ORIGINAL ARTICLE

Hyperbilirubinaemia in glucose-6-phosphate dehydrogenase deficient neonates: the role of haemolysis

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Abstract

Background: Hyperbilirubinaemia associated with varying complications is a common clinical presentation in Glucose-6-phospate dehydrogenase (G6PD) deficient neonates in our environment. This study is to determine the role of haemolysis in the pathogenesis of hyperbilirubinaemia in G6PD deficient neonates with a view to establishing an appropriate management strategy for acceptable neonatal outcome.

Materials and Methods: One hundred and fifty neonates admitted into the Special Care Baby Units (SCBUs) of the Jos University Teaching Hospital, Bingham University Teaching Hospital, and the Plateau State Specialist Hospital with neonatal jaundice were enrolled for this study between March 2013 and February 2014. The neonates were reviewed clinically and examined for fever, jaundice, cyanosis among other features and they had blood samples collected for laboratory investigations that include Full Blood Count (FBC), Reticulocyte Count, Serum Bilirubin (SB) and G6PD assay.

Results: Median age at presentation was 3 (IQR: 1-4) days. The mean haemoglobin concentration of the study subjects was 15.90 ± 2.23 g/dl while median reticulocyte count was 2.5 (IQR:2-3) %. Total serum bilirubin had a median concentration of 204.00 (IQR:168.25-255.50) μmol/L while median unconjugated bilirubin concentration was 184.50(IQR: 144.50-233.71) μmol/L. Sixty-one (40.7 %) of the studied neonates were G6PD deficient with mean G6PD activity of 3.99(IQR: 2.72-4.94) IU/gHb

Conclusion: Hyperbilirubinaemia is a common clinical finding in G6PD deficient neonates in our environment but haemolysis is not a major event in its pathogenesis.

Key words: Glucose-6-phosphate dehydrogenase, Haemolysis, Hyperbilirubinaemia, Neonates

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Introduction

Hyperbilirubinaemia affects about 60-70% of otherwise healthy full term neonates and 80% preterm neonates, with increasing concentration of total serum bilirubin (TSB) during the first week of life. [1] It presents clinically as jaundice, a term used to describe the yellowish discoloration of the skin, sclera and mucous membrane due to the deposition of bile pigments. [1, 2]

Hyperbilirubinaemia develops when the rate of bilirubin production exceeds the rate of elimination primarily by conjugation resulting in high levels of lipid soluble unconjugated bilirubin with the potential for neonatal neurotoxicity[1,2]. Various genetic, environmental, and racial factors play significant roles in the aetiology of hyperbilirubinaemia and these include breast feeding, congenital hypothyroidism, pyloric stenosis, sepsis, blood group incompatibilities and glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked recessive disorder. [1, 2] Neonates of Oriental, African, North American and Jewish descent tend to have more severe jaundice with as many as 24-54% of them developing hyperbilirubinaemia with serum bilirubin ≥ 200 μmol/L.[2] Studies amongst Nigerian children, have shown that neonatal jaundice is a significant cause of morbidity and mortality in paediatric age group and an important contributing cause of cerebral palsy in the country. [3]

G6PD deficiency is the commonest inherited red cell enzymopathy affecting an estimated 400 million people globally. G6PD deficiency has a worldwide prevalence of about 4.9% varying between countries but highest in the tropics and sub-tropics. [4] The prevalence of G6PD deficiency is as high as 60-70% among Kurdish and Sephardic Jews, 12.8% in the United States of America (USA) while less than 0.1% prevalence has been reported in Japan attributed to spontaneous mutations among the natives. [5-8]

The prevalence of G6PD deficiency in the region of African is between 5-25%. G6PD deficiency is believe to confer selective advantage against *Plasmodium falciparum* malaria and hence its high prevalence in malaria endemic areas. [5-8] Prevalence of 28.1% was reported in South-West, Nigeria where 21% of the male population were G6PD deficient. [9, 10]

