Glucose-6-phosphate dehydrogenase deficiency in neonatal hyperbilirubinemia and its relationship with severity of hyperbilirubinemia

Naseem Akhter[1], Umme Habiba[2], Nazish Mazari[3], Sabeen Fatima[4], Maliha Asif[5], Yasmeen Batool[4]

ABSTRACT

Objective: To determine the frequency of Glucose-6-Phosphate dehydrogenase deficiency in neonates with hyperbilirubinemia and to find the association between level of G6PD deficiency and severity of hyperbilirubinemia.
Study Design: Cross sectional Descriptive study
Place and Duration: Hematology and Pediatrics Departments of Shaikh Zayed Hospital, Lahore from November 1st, 2012 to January 1st, 2014.
Methodology: We included 100 neonates with hyperbilirubinemia after excluding the other risk factors. Initially screening for G6PD deficiency done with qualitative methods and deficient neonates were confirmed with quantitative method. History, examination and investigations like routine hematological investigations and total and direct bilirubin, Coomb's test, G6PD qualitative and quantitative assay were done in all subjects.
Results: Out of 100 neonates 6% were G6PD deficient and in majority jaundice appeared on third day. For G6PD deficient neonates maternal age was found to be higher (29.83±1.17 years) as compared to that for normal G6PD neonates. Bilirubin was higher with a mean value of 24.48±7.0 mg/dl and platelets were lower in deficient group with a mean value of 166.33±58.64 x 10^9/L.
Conclusion: In newborns presenting with neonatal jaundice, screening for G6PD deficiency should be considered. There is association between lower enzyme activity and hyperbilirubinemia, and an early recognition followed by treatment to avoid complications is advisable.
Keywords: Glucose-6-phosphate dehydrogenase, Deficiency, Neonate, Hyperbilirubinemia, Direct bilirubin, Coomb's test

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1. Assistant Professor of Pathology, Ibn e Sina Hospital, Multan Medical & Dental College, Multan
2. Senior Demonstrator of Pathology, University of Lahore, Lahore.
3. Assistant Professor of Pathology, University of Health Sciences, Lahore.
4. Assistant Professor of Pathology, Nishtar Medical University & Hospital, Multan.
5. Assistant Professor of Pathology, Rahber Medical and Dental College, Lahore

Correspondence:
Dr. Naseem Akhtar
Assistant Professor of Pathology, Ibn e Sina Hospital, Multan Medical & Dental College, Multan
Email: naseem.akhter2009@gmail.com

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INTRODUCTION

G6PD is a housekeeping enzyme and has many important functions in the body[1]. It catalyzes the first step in Pentose phosphate pathway and Nicotinamide adenine dinucleotide phosphate (NADPH) is produced. Nicotinamide adenine dinucleotide phosphate is important reducing agent and is required for the detoxification of reactive oxygen species and hydrogen per oxides. It is done through two oxidative defense mechanisms involving catalase and glutathione peroxidase and reductase. For red blood cells Nicotinamide adenine dinucleotide phosphate is essential and Pentose phosphate pathway is the only pathway for NADPH production in red blood cells[2].
G6PD deficiency, the most common red cell enzymopathy, is an X linked disorder affecting almost 400 million people worldwide[3]. Areas of high prevalence are Africa, Southern Europe, Middle East, Mediterranean and Asia. But due to migration and traveling, it is distributed to whole world[4]. Almost 7.5% of world population is carrier of G6PD deficiency with range of 35% in Africa to 0.1% in Japan and some parts of Europe[5]. 10% of adult Black Americans are G6PD deficient[6]. In Pakistan regional variability has been reported in various studies[7].

G6PD deficiency causes a spectrum of diseases including neonatal hyperbilirubinemia, acute hemolytic and chronic hemolytic anemia[8]. While majority of the individuals are asymptomatic throughout their life, in others it may present as acute hemolysis secondary to oxidative stress triggered by drugs, infections or ingestion of fava beans. Other manifestations of G6PD deficiency include chronic non spherocytic hemolytic anemia and neonatal jaundice[9].

