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STUDY FROM EAST INDIA ON NEONATAL HYPERBILIRUBINEMIA AND G6PD DEFICIENCY.

Neonatology

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ABSTRACT

Background- G6PD deficiency is one of the important causes of pathological jaundice in neonates. Undetected neonates can land up with increased morbidity (kernicterus, exchange transfusion etc) and mortality. Universal Screening in neonates for G6PD deficiency is still not established in India.

Aims and objectives-

a) Ascertain different causes of neonatal jaundice in our institution requiring therapeutic interventions.
b) Estimating the prevalence of Glucose-6-phosphate dehydrogenase deficiency in neonatal jaundice patients of West Bengal, India.
c) To find the relation between Glucose-6-phosphate dehydrogenase level and degree of jaundice in the patients.
d) Determining the importance of routine neonatal screening for G6PD in newborns born in this geographical area.

Patient and method- A cross-sectional study was done on 242 neonates admitted with hyperbilirubinemia (bilirubin >15mg/dl) in baby nursery , Department of Pediatrics, R.G.Kar Medical College & hospital Kolkata, from December 2010 to October 2011 . Only the neonates with congenital abnormalities were excluded from the study.

Different parameters that were studied included:

i. Detailed history.
ii. Physical examination.
iii. Complete hemogram.
iv. C reactive protein.
v. Total and conjugated bilirubin.
vi. Direct Coomb's test
vii. ABO and Rh group of mother and baby.
viii. Glucose-6-phosphate Dehydrogenase quantative assay.

Results- ABO incompatibility was found to be the most common cause of neonatal hyperbilirubinemia. G6PD deficiency was found in 6.61% (16 out of 242 neonates studied). The sex distribution was male 10(81.25%) and female 6 (18.75%). G6PD deficiency was found to be of mild degree(3.84-6.4U/gm Hb) in 37.5% of cases, moderate(0.64-3.84U/gm Hb) in 62.5% cases whereas no case was found to be having severe degree(<0.64U/gmHb) of G6PD deficiency. Total serum bilirubin level in G6PD deficient group was found to be significantly higher ($p<0.05$) than blood group incompatibility group or other causes of pathological jaundice combined. Requirement of Exchange transfusion as well as prolonged Phototherapy was significantly higher (p value<0.05) in G6PD deficient neonates than due to any other causes. Neither Sex nor any clinical nor any routine biochemical parameters were found to be of any predictive value for detection of G6PD deficiency in neonates. G6pd level of neonates with gestational age 30-32 weeks were found to be significantly higher than neonates with gestational age 38-40weeks and 40-42weeks (pvalue <0.05)

Conclusion and recommendations- G6PD is one of the leading cause of Hyperbilirubinemia (Bilirubin>15mg/dl) in neonates. They usually present with very high bilirubin level requiring exchange transfusion or prolonged phototherapy or both adding to their morbidity and mortality. No clinical or routine biochemical predictors are available to detect G6PD deficiecy. Preterm babies < 32 weeks gestation may show higher G6PD level initially after birth thus level may be repeated later if suspicion of deficiency is there.

G6PD level screening is available in every laboratory. As undetected G6PD deficiency carries a high morbidity- mortality and thus medicolegal implications. This study recommends routine newborn screening for G6PD deficiency in every neonatal units in India. We have started routine screening in our unit and requirement of exchange transfusion in our unit is on the decrease.

KEYWORDS

neonatal hyperbilirubinemia,G6pd deficiency, neonatal screening

INTRODUCTION:

Jaundice is the most common condition that requires medical attention in newborns. Most of them are physiological and requires no intervention. Only monitoring is required in most of them.

Common causes of pathological jaundice are Rh incompatibility, ABO incompatibility, Neonatal sepsis, Cephalohematoma or other birth injuries and Polycythemia.

Among the remaining cases in which no definitive diagnosis can be made with the help of routine first line routine laboratory investigations (blood group, direct comb's test, septic screen and complete hemogram) Glucose-6-phosphate Dehydrogenase deficiency is one of the commonest. Followed by other conditions like Hypothyroidism, pyruvate kinase deficiency, breastmilk jaundice, biliary atresia etc.