Reports from many countries of the world including Nigeria have indicated G6PD deficiency to be a major risk factor for neonatal jaundice, due to haemolysis which may occur after exposure to oxidant agents like infection, drugs, chemicals or fava beans ingestion. [9, 10] Neonates with variants of severe G6PD deficiency develop hyperbilirubinaemia that may be sufficient to cause kernicterus and death if untreated. [11, 12] Other studies have however shown that hyperbilirubinaemia can occur in G6PD deficient neonates in the absence of

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haemolysis due to variants of this enzyme whose deficiency may not be associated with haemolysis but insufficient hepatic bilirubin conjugating function. [13-16]

To the best of our knowledge there are no studies in our environment on the relationship of haemolysis and hyperbilirubinaemia in G6PD deficient neonates despite its high prevalence. This study was aimed at determining the role haemolysis plays in the pathogenesis of hyperbilirubinaemia among G6PD deficient neonates in our environment. This will enable managing physicians' tailor their management strategies appropriately and our finding can also serve as a template for advocacy for molecular characterization of G6PD enzyme variants in our environment.

Materials and Methods

Study design and setting

A cross-sectional study involving 150 icteric neonates admitted into the Special Care Baby Units of Jos University Teaching Hospital, Bingham University Teaching Hospital, and Plateau State Specialist Hospital, Jos between March 2013 and February 2014. The neonates were recruited using the non-probability convenience sampling technique, questionnaire was self administered to obtain relevant clinical information and the neonates were examined for fever, pallor, cyanosis, and cephaelhaematomas among other clinical features. Neonates whose parents did not consent, those with cephalhaematomas, bleeding tendencies, birth asphyxia and those recently transfused were excluded.

Laboratory Procedures

Five milliliters of venous blood was taken into an ethylene diamine tetra acetic acid (EDTA) and plain sample bottle for full blood count (FBC) using the 3-part Sysmex (KX-21N 2007 model) haematology auto-analyser, peripheral blood film morphologic analysis and reticulocyte count by manual methods as described by Dacie and Lewis. [17] Bilirubin assay was carried out by the Jendrassik and Grof method using the Roche/Hitachi 902 SN 1694-019-1996 auto analyzer while G6PD enzyme assay was carried out using reagents and control samples manufactured by the Pointe Reagent Company (USA). Red blood cell G6PD enzyme activity < 6.0 IU/gHb was considered deficient. [18]

Statistical Analysis

Information gathered was analyzed using Epi Info Version 6 software (Epi Info™, Atlanta, Georgia, USA). Continuous variables uniformly distributed were described using mean with standard deviation (SD), while the non uniformly distributed continuous variables were reported as median with interquartile (IQR) range and compared using the Kruskal Wallis test. Chi square test was used to compare categorical variables. P value <0.05 was considered statistically significant.

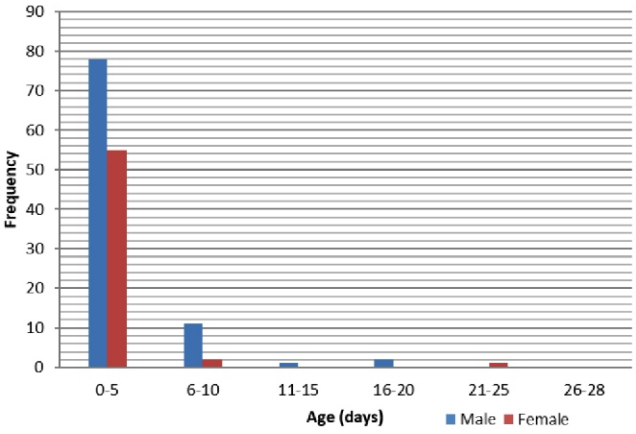
Ethical Consideration

Ethical approval was obtained from the Health Research Ethics Committees of Jos University Teaching Hospital, Bingham University Teaching Hospital, and Plateau State Specialist Hospital while written informed consent was obtained from parents/care givers of the participating neonates

Results

A total of 150 icteric neonates (92 males and 58 females) were studied. Median age at presentation was 3 (IQR: 1-4) days with 78 of the males and 55 of the females in the 0-5 days age group (Figure 1).

