficient participants compared with G6PD normal. Neonates should be screened for G6PD deficiency when family history, ethnic or geographic origin on the timing of the appearance of neonatal jaundice suggests the possibility of G6PD deficiency.

INTRODUCTION

G6PD is the first enzyme of the pentose phosphate pathway and catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone, with the concomitant reduction` of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form (NADPH). Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency in the world; clinically, it affects an estimated 400 million people (Frank, 2005).It is most commonly prevalent in African, South East Asian, Mediterranean and Middle Eastern populations. It affects about 1 in 10 African-American males in the United States (Chinevere et al; 2006). G6PD deficiency is an X-linked disorder, hemizygous males and homozygous females are the ones that are mainly affected. However, approximately 10% of heterozygous females may also be at risk; causing hemolytic anaemia and neonatal hyperbilirubinaemia. 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. 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 (Lukens and Glader, 1999). It is also possible that some patient could have been G6PD deficient with normal levels during hemolysis, repeating the G6PD screen could have identified these individuals. G6PD deficiency is also known to be associated with neonatal jaundice, kernicterus and even death (Valaes, 1994). Hemolytic anaemia and neonatal jaundice are the two major pathologies associated with G6PD deficiency in the newborn; during the neonatal period, the disease may manifest as neonatal jaundice, which may cause severe neurological complications and even death in some populations (Slusher , 1995; Yaish *et al.*, 1991), although the mechanism of jaundice in G6PD deficient neonates is not completely defined, haemolysis does not seem to contribute as much as impaired bilirubin conjugation and clearance by liver (Cappellini and Fiorelli 2008). There is a remarkable association between neonatal jaundice, G6PD deficiency and Gilbert syndrome. The newborns that are homozygous for both abnormalities are particularly at risk for neonatal jaundice with pathogenesis of decreased bilirubin conjugation and elimination (Kaplan *et al.*, 1997; Kaplan and Hammerman 1998). In patients with acute haemolysis, testing for G6PD deficiency may be falsely negative because older erythrocytes with a higher enzyme deficiency have been haemolyzed; young erythrocytes and reticulocytes have normal or near-normal enzyme activity. Female heterozygotes may be hard to diagnose because of X-chromosome mosaicism leading to a partial deficiency that will not be detected reliably with screening tests (Reclos *et al.*, 2000; Ainoon *et al.*,

2003; Gregg and Prchal, 2000). 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 released from the breakdown of the cell. It leads to accumulation of the unconjugated bilirubin there by causing jaundice which is characterized by yellow pigment seen on the skin and eye. G6PD deficiency is one of a group of congenital hemolytic anaemia, and its diagnosis should be considered in children with a family history of jaundice, anaemia, splenomegaly, or cholelithiasis, especially in those of Mediterranean or African ancestry (Hermiston and Mentzer, 2002). About 60% of term babies and 80% of pre-term infants develop some degree of jaundice during their first week of life (Parthasarathy *et al.,* 2004) but, the jaundice due to G6PD deficiency occurs in the first day of life and usually severe (pathological) in nature (Mohanty, 2004). The aim was to study the prevalence of G6PD deficiency in relation to neonatal jaundice and degree of anaemia.

MATERIALS AND METHODS

STUDY AREA

The study site was Ido town, the headquarters of ido-osi local government in Ekiti State, Nigeria. The secretariat sited in between Ido town and Usi town. It is very close to other local government districts, (Moba, Ijero, Ilejemeje and Ado). The local government comprises rural towns: Aaye, Ido, Usi, Ayetoro, Ilogbo, Osi, Ifaki, Orin, Ora, Igbole and some other smaller villages, inhabited mainly by the Ekitis, but with some non-Ekitis fund living peacefully among the people. People in Ido - Osi cherish farming, education, trading and practicing majorly Christianity religion. According to 1991 Census, the Local government has a total population of 107,000 people with eleven electoral wards in the Local government.

