PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AMONG NEONATES IN USMANU DANFODIYO UNIVERSITY TEACHING HOSPITAL (UDUTH), SOKOTO, NIGERIA: TOTAL ANTIOXIDANT CAPACITY AND LIPID PEROXIDATION IN G6PD DEFICIENT NEONATES

ABSTRACT

Backgroud: Glucose-6-phosphate dehydrogenase deficiency is one of the most common enzyme defects affecting all races and particularly in malaria-endemic areas. This study aimed at determining G6PD deficiency, bilirubin and oxidative stress biomarkers in G6PD deficient neonates among neonates in UDUTH, Sokoto.

Methods: Samples of cord blood were collected at delivery, in the Labour Room, from 300 neonates made up of 131 (43.7%) males and 169 (56.3%) females. Methaemoglobin reduction method was used for the screening of G6PD deficiency; total bilirubin level was estimated using bilirubinometer, total antioxidant capacity (TAC) was measured using TAC Assay Kit, and malondialdehyde (MDA) using thiobarbituric acid method.

Results: Of the 300 neonates tested, a total of 90(30%) were G6PD-deficient while 210(70%) had normal G6PD status. Of the 90 G6PD-deficient neonates, 41(45.6%) were males and 49(54.4%) were females. The prevalence was 31.3% among male population and 29.0% among female population. The mean ± standard error of total bilirubin (mg/dL), TAC (uM), and MDA (Mmol/L) in G6PD-deficient and G6PD-normal neonates were 6.63 ± 0.12 and 6.11 ± 0.06, 364.34 $\pm$ 18.76 and 390.99 $\pm$ 24.18, 26.15 $\pm$ 1.22 and 23.35 $\pm$ 1.15. The total bilirubin was significantly higher ($p<0.05$) in G6PD-deficient neonate than in G6PD-normal

neonates, both TAC and MDA values showed no significant difference between the G6PD deficient and G6PD normal neonates.

Conclusion: From this study, there is a high prevalence of G6PD deficiency among neonates in UDUTH, Sokoto. G6PD deficiency is a known cause of neonatal jaundice hence it is recommended G6PD screening be made routine for all neonates born in UDUTH, Sokoto.

Key words: G6PD, prevalence, lipid peroxidation, bilirubin, neonatal jaundice

INTRODUCTION

Glucose-6-phosphate-dehydrogenase (G-6-PD) deficiency is the most common enzyme defect, being present in more than 400 million people worldwide [1, 2]. G6PD deficiency is described as a widespread, heritable X-chromosome linked abnormality [3]. It is seen most frequently in approximately all of Africa, Asia, and the countries near the Mediterranean Sea [4]. Glucose-6-phosphate-dehydrogenase deficiency is an important disorder of hexose monophosphate shunt in erythrocyte metabolism [5, 6]. G6PD enzyme activity is necessary for RBC survival as it catalyses the only metabolic pathway capable of generating reducing power to these cells lacking mitochondria [7]. Reducing power, supplied in the form of NADPH, is necessary as an electron donor for detoxifying oxidative challenges to cells. The metabolic reactions concerned are part of the pentose phosphate pathway, the first and rate-limiting step of which is catalysed by the G6PD enzyme: the oxidation of glucose-6-phosphate into 6-phosphoglucono-δ-lactone, which simultaneously reduces NADP to NADPH. The electron of NADPH passes to abundant glutathione dimers (GSSG) via another enzyme, glutathione reductase. Reduced glutathione monomers (GSH) represent the primary defense against hydrogen peroxides, organic peroxidises, and free radicals. When G6PD functions normally, the drain of electrons from the NADPH pool caused by oxidative challenge within the cell prompts the PPP to accelerate according to need, i.e. maintaining an NADP–NADPH equilibrium that strongly favours NADPH. This in turn maintains the

oxidised–reduced glutathione (GSSG–2GSH) equilibrium strongly in the direction of the reduced state [8]. Thus, G6PD serves as dominant cellular defense against oxidative stress [9]. In G6PD deficiency, acute hemolytic anemia usually begins within hours of an oxidative stress and ends when G6PD deficient erythrocytes have haemolyzed; therefore, the severity of the anemia associated with these acute hemolytic episodes is proportionate to the deficiency of G6PD and oxidative stress [10]. Viral and bacterial infections are the most common triggers, but many drugs, foods and toxins can also precipitate hemolysis[10]. The most clinically serious public health burden of G6PD deficiency is neonatal jaundice as a result of hyperbilirubinaemia, and puts infants at risk of kernicterus within the first few days of life. Kernicterus can lead to hearing deficits, behaviour problems, and permanent neurological damage or death [1]. Previous studies in Nigeria documented a prevalence of 4-26% for G6PD deficiency [11]. It has been documented that G6PD deficiency is implicated as the major factor associated with high prevalence of severe neonatal hyperbilirubinaemia, acute bilirubin encephalopathy, kernicterus, and cerebral palsy among Nigeria infants; hence this study is designed to establish the prevalence of G6PD deficiency in neonates born in UDUTH, Sokoto in order to take preventive measures if the need arises.