Figure 1: Age at presentation and sex distribution of study subjects



G6PD deficient icteric neonates from this study had a mean haemoglobin concentration of 16.32 ± 2.64 g/dl with a haematocrit of 0.47 ± 0.07 while the G6PD normal subjects had mean haemoglobin concentration and haematocrit of 15.61 ± 1.86 g/dl and 0.46 ± 0.05 respectively (Table 1). Mean values of both the haematocrit and haemoglobin concentration in relation to G6PD activity level showed no statistically significant difference (p value of 0.06 and 0.56 respectively). One hundred and thirteen (75.33%) neonates had normocytic normochromic red blood cell morphology, 65 (43.33%) of them were G6PD normal. Nucleated red blood cells were seen in 6 (9.8%) of the G6PD deficient icteric neonates. Median reticulocyte count for the G6PD deficient neonates was 2.4 (IQR: 2-3) % and 2.5(2-3) % for the G6PD normal neonates (Table 1). The difference was however not statistically significant (p value of 0.67)

Median total serum bilirubin of all the neonates was 204 (IQR: 168.25-255.50) with the median unconjugated serum bilirubin concentration of the G6PD deficient icteric neonates being 190.40 (IQR: 149.60-261.20), Table 1.

Table 1: Demographic and Haematologic parameters of study subjects and their G6PD status

Parameters	All Subjects n= 150	G6PD deficient n= 61	G6PD Normal n= 89	P value
Age: Median(IQR) In days	3(1-4)	2(1-4)	3(1-4)	0.52
Sex				
Male	92	45	47	0.01
Female	58	16	42	
G6PD activity level: Median(IQR) IU/gHb	7.19(4.56-10.72)	3.99(2.72-4.94)	9.44(7.57-13.63)	
Serum Bilirubin: Median(IQR) μmol/L				
Total Serum Bilirubin	204.0(168.25-255.5)	205.0(170.0-281.6)	200.0(168.0-244.0)	0.14
Unconjugated Bilirubin	184.5(144.5-233.17)	190.4(149.6-261.2)	179.48(144.5-223)	0.22
Haemoglobin Conc. g/dL	15.90±2.23	16.32±2.64	15.61±1.86	0.06
Haematocrit	0.47±0.06	0.47±0.07	0.46±0.00	0.56
PF RBC Morphology n (%)				
Normocytic	113(75.33)	48(78.7)	65(73)	0.79
Macrocytes	26(17.33)	9(14.8)	17(19.1)	
Nucleated RBC	13(8.67)	6(9.8)	7(7.9)	
Spherocytes	26(17.33)	26(42.6)	0(0.0)	
Bite cells	26(17.33)	26(42.6)	0(0.0)	
Polychromatic cells	34(22.67)	13(21.3)	21(23.6)	
Heinz Bodies	4(2.67)	4(6.6)	0(0.0)	
Reticulocyte Median(IQR) %	2.5(2-3)	2.4(2-3)	2.5(2-3)	0.67

G6PD: glucose-6-phosphate dehydrogenase; IQR: interquartile range; RBC: red blood cell

Discussion

The mean haemoglobin concentration, haematocrit, peripheral blood film finding and reticulocyte count in this study showed similar figures in G6PD deficient and G6PD normal icteric neonates. There was no statistically significant difference established in these parameters in relation to the G6PD status of the neonates in the presence of neonatal jaundice. These findings are similar to reports from other studies. [19,20]