Jaundice usually appears at the same time as or slightly earlier than physiological jaundice. Mostly it peaks two to three days after birth[10]. Infants with the severe variant of G6PD deficiency may develop hyperbilirubinemia severe enough to cause kernicterus[10].

The study was conducted with an objective to determine the frequency of Glucose-6-Phosphate dehydrogenase deficiency in neonates with hyperbilirubinemia and to find the association between level of G6PD deficiency and severity of hyperbilirubinemia.

METHODOLOGY

This cross sectional descriptive Study was conducted in the Departments of Hematology and Pediatrics at Shaikh Zayed Medical Complex, Lahore from November 1st, 2012 to January 1st, 2014. Our study included 100 neonates born with gestational age of ≥35 weeks and admitted in the hospital with hyperbilirubinemia with a total serum bilirubin ≥ 10 mg/dl. Any neonate with other possible etiology of hyperbilirubinemia such as infant of diabetic mother, perinatal infection/sepsis, gastrointestinal obstruction, prematurity <35 weeks and ABO incompatibility were excluded from the study. Also excluded were those who received intensive phototherapy, those who had signs and symptoms of severe illness, cephalhematoma and neonates with conjugated hyperbilirubinemia. Female neonates and neonates presenting after 10 days of birth were excluded as well. Written informed consent was taken from parents/guardians. After taking history, 8ml of blood sample was collected in disposable syringe from the peripheral vein using aseptic technique by a trained phlebotomist. The sample was divided; 3ml for complete blood count, reticulocyte count, peripheral blood smear and G6PD qualitative screening, 1ml for direct coombs test and blood grouping, 2 ml for total and direct bilirubin, 2 ml for G6PD quantitative assay.

Data Analysis: All data was entered and analyzed by using SPSS 20. Quantitative Variables were presented by percentage, mean and standard deviation. Qualitative variables including deficiency of G6PD, type of feeding and outcome were expressed by frequency and percentage. Association of glucose-6-phosphate dehydrogenase deficiency with bilirubin and outcome was obtained by using Chi Square likelihood ratio. Odd ratio with 95% confidence interval was calculated. P value ≤0.05 was considered statistically significant.

RESULTS

A total of 100 neonates with hyperbilirubinemia were included in our study. All neonates were male. The mean age was 5.34±1.58 days (range1-8 days). Their birth weights ranged from 1.5 to 3.6 kg with an average birth weight of 2.51±0.46 kilograms. The mean Apgar score at birth was 7.80±0.738 (range, 5-8). The mean maternal age was 28±3.73 years (range, 18-37 years). 51(51.0%) of neonates were fed on breast milk while 49(49.0%) were fed on formula milk.

The time of appearance of jaundice ranged from zero to 6 days. The mean time for appearance of jaundice was 2.79±0.99 days. Fever was the presenting complaint of only one (1.0%) neonate. Five (5.0%) came with drowsiness, three (3.0%) with high pitched cry and two (2.0%) with lethargy.

The means of values of baseline laboratory parameters are given in Table-I. Here it is important to note that the mean reticulocyte count for the neonates included in the study was 1.92±0.78 % (range, 0.1-4.1%). The reticulocyte count of deficient neonates ranges from 0.8% to 3%. So there was no reticulocytosis that could interfere with screening method, as reticulocytes have higher enzyme level. (Table-I)

Table-I: Baseline laboratory parameters of neonates (N=100)

Variable	Mean	Range
Hemoglobin	14.85±2.75	(9.6 – 19.5) g/dl
WBC ($\times 10^9$/L)	10.22±4.31	(2.5 – 22.9)
Platelet count ($\times 10^9$/L)	227±83	(40 – 384)
Hematocrit	43.8±8.4	(26.3 – 56.9)
Mean corpuscular volume	101±5.8	(89 – 121)fL
Red cell distribution width	12.67±1.06	(11.0 – 16.7%)
Reticulocyte counts	1.92±0.78%	(0.1 – 4.1%)
Total bilirubin	19.9±5.26	(10.7 – 36.2) mg/dl
Direct (Conjugated) bilirubin	1.83±1.24	(0.1 – 5.0) mg/dl

Glucose-6-Phosphate Dehydrogenase of all the neonates included in the study was evaluated with qualitative assay. Among these neonates, 6(6.0%) were found to be deficient for G6PD (Fig-1). Quantitative analysis of G6PD was performed for the 6 G6PD deficient cases.