METHODS

Study design

This was a prospective observational study conducted in the labor ward of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria between March and June, 2015.

Subjects

The study population consisted of three hundred male and female term neonates delivered by normal vaginal delivery or by caesarian section. Intra-uterine fetal distress (IUFD), and still birth were excluded from the study. The sample size was calculated based on prevalence rate

of G6PD deficiency in neonates from a previous study [12]. Ethical approval was obtained from Ethics and Research Committee of the Hospital and informed consent was obtained from the mother each neonate prior to delivery.

Blood collection and analysis

Five milliliter of cord blood from each neonate was collected into a clean lithium heparinised sample container and was mixed gently to prevent clotting. G-6-PD screening was performed using Methaemoglobin Reduction Method [13]. The screening was carried out on the day of blood collection. Total plasma bilirubin was determined using Bilirubinometer (Neo-bil Plus) [13], lipid peroxidation by plasma malondialdehyde estimation colorimetric method of Shah and Walker [14] and total antioxidant potential by copper reducing antioxidant assay method of Sashindran et al [15]. The data generated from this study were analyzed using the statistical package for social sciences (SPSS) version 20.0. Values were presented as the mean ± standard error of mean (SEM). Statistical comparisons of the parameters were made between G6PD normal and G6PD deficient neonates using student t-test.

RESULTS

A total of 300 neonates made up of 131 (43.7%) males and 169 (56.3%) females were screened for G6PD deficiency. Of this number, 90 (30%) were G6PD-deficient while 210(70%) were G6PD normal. Of the 90 G6PD-deficient neonates, 41(45.6%) were males and 49(54.4%) were females (Table 1). Table 2 shows the prevalence based on gender of the neonates. The prevalence was 31.3% among male population and 29% among female population. Table 3 shows the Bilirubin and oxidative stress biomarkers in G6PD deficient neonates and G6PD normal neonate (controls). The mean ± standard error of mean of total bilirubin (mg/dL) for the G6PD-deficient neonates and G6PD-normal neonates were 6.63 ± 0.12 and 6.11 ± 0.06 respectively. The mean ± standard error of mean of TAC (uM CRE) for

the G6PD-deficient neonates and G6PD-normal neonates were 364.34 ± 18.76 and 390.99 ± 24.18 respectively.

The mean ± standard error of mean of MDA (nmol/L) for the G6PD-deficient neonates and G6PD-normal neonates were 26.15 ± 1.22 and 23.35 ± 1.15 respectively.

Table 1 Frequency of G6PD deficiency among the neonates

G6PD Status	Frequency	Percent	Valid Percent	Cumulative Percent
Deficient	90	30	30	30
Normal	210	70	70	70
Total	300	100	100	100

Table 2 Prevalence of G6PD Deficiency based on gender

G6PD Status	Female	Percent	Male	Percent	Total
Deficient	49	29	41	31.3	90
Normal	120	71	90	68.7	210
Total	169	100	131	100	300

Table 3 Bilirubin and oxidative stress biomarkers in G6PD deficient neonates

Parameters	Control n(50)	Deficient n(90)
Total Bilirubin(mg/dL)	6.11 ± 0.06	6.63 ± 0.12**
MDA(nmol/L)	23.35 ± 1.15	26.15 ± 1.22
TAC(µM CRE)	390.99 ± 24.18	364.34 ± 18.76

Values are presented as mean ± SEM. ** statistically significant ($p<0.01$) as compared to control.

Abbreviation: CRE = Copper reducing equivalence.

