DETERMINANTS OF SEVERITY OF HYPERBILIRUBINAEMIA AMONG GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT NEONATES IN JOS NORTH CENTRAL NIGERIA

Jatau, E.D[1]., Damulak, O. D[1]., Toma, B.O[1]., Ma'an V. T[1]., Adeyemi O. A[1]., Egesie, O. J[1].
1. Department of Haematology and Blood Transfusion, Jos University Teaching Hospital,
2. Department of Paediatrics, Jos University Teaching Hospital

Corresponding Author
Email: ezradjatau@gmail.com, Jatauez@unijos.edu.ng
Phone: +2348065403375

ABSTRACT

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited disorder capable of causing severe neonatal hyperbilirubinaemia, kernicterus and death. Identifying such neonates and other factors that could aggravate their clinical states have definite place in managing them for favourable outcomes.

Materials and Methods: One hundred and fifty (150) icteric neonates admitted into the Special Care Baby Units of the Jos University Teaching Hospital, Plateau State Special Hospital and the Bingham University Teaching Hospital were recruited for this study. It was a cross sectional descriptive study conducted between March 2013 and February 2014. Parental consents were obtained and Clinical information was gathered using a questionnaire, weight were measured in grams while laboratory investigations that included Full Blood Count (FBC), Reticulocyte Count, Serum Bilirubin (SB) Assay and G6PD activity levels were carried out.

Results: Mean age of the studied neonates at presentation was 3.28 ± 3.11 days while mean age of detection of jaundice was 2.86 ± 1.67. One hundred and five (70%) were delivered at full-term gestation (>37weeks) while 45 (30%) were delivered preterm (<37 weeks) with twenty-nine (19.3%) having history of jaundice in siblings. Fifty (35.7%) had birth weight of less than 2500g while the birth weight of 10 (6.7%) were unknown. Sixty-one of these neonates (40.7 %) were G6PD deficient with mean total serum bilirubin of 205.01 ± 96.57µmol/L.

Conclusion: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common enzyme disorder among neonates presenting with hyperbilirubinaemia which can be aggravated by other factors.

Key words: Determinants, Hyperbilirubinaemia, Glucose-6-phosphate dehydrogenase deficiency, Neonates

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked disorder and a common cause of haemolytic anaemia with an estimated 400 million people affected worldwide. [1] G6PD is a cytoplasmic enzyme distributed in all cells for the purpose of catalyzing glucose-6-phosphate oxidation to 6-phosphogluconolactone required for regeneration of reduced glutathione which protects these cells against peroxide and other reactive oxygen species. [1] Triggers of oxidative stress on G6PD deficient red blood cells (RBC) like acute illnesses, infections, fava beans, naphthalene balls, antibiotics as well as some antimalarials commonly used in our environment are documented causes of haemolysis, anaemia and neonatal hyperbilirubinaemia, a major cause of morbidity and mortality in neonates [2]

The G6PD enzyme deficiency is characterized by markedly diminished activity in the range of 8-20 percent of the normal reference range of 65-150 percent activity. [2] Prevalence of G6PD deficiency are higher in persons of African, Asian, Semitic or Mediterranean descent. [2] In the United States of America, African-American males have a prevalence of about 10% in the general population and 22.5% among jaundiced neonates. [3] Among the Kurdish Jews, a prevalence rate as high as 70% was

reported, 5% among the Chinese and as low as 0.1% in Japanese. [3]Prevalence of G6PD deficiency in the general population in Nigeria range between 4-26% with a report of 20.5- 35.3% prevalence amongst jaundiced neonates. [3] A study in Jos, Nigeria reported a prevalence rate of 20% among males. [4]

More than 60% of full term neonates and 80% of preterm neonates develop clinical jaundice during the first week of life noted by yellowish eyes and skin. [5, 6] Factors implicated in the mechanism of jaundice in G6PD deficient neonates apart from haemolysis includes, slower liver enzyme system maturation for bilirubin conjugation and excretion, neonatal maturity, presence of infection, method of feeding among others. [5,6]

This study is aimed at establishing the presence of some factors associated with developing jaundice in G6PD deficient neonates in our environment and their contribution to the severity of hyperbilirubinaemia in these subjects for the purpose of preventing neonatal morbidity and mortality due to neonatal hyperbilirubinaemia.