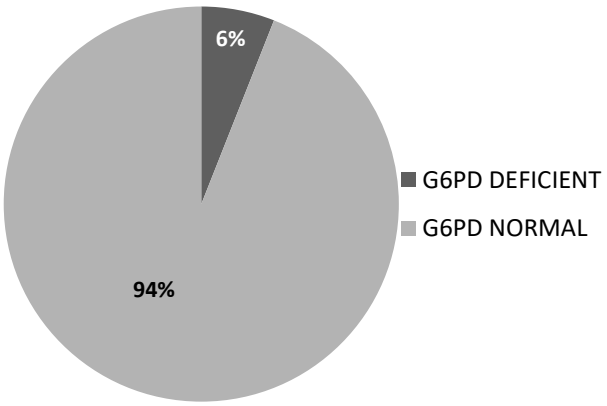

Fig-1: Status of G6PD in neonates with hyperbilirubinemia (N=100)

It was noted that 2(33.33%) of G6PD deficient neonates had G6PD levels below 1.0, 2(33.33%) neonates had G6PD levels between 2 and 3, 1(16.67%) neonate had G6PD level between 1.0 and 2.0 and 1(16.67%) neonate had G6PD level above 3.0.

An interesting observation was that the mothers of G6PD deficient were of higher age (29.83±1.17 years) as compared to maternal age of normal G6PD neonates (27.91±3.81 years). The difference was highly significant with a p-value 0.008. The mean total bilirubin level in G6PD deficient jaundiced neonates was found to be higher as compared to the mean total bilirubin level in normal G6PD jaundiced neonates. (p-value 0.027).

Likewise, the mean direct bilirubin level of the G6PD deficient neonates was higher (p-value 0.036). Mean platelet count was significantly lower in G6PD deficient group when compared to platelet count in normal G6PD neonates with p-value 0.052.No statistically significant difference was found for other parameters used in the study including birth weight, Hemoglobin level, WBC count, Hematocrit, MCV, RDW and Reticulocyte count. (Table-II)

Table-II: Comparison of various parameters for the children with deficient andnormal G-6-P levels (N=100)

	Glucose -6- phosphate dehydrogenase assay				P-value
	Deficient		Normal		
	Mean	SD	Mean	SD	
Birth weight (Kgs)	2.25	0.23	2.53	0.46	0.145
Mother Age (years)	29.83	1.17	27.91	3.81	0.008
Hemoglobin(g/dl)	14.07	3.46	14.89	2.71	0.477
White Blood cell counts($\times10^9$/L)	9.10	5.23	10.29	4.27	0.514
Platelet count($\times10^9$/L)	166.33	58.64	232.27	80.59	0.052
Hematocrit (%)	42.20	11.50	43.87	8.28	0.668
Mean corpuscular volume(fL)	100.67	2.88	100.85	5.93	0.939
Red cell distribution width	12.48	0.78	12.68	1.08	0.660
Retic count (%)	1.72	0.72	1.93	0.78	0.519
Total bilirubin(mg/dl)	24.48	7.00	19.59	5.04	0.027
Direct bilirubin(mg/dl)	2.85	1.66	1.76	1.19	0.036

All the jaundiced neonates included in the study were followed for the outcome of treatment of their jaundice. Among neonates with G6PD deficiency, two out of six were recovered and discharged, two neonates left against medical advice, one developed sign and symptoms of kernicterus and one neonate died. Out of 94 neonates with normal G6PD level 90 recovered and were discharged, two developed sign and symptoms of kernicterus, one expired and one left against medical advice (Fig-2). It was found that the rate of development of complications and death were higher in G6PD deficient neonates as compared to neonates with normal G6PD levels. The difference was significant with a p value 0.001.