DISCUSSION

It has been established that Glucose-6-phosphate dehydrogenase deficiency is the most easily identified inherited disorder that causes newborn jaundice, severe hyperbilirubinemia, and bilirubin encephalopathy. Furthermore, acute bilirubin encephalopathy (ABE) and its posticteric chronic sequelae (kernicterus, in its classic form) are the most severe, life-threatening manifestations of neonatal G6PD deficiency that should be preventable [9]. Its prevalence in neonates with indirect hyperbilirubinemia varies in different parts of the world according to ethnic variations. Studies from different parts of the world report different prevalence rates. In Spain, France and Singapore the prevalence rates (1.57, 2.1 and 1.62% respectively) were low, while that of Saudi Arabia, Nigeria and in American Blacks (18.4, 40 and 14% respectively) were high [16]. In an earlier study, the prevalence of G-6-PD deficiency in apparently healthy individuals in Sokoto was established to be 37.6% [17]. In the present study, the prevalence of G6PD deficiency amongst neonates born in UDUTH, Sokoto, Nigeria; was determined and found to be 30%. Strong relationship between malaria and G6PD deficiency state has been widely reported, prevalence of G6PD deficiency is high in malaria endemic region [11]. It has also been documented that G6PD deficiency provides

131 great protection from malaria infections especially for falciparum infections. Nigeria being a
132 malaria endemic country, might have accounted for the high prevalence of G6PD deficiency.
133 G6PD deficiency, being an X-linked condition, the G6PD deficiency was found to be more in
134 male than the female from this study and this finding is consistent with previous reports [18].
135 In the present study, the mean bilirubin level of G6PD deficient neonates was significantly
136 higher than G6PD normal neonates. Our finding is consistent with that of Isa *et al* [18]
137 Badejoko *et a*l [19]. Significant association of G6PD deficiency with neonatal
138 hyperbilirubinaemia in the immediate perinatal period has been documented [20]. It has also
139 been reported that significant hyperbilirubinaemia poses a potential threat for permanent
140 neurological deficit or kernicterus. Studies have revealed that insufficient hepatic metabolism
141 of unconjugated bilirubin[21] rather than increased hemolysis [22] is the major contributor to
142 neonatal hyperbilirubinaemia.
143 MDA level is a sensitive indicator of lipid peroxidation and thus of oxidative stress.
144 Increased concentrations of free oxygen radicals in newborns damage the cell membrane
145 through lipid peroxidation, and this damage may be associated with various pathologies such
146 as hypoxic ischemic encephalopathy, intraventricular hemorrhage, necrotizing enterocolitis,
147 and bronchopulmonary dysplasia Bilirubin is an effective scavenger of oxidant radicals, and
148 its concentration is increased during oxidative stress [23].The level of MDA was higher in
149 G6PD deficient neonates than G6PD normal neonates though the increase was not
150 statistically significant, this is consistence with a study by Alkhotani *et al* [23] and Nassef *et*
151 *al* [24]. Total antioxidant capacity concentration was also higher in G6PD normal than G6PD
152 deficient neonates; the difference was also not statistically significant.
153 In conclusion, there is a high prevalence of G6PD deficiency among neonates in UDUTH,
154 Sokoto, which may lead to neonatal hyperbilirubinaemia which can result to kernicterus. In
155 UDUTH, neonates are not routinely screened for G6PD deficiency, and the common practice

of early discharge means that newborns are discharged before the onset of jaundice. Therefore, it is recommended that all neonates should be screened for G6PD deficiency in order to take appropriate measures to prevent complications of hemolysis and jaundice; as well as the bilirubin level before postnatal discharge. All patients that are malaria positive must be screened to know their status prior to treatment so as to avoid antimalarial and all other oxidative agents that can trigger hemolytic crisis in G6PD deficient neonates.

REFERENCES

1. Williams O, Gbadero D, Edowhorhu G, Brearley A, Slusher T, Lund TC. Glucose-6-phosphate-dehydrogenase deficiency in Nigerian Children. PLoS ONE, 2013; 8(7), e68800
2. Hsieh YT, Lin MH, Ho HY, Chen LC, Chen CC, Shu WC. Glucose-6-Phosphate Dehydrogenase (G6PD)-Deficient Epithelial Cells Are Less Tolerant to Infection by *Staphylococcus aureus. PLoS ONE*. 2013; 8(11): e79566
3. Stadem PS, Hilgers MV, Bengo D, Cusick SE, Ndidde S, Slusher TM, Lund TC. Markers of oxidative stress in umbilical cord blood from G6PD deficient African newborns. PLoS ONE, 2017; 12(2): e0172980.
4. Frank, JE. Diagnosis and management of G6PD deficiency. *American family physician,* 2005; 72(7):1277.
5. Segel GB. Enzymatic defects. In: Behrman RE, Kliegman RM, Jenson HB. Nelson Textbook of Pediatrics. 17th ed. Philadelphia; Saunders, 2004:635-638.
6. Azma RZ, Hidayati N, Farisah NR, Hamidah NH, Ainoon O, G6PD enzyme activity in normal term Malaysian neonates and adults using a Osmmr 2000-d kit with HB normalization. *Southeast Asian J. Trop. Med. Public Heal*, 2010; 41: 982-988.