Gucose-6-phosphate dehydrogenase deficiency is the most common enzyme deficiency involving more than 400 million people worldwide[1]. G6PD deficiency was first reported in India as early as in 1961. The exact incidence in India is not known, however several studies has reported incidence as high as 0-27.9 [2] There is no established routine newborn screening protocol established in major parts of the country.

AIMS AND OBJECTIVES:

a. Ascertain different causes of neonatal hyperbilirubinemia in our institution requiring therapeutic interventions.
b. Estimating the prevalence of Glucose-6-phosphate dehydrogenase deficiency in neonatal hyperbilirubinemia patients of West Bengal, India.
c. To find the relation between Glucose-6-phosphate dehydrogenase level and degree of hyperbilirubinemia in the patients.
d. Determining the importance of routine neonatal screening for G6PD in newborns born in this geographical area.

MATERIALS AND METHOD:

A cross-sectional study was done on all neonates admitted with hyperbilirubinemia (bilirubin >15mg/dl) in baby nursery , Department of Pediatrics, R.G.Kar Medical College & hospital Kolkata, from December 2010 to October 2011 . Only the neonates with congenital abnormalities were excluded from the study.

Different parameters that were studied included:

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viii. Glucose-6-phosphate Dehydrogenase quantative assay.

The parents of the jaundiced neonates admitted in nursery were informed about the study and consent were taken from them to include the neonate in the study. After proper history taking and physical examination blood drawn from the jaundiced neonates were sent for complete hemogram, liver function test, direct coombs test, blood grouping of mother and baby and quantitive assay of Glucose-6-phosphate dehydrogenase. All the data obtained were used to find correlation between them. The diagnosis with which those jaundiced neonates discharged were noted.

All the data collected during the given time period were tabulated in a Microsoft Excel 2007 sheet. The data were compiled and statistical analysis was done using SPSS 17 and EPI info 7 softwares. All the charts were prepared using Microsoft Excel.

RESULTS:

ABO incompatibility was the most common cause of neonatal hyperbilirubinemia (bilirubin level>15mg/ dl) in the study. G6PD deficiency was found in 6.61% of cases (Table 1).

Table 1 Different causes of neonatal hyperbilirubinemia in the study population

Cause of hyperbilirubinemia	Number of cases	Percentage of total
ABO incompatibility	75	30.99%
Physiological jaundice	60	24.79%
G6PD Deficiency	16	6.61%
Sepsis	9	3.71%
Polycythemia	6	2.47%
Baby of diabetic mother	5	2.06%
Rh incompatibility	3	1.23%
Intrauterine infection	3	1.23%
Congenital haemolytic anemia	3	1.23%
Cephalohematoma + hemorrhage	2	0.82%
Unknown	60	24.79%
Total	242	100%

G6PD deficiency was found to be of mild degree in37.5% cases, moderate in 62.5% cases whereas no case was found to be having severe degree of G6PD deficiency (Table no. 2)

Table 2. Distribution according to degree of severity of G6PD deficiency

Degree of severity	Number	Percentage
Severe (<10% of normal i.e.0.64U/gm Hb)	0	0.0
Moderate (10-60% of normal i.e. 0.64-3.84 U/gm Hb)	10	62.5
Mild (60-100% of normal i.e. 3.84- 6.4 U/gm Hb)	6	37.5

One Way ANOVA and Post HOC analysis revealed Total serum bilirubin level at presentation in G6PD deficient group is significantly higher ($p<0.05$) than blood group incompatibility group or other causes of pathological jaundice combined (Table. 3)

Table 3. level of bilirubin in different causes of pathological jaundice

Cause of hyperbilirubinemia	Mean (mg/dl)	Standard deviation (mg/dl)
G6PD deficiency	21.05	4.20
Blood group incompatibility (ABO, Rh)	18.42	4.37
Other causes	18.00	4.28

Requirement of Exchange transfusion as well as prolonged phototherapy was significantly higher (p value<0.05) in G6PD deficient neonates than due to any other causes (Table 4).

Table 4. Requirement of exchange transfusion or phototherapy in the study population.