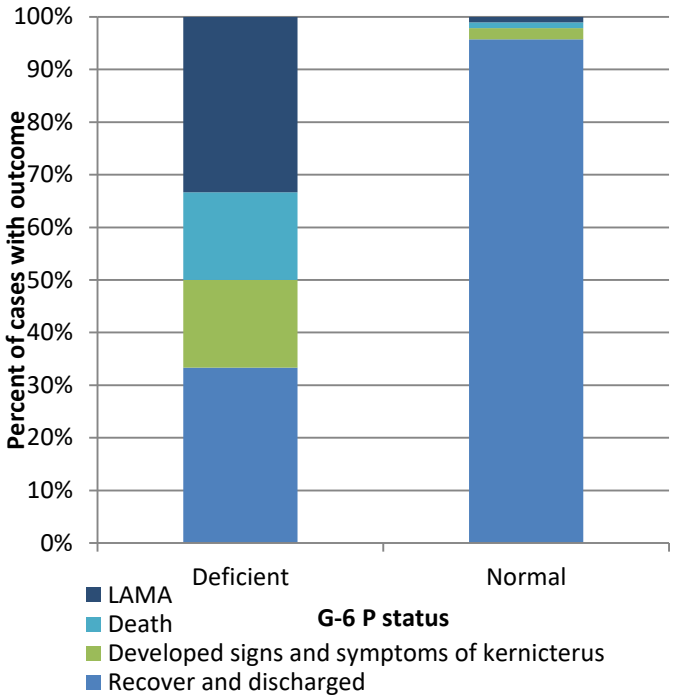

Fig-2: Outcome of the treatment (N=100)

DISCUSSION

Among the red cell enzymopathies, G6PD deficiency affects almost 400 million population with global prevalence of 4.9%. [11] There is increased frequency of G6PD in Africa, Asia, the Mediterranean and the Middle East[4]. In our study frequency of G6PD deficiency was found to be 6 % among jaundiced neonates. There are differences in G6PD deficiency frequency reported in different studies country wide. Munir et al reported G6PD deficiency 6.4% in neonates with jaundice, and these results were in conformation with our study[12]. Rehman et al reported in their study 8.2% of the neonates with jaundice were G6PD deficient respectively[12]. In another study from Peshawar, Imran et al found that 12%[13] and 11% by Khan S et al in different districts of Khyber Pakhtunkhwa[14] respectively. Studies have shown that there is increased frequency of G6PD deficiency in Pathans as compared to Punjabis[15]. The higher frequency of G6PD deficiency may be due to high incidence of inter-family marriages, consanguineous marriages and environmental factors[13].

The international frequency of G6PD deficiency varies a lot in different areas of world and ethnic differences are also prominent. The incidence rates have been reported as 8.65% - 16.4% in Iran[16], 0-27 % in India[17], 62% among Kurdish Jews to as low as 0.1% in Japan and Europe[8]. The relatively low frequency seen in our study could be due to the fact that we included only male neonates in our study whereas majority other work included both male and female neonates and

another factor may be the regional variation and environmental factors.

Our study has also found out that G6PD deficient neonates have severe hyperbilirubinemia as compared to those with normal G6PD enzyme level. These results are in conformity with the international data. A significant correlation between bilirubin levels and G6PD activity in infants of both sexes with severe hyperbilirubinemia was reported by Kilicdag et al[18] and Paneliya et al.[19] Hyperbilirubinemia in deficient infants was found to be due to gene interaction between G6PD enzyme and bilirubin conjugating enzyme UGT1A1[15].