Materials and Methods

This descriptive cross-sectional study was carried out at the Special Care Baby Units (SCBU) of Jos University Teaching Hospital, Bingham University Teaching Hospital, and Plateau State Specialist Hospital, Jos with ethical approval from the Health Research Ethics Committees of these institutions. All Jaundiced neonates admitted into the SCBUs with parental consent and satisfying all inclusion criteria were enrolled while those whose parents refused consent, recently transfused neonates or those with cephalhaematomas, bleeding tendencies and birth asphyxia were excluded. Questionnaires were administered and the subjects assessed clinically for fever, pallor, jaundice, congenital malformations and muscle tone among others. Five milliliters of venous blood was taken into an EDTA and plain sample bottle forfull blood count (FBC) using the 3-part Sysmex (KX-21N 2007 model) haematology autoanalyser, Reticulocyte count by manual methods as described by Dacie and Lewis, bilirubin assay by the Jendrassik and Grof method using the Roche/Hitachi 902 SN 1694-019-1996 auto analyzer and G6PD enzyme assay was carried out using reagents and control samples manufactured by the Pointe Reagent Company (USA).

Data Analysis

Data analysis was with Epi Info Version 6 software. Relationship between categorical variables was tested using Chi-square while Student t-test was used to assess the significance between means of two groups. The results were reported in tables, proportions, and percentages. P value ≤ 0.05 was considered statistically significant.

Results

One hundred and fifty icteric neonates made-up of 92 (61.3%) males and 58 (38.7%) females (M: F= 1.6:1) with a mean age at presentation of 3.28 ± 3.11 days were studied. The mean age of detection of jaundice was 2.86 ± 1.67, median of 2.00 days within a range of 1-12 days. One hundred and five (70%) were delivered at full-term gestation (>37weeks) while 45 (30%) were delivered preterm (<37 weeks). Twenty-nine (19.3%) had history of jaundice in siblings compared to 121 (80.7%) with no such history. Fifty (35.7%) had birth weight of less than 2500g while 90 (64.3%) were of normal birth weight. Birth weight of 10 (6.7%) of the study subjects was unknown because they were delivered at home. G6PD activity ranged from 0.54-24.18 IU/gHb with a mean activity of 8.02 ± 4.87 IU/gHb while 61(40.7%) of the icteric neonates were G6PD deficient. The mean total serum bilirubin of the 150 subjects was 205.01 ± 96.57µmol/L, mode of 184.50µmol/L in a range of 86.70-606.00µmol/L.

Table-1 Clinical parameters of all subjects and G6PD status

Parameters	G6PD deficient; n=61	G6PD normal; n=89	t	χ^2	P value
Age (days)	3.39 ± 3.05	3.20 ± 3.17	0.37		0.71
Sex					
Male	45	47			
Female	16	42		6.71	0.01
Duration of pregnancy (weeks)					
Full-term	45	60			
Pre-term	16	29		0.70	0.40
Age jaundice was noticed	3.08 ± 2.09	2.70 ± 1.29	1.35		0.56
History of jaundice in siblings	10	19		0.57	0.45
Weight at birth g; n (%)					
< 2500	16	34			
≥ 2500	41	49		2.45	0.12
Weight at presentation g; n (%)					
<2500	24	41			
≥2500	37	48		0.67	0.41

Twenty two (36.1%) of the G6PD deficient neonates had mild hyperbilirubinaemia with a mean total serum bilirubin of 132.3 ± 24.6 µmol/L while 40 (44.9%) of the G6PD normal neonates with mild hyperbilirubinaemia had a mean total serum bilirubin of 135.8 ± 28.9 µmol/L. Development of hyperbilirubinaemia based on G6PD status was not statistically significant with P values of 0.49, 0.78 and 1.10 for mild, moderate and severe hyperbilirubinaemia respectively. (Table 2)

Table 2: G6PD status and severity of hyperbilirubinaemia

Hyperbilirubinaemia	G6PD status							
	Deficient			Normal				
	n	%	mean TSB	n	%	mean TSB	t	P
Mild	22	36.1	132.3 ± 24.6	40	44.9	135.8 ± 28.9	0.70	0.49
Moderate	24	39.3	205.6 ± 26.6	36	40.4	203.7 ± 24.2	0.29	0.78
Severe	15	24.6	383.1 ± 110.1	13	14.6	338.9 ± 100.4	1.10	0.28
Total	61	100		89	100			

TSB= Total Serum Bilirubin in µmol/L

One hundred and five (70.0%) full term neonates involved in this study had a mean total serum bilirubin of 216.1 ± 106.7 µmol/L while 45(30.0%) preterm neonates had a mean serum bilirubin of 179.4 ± 59.8 µmol/L. Duration of pregnancy in relation to hyperbilirubinaemia showed significant statistical difference with a P value of <0.01 for moderate and severe hyperbilirubinaemia (Table 3).

