GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN JAUNDICED NEONATES

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ABSTRACT

Objectives: *To detect the frequency of G-6-PD deficiency in 200 consecutive neonates admitted with jaundice to the neonatal unit, LRH, Peshawar.*

Material and Methods: *This descriptive study was conducted in the Neonatal Unit of Lady Reading Hospital, Peshawar from March 2009 to August 2009. Two hundred consecutive cases of neonatal jaundice of both sexes, admitted to the Neonatal Unit, Lady Reading Hospital, Peshawar were enrolled in the study. Detailed history and clinical examination was recorded. All the neonates were subjected to be estimation of Serum Bilirubin levels (Total, Direct and Indirect), G-6-PD detection via Hemolysate Decolorization time test and blood groups of both the mother and the baby besides looking at the peripheral smears and other relevant investigations.*

Results: *Out of the 200 icteric neonates, 145 (72.5%) were males while 55 (27.5%) were females. Thirty two (16%) babies were found to be G-6-PD deficient. No specific precipitating factor for haemolysis was found. Majority of the patients 188 (94%) presented with neonatal jaundice in the first week of life, while the age of presentation amongst the G-6-PD deficient neonates was between 2nd to 4th day of life. An Indirect serum bilirubin level of 9 to 40mg% was found in these G-6-PD deficient neonates.*

Conclusion: *G-6-PD deficiency is a common cause of neonatal jaundice and has more preponderance for male sex. Babies suffering from G-6-PD deficiency present with jaundice relatively earlier than the other causes of neonatal jaundice like ABO/Rh incompatibility and idiopathic causes.*

Key words: *Neonatal Jaundice (NNJ), Glucose 6 Phosphate dehydrogenase (G6PD) enzyme deficiency, Hyperbilirubinemia, Total serum bilirubin (TSB)*

INTRODUCTION

More than 60% of otherwise healthy newborns develop clinical jaundice associated with increased concentration of total serum bilirubin (TSB) during the 1st week of life. Hyperbilirubinaemia typically resolves by 7-10 days of age and the outcome is usually benign, this is termed as physiological jaundice. However, severe hyperbilirubinemia, defined as total bilirubin (TSB) above the 95th percentile for age in hours (high risk zone), occurs in 8-9% of infants during the first week, with approximately 4% affected after 72 hours[1, 2].

Without appropriate intervention, progressive increase in total serum bilirubin (TSB) to values > 25 or 30mg/dl (greater than 99th percentile for age in hours) places otherwise healthy babies at risk of bilirubin – related brain damage (Kernictrus)[2].

Unconjugated bilirubin is fat soluble; it crosses cell membranes and is potentially neurotoxic. However, toxicity is generally avoided because most un-conjugated bilirubin is bound to albumin. Hyperbilirubinaemia develops when rate of bilirubin production via the breakdown of heme by the reticuloendothelial system exceeds the rate of elimination, primarily by conjugation. Various genetic, environmental and racial factors affect the equilibrium between the processes of production and elimination[3].

The etiology in the majority of neonatal jaundice include: a) - Hemolytic disorders (rhesus disease ABO isoimmunization, minor blood group incompatibilities, intrinsic red blood cell defect etc). b) - G6PD deficiency (associated with both hemolysis and impaired bilirubin conjugation). c) - Idiopathic causes (presumably from delayed or impaired function of glucoronyl transferase

enzyme system), coupled with, d) - Breast feeding and e) - Inadequate nutritional intake.

G6PD is a central enzyme in the hexose monophosphate shunt of glucose metabolism and catalyzes the conversion of Glucose-6-phosphate to 6 phosphogluconic acid. Reduced from of nicotinamide adenine dinucleotide phosphate (NADPH) is produced in this process and is necessary to maintain glutathione in the reduced state. Glutathione is present in the red cells and acts to neutralize agents that potentially oxidize either haemoglobin or components of the red cell membrane. Without sustaining reduced glutathione to remove oxygen radicals generated by oxidant drugs, the hemoglobin precipitates forming Heinz bodies, and the red cell membrane is critically damaged, leading to haemolysis[4].

Synthesis of Red Blood Cell G6PD is determined by a gene on the x-chromosome. G6PD deficiency, therefore, occur more frequently in males than in females[5]. G6PD deficiency is responsible for a major proportion of neonates suffering from severe neonatal jaundice. About 12.8% of African – American males[6] and much higher percentage of Pakistani[7] males have G6PD deficiency. In this study we wanted to see the occurrence of G6PD deficiency in the jaundiced neonates admitted to Nursery, Lady Reading Hospital Peshawar.