Haemoglobin concentration and haematocrit were not found to be low in the G6PD deficient neonates in this study. This was supported by the fact that reticulocyte count known to increase in haemolysis was within normal ranges and there was no significant difference between the G6PD deficient and the G6PD normal neonates. Alternative factor in defective G6PD activity in the hepatocytes has been suggested as a cause of reduced hepatic conjugation of bilirubin and its excretion contributing to the development of hyperbilirubinaemia.[21-24] This appears to be the case in this study and it further supported the conclusion that jaundice rather than anaemia predominates when neonates are exposed to haemolytic agents. [24] Valaes *et al.* [24] achieved a reduction in serum bilirubin values in G6PD deficient neonates when he administered Phenobarbital, a potent liver enzyme inducer, to pregnant mothers with the intention of inducing uridine diphosphate glucuronosyl transferase (UDPGT) while Oluboyede *et al.* [25] reported reduced G6PD activity in liver tissue of G6PD deficient adults with resultant reduction in hepatic function. A more reasonable explanation for hyperbilirubinaemia in G6PD deficient neonates may be that G6PD deficiency is just one risk factor for neonatal jaundice and that in many neonates, other factors have to be present at the same time to cause significant hyperbilirubinaemia. [26] In contrast to the finding in our study, Slusher *et al.* [27] however found significantly lower haematocrit values at a median age of four days in hyper-bilirubinaemic G6PD deficient Nigerian neonates compared with control subjects. The G6PD deficient neonates with kernicterus in that study had lower haematocrit values than those without kernicterus and carboxyhaemo-globin (COHb) values were higher in the deficient neonates than the controls supporting the fact that haemolysis had occurred. However, the influence of other possible haemolytic causes of hyper-bilirubinaemia was not excluded in that study so also in our study.[27] Three case reports from Birmingham by Dhillon *et al.* [28] revealed episodes of massive acute haemolysis with anaemia in all three G6PD deficient patients in the absence of blood group incompatibilities, infection, or ingestion of oxidizing agents known to trigger haemolysis. These three cases supported the theory that massive haemolysis, although rare, does occur in neonates with G6PD deficiency and can present with severe anaemia and hyper-bilirubinaemia contrary

to the finding in our study.[28] The consequences of red cell enzymopathies are diverse and often dependent on the enzyme variant with some causing varying degrees of haemolysis and anaemia while others have no adverse effect. [29] G6PD B is the predominant variant encountered in all population and is not associated with haemolysis. [29] Similarly, G6PD A^+, a mutant enzyme variant prevalent among persons of African descent has normal catalytic activity and does not cause haemolysis. [29] There is therefore the possibility that the enzyme variants in the G6PD deficient neonates in this study are predominantly the variants not known to cause haemolysis. However, G6PD A^- variant, seen in 22% of Nigerians is associated with haemolysis and may cause kernicterus. [29] It is therefore, more likely that the jaundice in the G6PD deficient group in this study, occurred as a result of reduced activity of the G6PD enzyme in the liver leading to conjugation defect more so that unconjugated bilirubin account for greater proportion of the total serum bilirubin. The jaundice could also be from exaggerated physiologic jaundice and other causes of neonatal hyperbilirubinaemia known to occur in both term and preterm neonates. [30-32]

Conclusion

The findings in this study demonstrated that G6PD deficiency is a major cause of neonatal hyperbilirubinaemia that is more likely due to insufficient hepatic function rather than haemolysis as is also supported by higher concentration of unconjugated bilirubin.

Limitations

Inability to evaluate for common defect of bilirubin conjugation and determine the enzyme variants in our environment. Assay of lactate dehydrogenase, an indicator for haemolysis among other indicators would have also been supportive.

Recommendations

Medical practitioners should be alerted to the current ease of travel, migration of population groups, and intermarriage allowing a World Wide spread of other variants of the G6PD deficient gene, manifesting with variable laboratory findings with or without haemolysis. Determination of the predominant enzyme variants in our environment, assessment of neonatal hepatic function and screening for other risk factors including Gilbert syndrome is advocated.

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Conflict of interest

None declared

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