In our study the hyperbilirubinemia is less likely to be associated with hemolysis. It is evident from reticulocyte count and hemoglobin values in our study. In majority of neonates the reticulocyte count was within normal limit for age. The other parameter supporting this is hemoglobin level. The G6PD deficient neonates have hemoglobin level similar to those neonates with normal G6PD status. Similar results were described by Isa et al[20] and Munir et al.[12]

The neonates with G6PD deficiency are at increased risk of hyperbilirubinemia and kernicterus even in the absence of other etiological factors[21]. Our study has also shown increased hyperbilirubinemia and kernicterus in G6PD deficient neonates. The frequency of kernicterus is high among deficient neonates with hyperbilirubinemia[22]. In our study one neonate in deficient group and two in normal developed the signs of kernicterus. The frequency among deficient group was 16.66% and 2.12% in neonates with normal G6PD level. The comparison between groups is not statistically appropriate because of the difference in sample size. However our results are in agreement with other studies.

Studies have shown that the neonates with G6PD deficiency develop maximal hyperbilirubinemia between third to fifth day which is not different from that of the physiological jaundice. In our study most of the patients developed jaundice at 3rd day of life followed by 30% at 2nd day. In deficient neonates 5 out of 6 developed jaundice at 3rd day while 6th one developed jaundice at 2nd day of life. The results are in agreement with the previous studies[23].

An interesting observation in our study was that the mothers of G6PD deficient were of higher age as compared to maternal age of normal G6PD neonates. However this parameter was not studies in previous studies. So, further studies are needed to evaluate the significance of this and to establish its validity. No statistically significant difference was found for birth weight, between G6PD deficient and normal G6PD group[24]. Among other parameters used in the study we found that Mean platelet count was almost significantly lower in G6PD deficient group as compared to platelet count of normal G6PD neonates. However this finding does not conform to work of other researchers. Stadem et al reported in their study that platelet counts were similar between the two groups[24].

One of the limitations of the study was the small sample size which is not true representative of the population. Moreover we excluded the preterm neonates and female neonates, so frequency may not be a true representative of population. Due to the fact that the number of G6PD deficient neonates in our study was low and we did not have predefined controls, therefore the comparison between the normal and deficient neonates could not be made. Screening was done with qualitative methods, so the patient with less severe deficiency might have been missed. The strengths of our study were that we effectively excluded the other potential risk factors for hyperbilirubinemia.

From the study we concluded that G6PD deficiency is not uncommon in Pakistan. The neonates with G6PD deficiency develop hyperbilirubinemia more commonly. These neonates are at increased risk of bilirubin encephalopathy. G6PD deficiency is not the only cause of hyperbilirubinemia, other factors are also involved. Lower the enzyme level increased the risk of complications.

So the threshold for phototherapy and exchange transfusion should be lower in children with early jaundice and rapidly increasing jaundice. The injudicious use of antibiotics, antimalarial and antituberculous drugs is common in Pakistan, so it is important to identify G6PD deficiency in individuals who are at risk. This will help to prevent undesirable effects and at times fatal acute hemolytic episodes. Comprehensive population studies are needed to know the prevalence and genetic variants and to devise national guidelines for early diagnosis and appropriate management.

CONCLUSION

In newborns presenting with neonatal jaundice, screening for G6PD deficiency should be considered. There is association between lower enzyme activity and hyperbilirubinemia, and an early recognition followed by treatment to avoid complications is advisable.

CONTRIBUTION OF AUTHORS

Akhter N: Designed research methodology, Data collection, Manuscript writing, Formulation of tables, Statistical analysis

Habiba U: Conceived idea, Statistical analysis, Data interpretation, Manuscript writing, Critical revision

Mazari N: Data collection, Statistical analysis, Data interpretation

Fatima S: Data collection, Manuscript writing, Formulation of tables, Data interpretation.

Asif M: Statistical analysis, Data interpretation, Manuscript writing.

Batool Y: Statistical analysis, Data interpretation, Manuscript writing, Critical revision of manuscript.

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