MATERIAL AND METHOD

This study was carried out at the Neonatal Unit, Postgraduate Medical Institute, Lady Reading Hospital, Peshawar, from March 2009 to August 2009. All jaundiced neonates who were full term and have Serum Indirect Bilirubin level of more than 9mg% were included in the study while Premature jaundiced neonates, those with neonatal sepsis and neonates with direct hyperbilirubinaemia were excluded from the study.

Total of 200 consecutive admitted jaundiced neonates, aging 1st day of life to 30 days were studied. Detailed history, examination and all the required investigations e.g. serum bilirubin (Total, Direct and Indirect), Haemoglobin, Peripheral smear, Reticulocyte count, Blood grouping (both mothers and neonates) and especially G-6-PD decolorization time were done in the main laboratory of Lady Reading Hospital, Peshawar. G6PD decolorization time was estimated by using "Sigma diagnostic G6PD regent" for the semi-quantitative, visual, calorimetric determination of G6PD deficiency in red cells procedure of the test.

Procedure of the Test

1. Red cell hemolysate is prepared by adding 2.5ml of water to 0.05ml of EDTA mixed blood. It is shaken thoroughly and than allowed to stand for about 5 minutes.
2. 0.5ml of buffer solution is mixed with powder dye and 1 ml of red cell hemolysate is mixed with it.
3. This mixture is layered with 2ml of mineral oil.
4. The tube is than placed in water bath at 37C and observed for a change in colour (from its original deep blue to a maroon or reddish endpoint) at 15 minutes intervals.

Interpretation of test result

Decolorization of time 20-60 minutes – Normal

Decolorization time more than 60 minutes – Deficient

Decisions regarding the treatment were made on the basis of serum Indirect bilirubin level and age of the babies.

RESULTS

A total of 856 patients were admitted in the Neonatal Unit of Lady Reading Hospital, Peshawar from March 2009 to August 2009.

These patients included 200 neonates admitted with neonatal jaundice (23.36%). Out of the 200 admitted jaundiced neonates 32 (16%) were G6PD deficient (Table 1)

Table 2: Break-up of the patients admitted to Nursery, LRH

Total number of patients	n = 856
NNJ amongst the total admission	200 (23.36%)
G6PD deficient amongst NNJ	32 (16%)

Of the total 200 cases of neonatal jaundiced studied 145 were males and 55 females (Table 2). Age ranged from 1 day to 6 day with mean age 3 days. Majority of the neonates having G6PD deficiency presented with jaundice between 2-4 days of birth (Table 3).

Table 2: Sex-wise distribution of the total NNJ neonates included in the study (n=200)

Sex	Number of patients	Percentage
Males	145	72.5%
Females	55	27.5%
Total number of patients	200	100%

Table 3: Age of presentation with NNJ in G6PD deficient neonates (n=32)

Age at presentation	Number of cases	Percentage
Upto 24 hours	2	6.25%
2nd day to 4th day of age	24	75%
After 4th day of life	6	18.75%
Total	32	100%

The etiologies of hyperbilirubinaemia in these cases are shown in Table 4, including G6PD deficiency (16%). Serum Indirect Bilirubin level range from 10mg % to 40mg %. Seventeen babies developed severe hyperbilirubinaemia (Serum bilirubin level of > 20mg %) and most of them were given exchange transfusion (Table 5). Red Blood Cells G6PD enzyme activity ranged from more than 60 minutes to 2 hours (Table 6). All G6PD deficient jaundice neonates received phototherapy. The duration of phototherapy was from 2 – 5 days. Total 17 exchange blood transfusions were performed in 14 babies. Two babies required more than one exchange transfusion (Table 7) while the remaining 3 babies with severe jaundice responded well to phototherapy and did not require blood transfusion.

Table 4: Etiology of the hyperbilirubinaemia in studied cases showing occurrence of G6PD deficiency.

Total number of admitted patients	N = 200
Number of patients with G6PD deficiency	32 (16%)
Number of patients with ABO incompatibility	65 (32.4%)
Number of patients with Rhesus disease	57 (28.6%)
Number of patients with idiopathic etiology	46 (23%)

Table 5: Indirect serum Bilirubin level (SBR) in G6PD deficient babies (n=32)

Grade	SBR (ID)	No of patients	%age
Mild to moderate	< 20 mg%	15	46.8%
Severe	> 20mg%	17	53.2%

Table 6: Red Blood Cells G6PD activity in G6PD deficient jaundiced babies (n=32)

G6PD decolorization time (minutes)	No of patients	Percentage
60-90	26	81.25%
> 120	6	18.75%

Table 7: Treatment given to G6PD deficient jaundiced babies (n=32)

Treatment modality	No of patients	Percentage
Phototherapy	32	100%
Exchange transfusion	14	43.75%

Two babies (6.2% developed Kernictrus). Average hospital stay was 4 days. No significant history of drug ingestion was noticed in the mothers. Five babies (15.6%) had received Vitamin-K injections.