Diagnosis	Exchange transfusion			Prolonged Phototherapy (>3 days)		
	Yes(%)	No(%)	total	Yes (%)	No (%)	total
ABo+Rh	15(19.23)	63(80.77)	78	47(60.25)	31(39.75)	78
Total	43(17.76)	199(82.23)	242	131(54.14)	111(45.86)	242
G6PD def	10(62.50)	6 (37.5)	16	15(93.75)	1(6.25)	16
Others	18(12.16)	130(87.84)	148	69(46.62)	79(53.38)	148

No significant correlation was found between any clinical as well as routine laboratory parameters between G6PD deficient and G6PD normal group except total peak serum bilirubin (Table 5). Thus no clinical or routine biochemical parameter are useful for screening of G6PD deficiency in neonates (Table 5)

Table 5. Clinical and laboratory findings in G6PD deficient versus G6PD normal cases.

Parameters		G6PD deficient neonates (n=16)		G6PD normal neonates (n=226)		P value
		number	%	number	%	
Sex	Male	13	81.25	134	59.29	X2=3.02 p=0.0821
	Female	3	18.75	92	40.70	
Gestational age	Preterm	3	18.75	86	38.05	X2=2.39 p=0.121
	Term	13	81.25	140	61.95	
Religion	Hindu	10	62.5	141	62.66	X2=.000 p=0.98
	Muslim	6	37.5	84	37.34	
Birth weight (kgs)		Mean= 2.6 SD= 0.679		Mean= 2.292 SD= 0.684		t=1.749 p=.082
Total peak serum bilirubin (mg/dl)		Mean= 21.05 SD= 5.195		Mean= 16.233 SD= 5.209		t=3.673 p=0.000
unconjugated bilirubin (mg/dl)		M=2.49 SD=.944		M=2.56 SD=2.112		t=.144 p=.885
Haemoglobin (mg/dl)		Mean= 15.62 SD= 2.816		Mean= 16.136 SD= 3.021		t=.663 p=.508

ONE WAY ANOVA was performed for comparison of G6PD level between different gestational age groups. No statistical significance was found($F=1.47, df=6, p=0.209$) .POST HOC analysis showed that the G6pd level of neonates with gestational age 30-32 weeks were significantly higher than neonates with gestational age 38-40weeks and 40-42weeks (pvalue <0.05) (Table 6)

Table 6. Level of G6PD in different gestational age group

Group	Gestational age	G6PD level Mean (U/gm Hb)	Standard deviation (U/gm Hb)
1	30-32	21.200	5.367
2	32-34	18.993	5.240
3	34-36	17.075	5.014
4	36-38	17.075	5.876
5	38-40	16.505	5.641
6	40-42	16.215	6.800
7	≥42	18.733	5.404

DISCUSSION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, a hereditary predisposition to hemolysis, is the most common of all clinically significant enzyme defects in the whole of human biology. It is estimated to affect approximately 400 million people worldwide [1]with the highest prevalence rates in tropical Africa, the Middle East, tropical and subtropical Asia, some parts of the Mediterranean and in Papua New Guinea. Several countries in Europe, South East Asia, the Middle East and the United States of America have successfully established a neonatal screening programme for this disorder. With almost 24 million children born annually in India, it is estimated that at least 390,000 children suffering from this disorder are born in India every year[4]. Several agents have been identified as triggers for hemolysis- viral and bacterial infections being the most common. Certain drugs and chemicals have been implicated as hemolytic triggers[5,6]. Hemolysis may also be triggered by ingestion of fava beans (*Vicia faba*) or even inhalation of its pollen[7]. Early detection and prevention of haemolytic episodes (by avoiding the triggers) is possible in this disorder. Hence, a neonatal screening program for G6PD deficiency in India is warranted with the increased availability of funds for the health sector.

Acute hemolytic anemia, neonatal jaundice and chronic non spherocytic hemolytic anemia are the major clinical manifestations associated with G6PD
deficiency.