7. Pandolfi PP, Sonati F, Rivi R, Mason P, Grosveld F, Luzzatto L . Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. *EMBO J*, 1995; 14:5209 – 5215.
8. Greene, L.S.. G6PD deficiency as protection against *falciparum*-malaria: an epide-miologic critique of population and experimental studies. *Yearb. Phys. Anthropol*, 1993; 36; 153–178.
9. Bhutani VK. Jaundice due to glucose-6-phosphate dehydrogenase deficiency. *Neoreviews,* 2012; 12(3). Available at htpp://neoreviews.aappublications.org
10. Leong A. Is there a need for neonatal screening of glucose-6-phosphate dehydrogenase deficiency in Canada? *McGill J. Med*, 2007; 10(1): 31-34.
11. Ibrahim B, Sani AM, Timothy B. Prevalence of glucose-6-phosphate dehydrogenase deficiency in children aged 0-5 years infected with Plasmodium falciparum in Katsina State, Nigeria. *Advances in Biochemistry*, 2016; 4(6): 66-73.
12. Ibrahim T, Sample size determination, In: Research methodology and dissertation writing for health and allied health Professionals. 1st Edition, Lucas, A.O (eds), Nigeria. 1997, P.74
13. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge, UK: Cambridge University Press; 2006:362–378
14. Shah JK, Walker's AM. Quantitative determination of MDA. *Biochemica et Biophysica Acta*. 1989; 11:207-211.
15. Sashindran R, Balasundaram M, Jegathambigai R, Kumar P. Evaluation of neuroprotective effect of quercetin and coenzyme Q10 in ethanol induced neurotoxicity in mice. *International Journal of Applied Biology and Pharmaceutical Technology*. 2015; 6(1):67-71.

16. Khodashenas E, Kalani-Moghaddam F, Araghi Z, Khodaparast M, Yazdani Z. Glucose-6-Phosphate Dehydrogenase Deficiency and Neonatal Hyperbilirubinemia. *Iranian Journal of Neonatology*, 2015, 6(3): 28-31.

17. Oduola1 T, Jelani I, Bolarin DM, Ndakotsu MA, Dallatu MK. Prevalence of Glucose -6- Phosphate Dehydrogenase (G-6-PD) Deficiency in Sokoto: Liver Function and Oxidative Stress Biomarkers in Deficient Individual. *BJMMR*, 2016; 13(11): 1-6.

18. Isa HM, Mohamed MS, Mohamed AM, Abdulla A, Abdulla F. Neonatal indirect hyperbilirubinemia and glucose-6-phosphate dehydrogenase deficiency.*Korean J Paediatr* 2017; 60(4): 106-111.

19. Badejoko BO, Owa JA, Oseni SBA, Badejoko O, Fatusi AO, Adejuyigbe EA. Early Neonatal Bilirubin, Hematocrit, and Glucose-6-Phosphate Dehydrogenase Status. *Paediatrics* 2014; 134(4): e1082-e1087.

20. Kaplan M, Algur N, Hammerman C: Onset of jaundice in glucose-6- phosphate dehydrogenase-deficient neonates. *Pediatrics* 2001, 108(4):956–959.

21. Kaplan M, Muraca M, Hammerman C, Vilei MT, Leiter C, Rudensky B, Rubaltelli FF: Bilirubin conjugation, reflected by conjugated bilirubin fractions, in glucose-6-phosphate dehydrogenase-deficient neonates: a determining factor in the pathogenesis of hyperbilirubinemia. *Pediatrics* 1998, 102(3):E37.

22. Jalloh S, Van Rostenberghe H, Yusoff NM, Ghazali S, Nishio H, Wahab NA, Matsuo M, Nik Ismail NZ. Poor correlation between hemolysis and jaundice in glucose 6-phosphate dehydrogenase-deficient babies. *Pediatr Int* 2005, 47(3):258–261.

23. Alkhotani A, Eldin EEMN, Zaghloul A, Mujahid S. Evaluation of neonatal jaundice in the Makkah region. *Sci. rep*. 2014; 4: 4802

24. Nassef YE, Fathy HA, Ali A, Hamed MA, Fathy GA. Evaluation of G6PD activity and antioxidants status in jaundiced Egyptian neonates. *Int. J. Med.Med. Sci*. 2013; 5(12): 550-559.