DISCUSSION

Results of this study show that neonatal jaundice makes about 1/4th of the total admissions to Nursery Unit (23.36 %). This fact is supported by other international and local studies[8-10] which is a true reflection of the importance of neonatal jaundice as a cause of neonatal morbidity.

Of the total cases studied 72.5% were males and only 27.5% were females. This male predominance may be attributed to the G6PD deficiency, as it frequently occurs in males than in females[5, 11-13].

Majority of the neonates with G6PD deficiency in our study presented with neonatal jaundice between 2nd and 4th day of life. This is supported by other similar studies conducted locally and internationally [15-17].

The causes of hyperbilirubinaemia that needed admission were attributed to three categories in nearly equal proportions: hemolytic disorders (Rhesus and ABO isoimmunization), G6PD deficiency and idiopathic causes. This is in conformity with results of other studies[3,10,18].

The frequency of G6PD deficiency in this study was 16%. This figure correlates with other local studies, like Rehman G et al[12] observed 14% incidence, Khan A et al[19] 13%, Imran et al [20] 12% and with that of Parveen et al[21] who reported an incidence of 12.1%. The results are, however, in contrast with another local study by Rehman H et al[22], which showed 8.2%.

This is also a relatively a high occurrence rate as compared to studies from India[11] (7.5%) Saudi Arabia[13] (2%) and Tehran[23] (2.1%). On the other hand, this frequency of G6PD deficiency in the jaundiced neonates is quite lower than the frequency reported from Thailand[24] (25.5%), China[25] (18.42%) and Nigeria[15] (38%). These variations may be due to demographic difference in the genetic make-up of societies. Socio-cultural differences, frequency of carrier individuals, sample size, method used for G6PD enzyme estimation and detection rate.

Both jaundiced neonates and their mothers had no significant history of drug ingestion. Injection Vitamin K was given only to about 15% of the babies. This finding supports the experience and observations of other workers, as hemolysis in G6PD deficient newborns is spontaneous, without significant drug exposure. Moreover, in some

G6PD deficient population groups, carboxy – haemoglobin studies have indicated exaggerated hemolysis but in others, increased hemolysis has no correlation with total serum bilirubin level. As hyperbilirubinaemia results from an imbalance between bilirubin production and its elimination, diminished bilirubin conjugation was suspected as a contributory factor in the pathogenesis of hyperbilirubinaemia. Serum conjugated bilirubin fraction, reflecting intra-hepatic bilirubin conjugation, are low in G6PD deficient neonates[26].

Phototherapy was given to all the babies and was found very effective in reducing the serum bilirubin levels. In our study 3 babies with severe hyperbilirubinaemia did not require exchange transfusion as they responded well to phototherapy. Total 17 exchange blood transfusions were performed in 14 babies. This procedure was highly effective in reversing the indirect hyperbilirubinemia.

CONCLUSION

G-6-PD deficiency is a common cause of neonatal jaundice and screening for this condition should be included in the schedule of investigations for neonatal jaundice. G-6-PD deficient babies present with jaundice relatively earlier than other causes of jaundice like ABO/Rh incompatibility and idiopathic causes. Hyperbilirubinaemia in these patients is indirect and may lead to bilirubin encephalopathy. Phototherapy and exchange blood transfusion are effective treatment for these patients.