Table 3: Duration of pregnancy and severity of hyperbilirubinaemia

Hyperbilirubinaemia	Duration of pregnancy							
	Full term			Preterm				
	n	%	mean TSB	n	%	mean TSB	t	P
Mild	23	51.1	135.5 ± 27.5	39	37.1	133.9 ± 27.5	0.27	0.82
Moderate	12	26.7	185.5 ± 9.7	48	45.7	209.2 ± 25.4	3.15	<0.01
Severe	10	22.2	272.8 ± 28.9	18	17.1	412.9 ± 100.3	4.27	<0.01
Total	45	100		105	100			

TSB= Total Serum Bilirubin in µmol/L

Jaundice was detected in 112 (74.7%) neonates recruited for this study within days 0-3 with 47 (42.0%) of them mildly hyperbilirubinaemic while 18 (16.0%) were severely hyperbilirubinaemic with a mean total serum bilirubin of 347.2 + 100.7 µmol/L. No statistically significant difference was established regarding the age at which jaundice was detected and severity of hyperbilirubinaemia (Table 4).

Table 4: Age of detection of jaundice and severity of hyperbilirubinaemia

Hyperbilirubinaemia	Age of detection of jaundice (days)										
	0-3			4-7			8-12				
	n	%	mean TSB	n	%	mean TSB	n	%	mean TSB	t	P
Mild	47	42.0	134.9 + 27.0	14	38.9	132.2 + 26.9	1	50.0	120.1 + 0.00	0.18	0.84
Moderate	47	42.0	206.8 ± 26.2	13	36.1	196.0 ± 18.5	0	0	0	1.40	0.17
Severe	18	16.0	347.2 + 100.7	9	25.0	373.5 +108.8	1	50.0	541.0 + 0.00	1.74	0.20
Total	112	100		36	100		2	100			

TSB= Total Serum Bilirubin in µmol/L.

Twenty nine (19.3%) had history of jaundice among siblings with 12 (19.4%) having mild hyperbilirubinaemia and a mean total serum bilirubin of 135.4 ± 27.8 µmol/L. This however showed no statistically significant difference when compared with those without history of jaundice in siblings (Table 5).

Table 5: History of jaundice among siblings and severity of hyperbilirubinaemia

Hyperbilirubinaemia	History of jaundice amongst siblings							
	Yes			No				
	n	%	mean TSB	n	%	mean TSB	t	P
Mild	12	19.4	135.4 = 26.1	50	80.6	134.3 ± 27.8	0.12	0.91
Moderate	13	21.7	208.9 ± 28.7	47	78.3	203.3 ± 24.1	0.71	0.48
Severe	4	14.3	362.5 + 81.1	24	85.7	362.6 + 111.2	0.00	1.00
Total	29	100		121	100			

TSB= Total Serum Bilirubin in µmol/L

Fifty (35.7%) had birth weight < 2500g (low birth weight) with twenty-Seven (54.0%) of them presenting with mild hyperbilirubinaemia with mean total serum bilirubin concentration of 136.2 ± 26.6 µmol/L. There was a statistically significant difference in severity of hyperbilirubinaemia with P value of 0.01 and <0.01 in moderate and severe hyperbilirubinaemia respectively (Table 6).

Table 6: Birth weight and severity of hyperbilirubinaemia

Hyperbilirubinaemia	Birth weight (g)							
	< 2500			≥ 2500				
	n	%	mean TSB	n	%	mean TSB	t	P
Mild	27	54.0	136.2 = 26.6	30	33.3	132.6 = 28.4	0.49	0.63
Moderate	13	26.0	187.5 ± 9.8	43	47.8	208.8 ± 26.9	2.86	0.01
Severe	10	20.0	286.2 ± 55.1	17	18.9	409.7 ± 105.9	3.41	<0.01
Total	50	100		90	100			