STUDY DESIGN

Three hundred and twenty five blood samples of neonates with Neonatal jaundice were collected for the study between the ages of 1–10days of both sexes at the Federal Teaching Hospital, Ido-Ekiti, Nigeria. The study was conducted with an informed consent of the patient's parents; ethical approval was obtained from ethical committee of Federal Teaching Hospital, Ido-Ekiti. About 4ml of blood sample was collected from newborn baby with jaundice, 2ml of blood sample was dispensed into di-potassium ethylenediaminetetracetic acid (K_2EDTA) bottles for packed cell volume and haemoglobin estimation using haematology analyzer (sysmex model KX-21N) also K_2EDTA blood sample was used for G_6PD status determination, remaining 2ml of blood sample was dispensed into heparin bottles for bilirubin estimation

RESEARCH PROCEDURES

ESTIMATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD)

Glucose-6-Phosphate dehydrogenase (G-6-PD) was determined using two standard methods; methaemoglobin reduction test and fluorescent spots test.

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 Mitchel, 1968).

BILIRUBIN ESTIMATION

Serum bilirubin level was estimated using Jendrassik and Grof bilirubin method.

PRINCIPLE:

Bilirubin reacts with diazotized sulphanilic acid to azobilirubin in the presence of caffeine in alkaline medium. The intensity of the coloured complex is proportional to the concentration of bilirubin in the serum

PROCEDURE

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 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 1978).

PACKED CELL VOLUME

PRINCIPLE

The aspirated blood sample is measured to a predetermined volume diluted at the specified ratio and then fed into each transducer chamber, which has a minute hole aperture and also contains electrodes through which direct current flows. Blood cells suspended in the diluents sample, pass through the aperture, causing direct current resistance to change between the electrodes, blood cell size is detected by electric pulses. Blood cell count is calculated by counting the pulses and the histogram determined by the pulse sizes

PROCEDURE

Sysmex machine was inspected (for instrument, reagents, waste bin and printer paper) before switch on the machine from power source, machine was calibrated before used and control sample was run along each batches of sample analysis. Well mixed EDTA blood sample was used for the analysis of complete blood count, blood sample was aspirated through the sample probe one after another by pressing start switch, sample was analyzed, rinsed and display the result on the LCD screen of the machine also printed the results out. After the analysis, machine was shut-down by aspirating cell clean which washed and rinsed the machine before finally shutdown and switch off from the power source

STATISTICAL ANALYSIS

Results obtained were analyzed using student t-test to compare the means. Analysis was performed using computer database software from the statistical package for social sciences (version 16.0 SPSS). A P-value of < 0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

RESULTS

Out of 325 newborn babies with neonatal jaundice, 96(29.5%) were G6PD deficient; 57 were male and 39 were femalewhile 229(70.5%) were G6PD normal; 112 were male and 117 were female. The prevalence of G6PD was high in age group 1-2days compared with other age groups. Mean±SD of total bilirubin (B_1) and conjugated bilirubin (B_2) were significantly higher in G6PD deficient compared with G6PD normal($P<0.05$); however, mean±SD of packed cell volume (PCV) and haemoglobin concentration (Hb) were significantly ($P<0.05$) lower in G6PD deficient compared with G6PD normal as shown in table 1-3.

Table 1: Age Distribution and G6PD Status among Neonatal Jaundice

G6PD	1-2days	3-4days	5-6days	7-8days	9-10days
G6PD Deficient (%) N=96	32(33.3)	28(29.2)	13(13.5)	13(13.5)	10(10.4)
G6PD Normal (%) N=229	60(26.2)	66(28.8)	23(10.0)	20(8.7)	60 (26.2)
TOTAL (%) N=325	92(28.3)	94(28.9)	36(11.1)	33(10.2)	70(21.5)