REFERENCES

1. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of pre-discharge hour-specific bilirubin for subsequent significant hyperbilirubinaemia in healthy term and near term newborns. Pediatrics 1999;103:6-14.
2. Stevenson DK, Fanaroff A, Maisels J, Young BW, Wong RJ, Vreman HJ, et al. Prediction of hyperbilirubinaemia in term and near term newborn infants. Pediatrics 2001;108:31-9.
3. Smitherman H, Stark AR, Bhutani VK. Early recognition of neonatal hyperbilirubinaemia and its emergent management. Semin Fetal Neonatal Med 2006;11:221-4.
4. Martinov MV, Plotnikov AG, Vitvitsky VM, Ataullakhanov FI. Deficiencies of glycolytic enzymes as a possible cause of hemolytic anaemia. Biochim Biopsys Acta 2000;1474:75-87.
5. Kaplan M, Beutler E, Vreman HJ, Hammerman C, Levy-Lahad E, Renbaum P, et al. Neonatal Hyperbilirubinaemia in glucose 6 phosphate dehydrogenase deficiency heterozygotes. Pediatrics 1999;104:68-74.
6. Kaplan M, Hereshel M, Hammerman C, Hoyer JD, Stevenson DK. Hyperbilirubinaemia among African American, G6PD deficient neonates. Pediatrics 2004;114:213-9.
7. Saha N, Ramzan M, Tay JS, Low PS, Basair JB, Khan FM. Molecular characterization of red cell G6PD deficiency in North West Pakistan. Hum Hered 1994;44:85-9.
8. Gvaran RL, Drew JH, Watkins AM. Jaundice – clinical practice in 88,000 live born infants. Aust N Z J Obstet Gynaecol 1992;32:186-92
9. Dzinovic A, Heljic S, Maksic H. Neonatal hyperbilirubinaemia evaluation and treatment. Med Arh 2002;56:44-5.
10. Hussain M, Gandapur AJ, Ahmad A, Hussain I. Indications of exchange transfusion in neonatal hyperbilirubinaemia at neonatal unit, Khyber Teaching Hospital, Peshawar: a retrospective cross sectional study. J Med Sci 2005;13:70-2
11. Iranpour R, Akbar Mr, Haghshenas I. Glucose-6-phosphate dehydrogenase deficiency in neonates. Indian J Pediatr 2003;70:855-7.
12. Rehman G. Shah SU, Khan K, Zia ullah, Talat A. Erythrocyte glucose –6- phosphate dehydrogenase deficiency: a cause of neonatal jaundice. J Postgrad Med Inst 2004;18:70-5.
13. Muzaffer MA. Neonatal screening of glucose-6-phosphate dehydrogenase in Yanbu, Saudi Arabia. J Med Screen 2005;12:170-1.
14. Hamid MH, Chishti AL, Mumtaz A, Hussain S, Maqbool S. Bilirubin estimation in neonatal jaundice: comparative study between autoanalyzer (Diazo-method) and Bilirubinometer (Direct photometric method). Pak Pediatr J 2003;27:68-73.
15. UKO EK, Agwunobi SN, Udoh JJ. Glucose 6 phosphate dehydrogenase level in jaundiced neonates in Calabar. Niger J Med 2003;12:98-102.
16. Ding G, Zhang S, Yao D, Na Q, Wang H, Li L, et al. An epidemiological survey on neonatal jaundice in China. Chin Med J (Engl) 2001;114:344-7.
17. Kaplan M, Algur N, Hammerman C. Onset of jaundice in G6PD deficient neonates. Pediatrics 2001;108:956-9.
18. Bhutani VK, Johnson LH, Shaprio SM. Kernicterus in sick and preterm infants (1999-2002): a need for an effective preventive approach. Semin Perinatal 2004;28:3129-5.

19. Khan A, Khawar N. Neonatal hyperbilirubinaemia secondary to erythrocyte glucose – 6 – phosphate dehydrogenase deficiency. J Postgrad Med Inst 2002;16:33-8.

20. Omran M, Rashid, Akbar A. Neonatal jaundice due to G6PD deficiency. Pak Pediatr J 1984:8:126.

21. Parveen A, Azra A, Ahman K. G6PD status and neonatal hyperbilirubinemai. Pak Pediatr J 1986;10:241.

22. Rehman H, Khan MA, Hameed A, Roghani MT, Ahmed A. Erythrocyte G6PD deficiency and neonatal jaundice. J Pak Med Assoc 1995;45:269.

23. Abolghasemi H, Mehrani H, Amid A. An update on the prevalence of glucose 6 phosphate dehydrogenase deficiency and neonatal jaundice in Tehran neonates. Clin Biochem 2004;37:241-4.

24. Thaithumyanon P, Visutiratinance C. Double phototherapy in jaundiced term infants with hemolysis. J Med Assoc Thai 2002;85:1176-81.

25. Weng YH, Chov YH, Lien RI. Hyperbilirubinemia in healthy neonates with glucose-6-phosphate dehydrogenase deficiency. Early Hum Dev 2003;71:129-36.

26. Kaplan M, Rehman P, Levy-Lahad E, Hammerman C, Lahad A. Beutler E. Gilbert syndrome and Glucose-6-Phosphage dehydrogenase deficiency a dose dependent genetic interaction crucial to neonatal hyperbilirubinemia. Proc Natl Acad Sci USA 1997;94:12128-32.

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