One of the common risk factors for pathologic hyperbilirubinemia in newborn infants is deficiency of G6PD enzyme[8]. Deficiency of this

enzyme is the most prevalent enzymopathy in red blood cells that causes hemolysis and hyperbilirubinemia[9]. Hyperbilirubinemia can be very severe in G6PDdeficiency and induces permanent damage to the brain causing kernicterus and death[9]. Overproduction of unconjugated bilirubin and lack of proper management of hyperbilirubinemia cause changes in the mitochondria of the basal ganglia and leads to impaired mitochondrial respiration, and also induce apoptosis, and cause bilirubin encephalopathy[10]. The pathogenesis of hyperbilirubinemia in G6PD deficient newborn babies is different from that in G6PD-normal ones. Meanwhile hemolysis is considered to be a principal cause of bilirubinemia in G6PD-normal neonates; but diminished bilirubin conjugation may be the main cause of hyperbilirubinemia in G6PD-deficient newborn infants[11].High levels of unconjugated bilirubin in G6PD-deficient neonates are the result of an interaction between G-6-PD deficiency and variant promoter for the gene encoding this enzyme, UDP glucuronosyltransferase[12]. Although there is a natural immaturity of bilirubin conjugation in neonates, the bilirubin conjugation ability of G6PD-deficient neonates who are also hyperbilirubinemic is even less efficient. Bilirubin conjugation ability in G6PD-deficient neonates may become worse due to increased hemolysis and more bilirubin production[12].

G6PD deficiency in India

In India, the spectrum of mutations causing G6PD deficiency has not been eluciated properly. However, studies[13] conducted revealed that the G6PD Mediterranean (563C→T) is the most common polymorphism in India followed by G6PD Kerala-Kalyan (949G→A) and G6PD Orissa (131C→G). G6PD Chatam (1003G→A) with undetected red cell enzyme activity[14]and G6PD Insuli (989G→A) with normal G6PD activity were found to be very rare in Indian population[15]. Mostly the mutations are seen in exon 6 and 7 which is in close proximity to G6PD binding site. G6PD Mediterranean is a severely deficient varitant associated with Drug Induced Hemolytic Anemia (DIHA), favism and neonatal jaundice. The presenc of G6PD Mediterranean in association with DIHA and Chronic Non Spherocytic Hemolytic Anemia(CNSHA) has been reported earlier from India[16,17,18]. G6PD Kerala-Kalyan mutation has been reported from Maharashtra, Kerala, Punjab and amongst Indo-Mauritians asccertained to be originating from Andhra Pradesh and Tamil Nadu in Southern India[20]. G6PD Orissa mutation was reported among the tribals of central, Eastern and Southern India[20]. However, it was found that this mutation was present both in the tribal and caste groups. G6PD Mediterranean was found in the context of two haplotypes in the population. European populations with G6PD Mediterranean have a T at 1311 whereas in South Italy and India have a C at 1311[21].

A higher incidence of G6PD deficiency are seen in the North and the West (15% in Parsees to 27% Angami Nagas) as compared to South India (1% to 2%) except in tribals of Tamil Nadu and Andhra Pradesh (5% to 13%)[14].

The populations in India comprises of numerous endogamous caste and tribal groups, each with characteristic physical, cultural and genetic traits. It has been estimated that more than 5,00,000 individuals are G6PD deficient and the irrational use of antimalarial drugs therefore causes concern in the medical fraternity about the occurrence of hemolysis in these individuals. Most of the individuals are underdiagnosed due to the lack of awareness and testing facilities.

CONCLUSION AND RECOMMENDATIONS

G6PD is one of the leading cause of Hyperbilirubinemia (Bilirubin.15mg/dl) in neonates. They usually present with very high bilirubin level requiring exchange transfusion or prolonged phototherapy or both, adding to their morbidity and mortality. This study did not detect any severe G6PD deficiency but majority were of moderate degree deficient. Preterm babies may show higher G6PD level thus level may be repeated after term if suspicion of deficiency is there. G6PD deficiency was also found in femal neonates (18.75% of all G6PD deficient cases)

It is one of the important causes of pathological jaundice in neonates. Significant hyperbilirubinemia is common in these neonates and unless treated promptly may lead to kernicterus and even death. There is no reliable clinical feature which can provide clue to the possibility of G6PD deficiency in a neonate. As many of the healthy neonates are discharged from hospital after 24hrs of observation, many G6PD deficient neonates will go unnoticed and develop significant morbidity and mortality from the same which carries significant medicolegal implications.

This study recommends routine neonatal screening for G6PD deficiency in every neonatal unit in India. We have started doing routine screening for G6PD deficiency in our unit and the results are encouraging.

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