Table 2: Sex Distribution and G6pd Status among Neonatal Jaundice

G6PD	MALE	FEMALE
G6PD Deficient (%) N=96	57(59.4)	39(40.6)
G6PD Normal (%) N=229	112 (48.9)	117(51.1)
TOTAL (%) N=325	169 (52.0)	156(48.0)

Table 3: MEAN±SD of Total Bilirubin (B1), Conjugate Bilirubin (B2), PCV AND HB Concentration G6PD Status among Neonatal Jaundice

G6PD (µmol/L)	Total Bilirubin (B1) (µmol/L	Conjugate Bilirubin (B2), (µmol/L	PCV %	HB (g/dl)
G6PD Deficient N=96	269.09 ±205.82	40.53 ±27.23	40.02 ±6.69	13.33 ±2.23
G6PD Normal N=229	116.08 ±67.01	18.13 ±9.99	45.31 ±7.71	15.11 ±2.57
(P-VALUE)	2.74	36.00	7.24	6.81
	(0.00)	(0.00)	(0.00)	(0.00)

DISCUSSION

Prevalence of G6PD deficient among newborn babies with neonatal jaundice in this study was 96(29.5%); 57 were male and 39 were female while 229(70.5%) were G6PD normal; 112 were male and 117 were female. Similar to Abbas, 1999 reported that 64(30.3%) were G6PD-deficient and 134(63.5%) were normal; 52 of the G6PD-deficient infants were males and 12 (19%) were females (Abbas, 1999). RaminIranpour, 2008 reported that out of 2501 newborns screened for G6PD, (1307 [52.3%] were males and 1194 [47.7%] were females; 79 neonates were found to have G6PD deficient (67 males, 12 females) (RaminIranpour , 2008). The prevalence of G6PD deficient in this study was high in age group 1-2days compared with other age groups, this was supported with the fact that jaundice due to G6PD deficiency occurs in the first day of life and usually severe (pathological) in nature (Mohanty *et al*., 2004). In this present study, hyperbilirubinaemia was observed in G6PD deficient compared with G6PD normal. This was supported by (Sukamal *et al.*, 2012) reported that G6PD deficient neonates comprises 16 (14.68%) out of 109 newborns, 5 (31.25%) of the 16 G6PD deficient babies developed severe jaundice as compared to the 16 (17.2%) of the 93 non-G6PD deficient babies who developed the same. 23.8% of the babies who developed severe jaundice were G6PD deficient (Sukamal *et al.,* 2012). Similarly, Tanphaichitr *et al.,* 1995 reported that 61(12.08%) out of 505 had G6PD deficiency (Group I) while the rest (444 cases) had normal G6PD (Group II). In Group I, 49.15% developed neonatal jaundice while in group II, 23.68% developed jaundice (Tanphaichitr *et al.,* 1995). Apart from G6PD deficiency that cause hyperbilirubinaemia in neonate; hyperbilirubinaemia might also be due to physiological jaundice, ABO and rhesus incompatibility or other pathological conditions like red blood cells membrane disorders such as hereditary spherocytosis, hereditary elliptocytosis which was not investigated in this study. The most common medical problem associated with glucose-6-phosphate dehydrogenase deficiency is hemolytic anemia, which occurs when red blood cells are destroyed faster than the body can replace them. This type of anemia leads to paleness, yellowing of the skin and whites of the eyes (jaundice), dark urine, fatigue, shortness of breath, and a rapid heart rate; this support why degree of anaemia was higher in G6PD deficient compared with G6PD normal in this present study(Valaes , 1994).

CONCLUSION

Neonates should be screened for G6PD deficiency when family history, ethnic or geographic origin on the timing of the appearance of neonatal jaundice suggests the possibility of G6PD deficiency. More importantly, it is necessary to determine G6PD status of all newborns with neonatal jaundice. Genetic counseling for G6PD families should be aimed at increasing the awareness of hemolytic triggers and advice individuals with G6PD deficiency to avoid exposure to oxidative drugs and ingestion of fava bean.

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