TSB= Total Serum Bilirubin in µmol/L

Discussion

Majority of the neonates in this study presented with jaundice within the first three days of life. Some studies done on cord blood have demonstrated that both non-haemolytic jaundice and jaundice attributed to haemolysis commence in-utero. [7, 8] In support of this theory, Kaplan *et al* demonstrated that G6PD deficient neonates had significantly higher serum bilirubin immediately after birth than those with normal G6PD activity. [8] He also reported a significantly higher serum bilirubin values evident on the third day probably reflecting the in-utero status. [8] Added to this is physiological jaundice attributed to neonatal immaturity known to appear between 24-72 hours of age, peaks at 4-5th day in term neonates and 7th day in preterm infants disappearing by the 10-14th day usually with serum bilirubin levels not exceeding 255 µmol/l. [8, 9] This may explain the early manifestation and presentation with jaundice in these neonates. Our finding fall short of statistical significance regarding age jaundice was detected, severity of hyperbilirubinaemia and G6PD status of the neonates but mean serum bilirubin concentrations were high. Other factors like sepsis, blood group incompatibilities and G6PD deficiency as demonstrated in our study are likely to have increased the serum bilirubin levels though not all risk factors for jaundice were assessed in this study.

Jaundice was detected in our subjects by most parents between days 0-3 after birth and our finding is supported by similar studies conducted locally and internationally. [10-12] The similarity of the age at presentation and when jaundice was detected in these neonates irrespective of their G6PD status, demonstrated the increased awareness most parents have on detecting jaundice early. Where parents lack the ability to recognize jaundice early, the resultant effect will be late presentation and delayed treatment of these G6PD deficient neonates increasing morbidity and mortality. Therefore, parents and care givers must be sensitized to have high index of suspicion for G6PD deficiency as a possible cause of neonatal jaundice in our environment especially with the variable triggers from baby care products to several medications. Development of jaundice early in life in most of our subjects and the fact that jaundice in the first 24 hours of life is often considered pathologic makes it imperative to rule out G6PD deficiency in neonates with jaundice at birth, especially in a high-risk population like ours. It also calls for the creation of a forum for follow-up of discharged neonates, for the purpose of assisting parents and relations detect jaundice early even after hospital discharge.

Preterm neonates in this study were found to have higher bilirubin concentration compared to full term neonates. This is attributed to the preterm babys inability to handle bilirubin load arising primarily from the immaturity of their conjugating enzyme systems. [10-12] The UDPGT activity of a term baby functions at 1% of adult values while that of a preterm at 34 weeks functions at less than 0.01%. [10-12] Intrauterine accumulation of meconium in the gut contains about 100-200mg of bilirubin per 100g of meconium at birth and 50% of this bilirubin is unconjugated. [10] Furthermore, preterm babys have immature enteric innervations that permit retention of this meconium within the gut giving rise to the full effect of enterohepatic circulation and thereby increasing the preterm babys bilirubin load. [10-12]

History of jaundice that required an intervention in siblings may help identify certain genetic factors contributing to development of hyperbilirubinaemia in certain families or population groups. G6PD deficiency is one of such familial conditions, however, no significant relationship was found between history of jaundice in siblings, G6PD status and severity of hyperbilirubinaemia. This finding is supported by a study in Singapore which also showed no significant relationship between G6PD deficiency and history of jaundice in siblings but agreed to the fact that the presence of additional genetic factors can increase the risk of developing severe hyperbilirubinaemia as a result of other gene interactions. [13, 14] Although, we did not establish a statistical relationship between history of jaundice in siblings, severity of hyperbilirubinaemia and G6PD status, it is important that this history is obtained to serve as a pointer to other risk factors for neonatal hyperbilirubinaemia so they can be monitored and managed early and appropriately.

Glucose-6-phosphate dehydrogenase status in relation to birth weight and severity of hyperbilirubinaemia did not demonstrate any statistical significance. Shah *et al* corroborated this finding by reporting that low birth weight is rather a risk factor for development of severe hyperbilirubinaemia as it is often exaggerated by inadequate breast milk intake known to cause breast feeding jaundice but has no effect on the

G6PD status of these individuals. [13] Few studies with inconsistent results have been carried out on G6PD deficiency in low birth weight neonates [13, 14] A research by Herz *et al* reported a contrasting finding of higher G6PD activity in neonates with low birth weight than their normal counterparts but the reason for this increase is still not clear. [15] Irrespective of the lack of relationship between weight and G6PD status in this study, weight should be monitored because its reduction may be associated with breast feeding jaundice that could further exacerbate hyperbilirubinaemia as demonstrated in this study. It is also important to identify those who are low birth weight because most of them are usually preterm babies who are more susceptible to developing bilirubin encephalopathy at lower serum bilirubin levels. [15]

Conclusion

Aetiology of hyperbilirubinaemia in neonates is multifactorial with the potential of interacting and a consequent severe hyperbilirubinaemia. Considering the deleterious effect it can have on neonatal outcome, factors with these potentials must be monitored and necessary action taking to avert neonatal morbidity and mortality.

Limitations

Several factors like sepsis, blood group incompatibilies and membranopathies can increase the severity of hyperbilirubinaemia in neonates and only few were considered this study.

Recommendations

Machinery for increased public awareness on the multifactorial causes, how to detect jaundice especially in neonates and its deleterious effect should be increased. Neonatal screening for sepsis, G6PD deficiency, red blood cell specific antigens and antibodies among many should also be instituted in our tertiary health care institutions with the aim of reducing morbidity and mortality among the neonatal age groups. Further studies' involving a larger population is recommended.

Acknowledgement

We remain grateful to the entire staff of the Special Care Baby Units, haematology and chemical pathology laboratories of the Jos University Teaching Hospital (JUTH), Bingham University Teaching Hospital (BhUTH), and the Plateau State Specialist Hospital (PSSH), Jos, for opening their doors. Our sincere appreciation also goes to Mr Pius, Managing Director of Trust Care Laboratory, Jos who provided a Spectrophotometer for the G6PD assay.

References

1. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: A historical perspective. Blood 2008; 111: 16-24.

2. Report of WHO Scientific Group Standardization of Procedures for the Study of Glucose-
6-phosphate dehydrogenase, WHO Tech Rep. Ser 366, 1966

3. Kaplan M, Hammerman C. Glucose-6-phosphate dehydrogenase deficiency: A Worldwide Potential Cause of Severe Neonatal Hyperbilirubinaemia. NeoReviews 2000; 1: 32-39.

4. Egesie OJ, Joseph DE, Isiguzoro I, Egesie UG. Glucose-6-phosphate dehydrogenase (G6PD) activity and deficiency in a population of Nigerian males resident in Jos. Niger J Physiol Sci 2008; 23: 9-11.

5. Ogunfowora OB, Daniel OJ. Neonatal jaundice and its management: Knowledge, Attitude, and Practice of Community health workers in Nigeria. BMC Public Health 2006; 6: 19-25.

6. Liaqat A, Liaqat A. Glucose-6-phosphate dehydrogenase deficiency in Jaundiced Neonates. J P M I 2010; 24: 122-126.

7. Tsuzuki S, Akahira-Azuma M, Kaneshige M, Shoya K, Hosokawa S, Kanno H, et al. A Japanese neonatal case of Glucose-6-Phosphate Dehydrogenase Deficiency presenting as Severe Jaundice and Haemolytic Anaemia with Apparent Trigger. SpringerPlus 2013; 2: 434-436.

8. Kaplan M, Algur N, Hammerman Cathy. Onset of Jaundice in Glucose-6-Phosphate Dehydrogenase Deficient Neonates. Pediatrics 2001; 108: 956-959.

9. Jarullah J, Aljaouni S, Sharma MC, Bushra MSJ, Kamal MA. Detection of Glucose-6-phosphate dehydrogenase deficiency in Heterozygous Saudi Female Neonates. EnzEng 2012; 1: 105-107.

10. Mohrenweiser HW, Fielek S, Wurzinger KH. Characteristics of enzymes of erythrocytes from newborn infants and adults: activity, thermo-stability, and electrophoretic profile as a function of cell

age. Am J Hematol 1981; 11: 125-136.

11. Jallo S, Rostenberghe HV, Yusoff NM, Ghazali S, Ismail NZ, Matsuo M, *et al*. Poor correlation between haemolysis and neonatal jaundice in Malaysian Glucose-6-phosphate dehydrogenase deficient babies. PaediatrInt 2005; 47: 1-4.

12. Kaplan M, Herschel M, Hammerman C, Hoyer J D, Stevenson DK. Hyperbilirubinaemia among African American, Glucose-6-phosphate dehydrogenase deficient neonates. Pediatrics 2004; 114: 213-219.

13. Najati N, Gharebaghi MM, Mortazavi F. Underlying Etiologies of Prolonged Icterus in Neonates. Pak J BiolSci 2010; 13: 711-714.

14. Shah VA, Yeo CL. Identifying Risk of Neonatal Hyperbilirubinaemia and Early Discharge for Glucose-6-Phosphate Dehydrogenase Deficient Newborns in Singapore. Ann Acad Med Singapore 2007; 36: 1003-1009.

15. Herz F, Kaplan E, Scheye ES. A Longitudinal Study of Red Cell Enzymes in Infants of Low Birth Weight. Z Kinderheilk 1975; 88: 328-404.