

## Association of Glucose-6-phosphate dehydrogenase deficiency and hyperbilirubinemia in neonates

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**Abstract: Background & objective** – Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most important disease of hexose monophosphate pathway. It is an X-linked recessive disease, where the deficiency of the enzyme causes a spectrum of clinical manifestations. The aim of this study was to see the occurrence of G6PD deficiency in neonates and its association with hyperbilirubinemia.

**Materials & Methods** – This retrospective study was carried out in a tertiary care centre between January to September 2016. Neonates of both gender with G6PD deficiency and normal G6PD having hyperbilirubinemia (TSB >5mg/dl) were included.

**Results** – Out of 300 neonates with hyperbilirubinemia, 44% were Glucose-6-phosphate dehydrogenase deficient. Male:female (M:F) ratio of G6PD deficient neonates was 1.6:1. Majority of G6PD deficient neonates had TSB >15 mg/dl; whereas G6PD non-deficient had mostly hyperbilirubinemia at TSB levels <15mg/dl (66.1%). Mean Total serum bilirubin (TSB) in G6PD deficient neonates were more as compared to normal G6PD icteric neonates and was statistically significant (P<0.05).

**Conclusion** – In G6PD deficient neonates' hyperbilirubinemia is very severe type which may be a risk factor for neurological complications like kernicterus. Therefore, implementation of National control program for screening of G6PD deficiency in neonates will reduce the complications and fatal conditions arising due to this inborn blood disorder.

**Keywords** - G6PD deficiency, hyperbilirubinemia, neonatal jaundice, Total serum bilirubin.

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### I. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked recessive enzymopathy, which is a well-known cause of hyperbilirubinemia that may be severe enough to cause kernicterus or death in neonates. The pathogenesis of G6PD deficiency associated neonatal hyperbilirubinemia is thought to be multifactorial.<sup>1</sup>

A higher incidence of G6PD deficiency is seen in tropical and subtropical zones of the world. Molecular analysis has revealed that each population has a characteristic profile of deficient variants. The G6PD A variant is mainly found in African populations while G6PD Mediterranean variant is predominant throughout the Mediterranean region, Middle East and India. In India, various studies have reported incidence of G6PD deficiency ranging from 2% to 27.9% in different communities.<sup>2,3</sup>

G6PD is a cytoplasmic enzyme in the pentose monophosphate pathway and catalyzes the conversion of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form, NADPH. It helps to protect the red blood cells from oxidative damage. G6PD deficiency causes neonatal hyperbilirubinemia and chronic hemolytic anemia. Although most affected individuals are asymptomatic, exposure to oxidative stresses such as certain drugs like antimalarial or sulfonamides, foodstuffs like fava beans, or with infections such as viral respiratory infection, hepatitis, and bacterial pneumonia can elicit acute hemolysis. The disease as such causes significant morbidity and mortality in childhood. There are no primary prevention interventions available for this disease and the only way to avoid the adverse outcomes is to recognize such children early in life and prevent exposure to agents which can trigger hemolysis.<sup>4,5</sup>

About 60% of term babies and 80% of pre-term infants develop some degree of jaundice during their first week of life. But, the jaundice due to G6PD deficiency occurs in the 1<sup>st</sup> day of life and usually is severe in nature. When total serum bilirubin rises to very high levels and for a prolonged period of time, it may cause acute bilirubin encephalopathy and kernicterus.<sup>3,6,7</sup>

The present study was aimed to see the occurrence of erythrocytic G6PD deficiency in neonates in this region of Upper Assam, India and its association with hyperbilirubinemia.

## II. Material And Methods

This retrospective hospital based study was carried out at Advanced Clinical Biochemistry Laboratory in Assam Medical College & Hospital, Dibrugarh, Assam, India from January to September 2016. A total of 300 newborns of either sex under the age group of day 1 to day 7 were included. All icteric neonates from Paediatrics department of Assam Medical College & Hospital who were screened for G6PD activity levels were included and the subjects were divided into two groups: G6PD deficient and G6PD non-deficient. Out of total 300 icteric neonates, 132 were G6PD deficient and 168 were G6PD non-deficient. These two groups were again compared based on total serum bilirubin levels (TSB) into 3 groups: TSB levels <15 mg/dl; 15-20 mg/dl and >20 mg/dl.

**Study Location:** This is a tertiary care teaching hospital based study done in Department of Biochemistry, at Assam Medical College & Hospital, Dibrugarh, Assam, India.

**Study duration:** January 2016 to September 2016.

**Sample size:** 300 neonates.

**Subjects and selection method:** The study population was drawn from the patients coming from Paediatrics department of Assam Medical College & Hospital who were screened for G6PD activity levels and were hyperbilirubinemic (TSB >5mg/dl). The subjects were divided into G6PD deficient and G6PD non-deficient groups. Based on TSB levels, again the subjects were divided into three groups: TSB <15mg/dl; TSB 15-20 mg/dl and TSB >20 mg/dl.

### Inclusion criteria:

1. Newborns from age Day 1 to day 7 of either sex.
2. Neonates screened for G6PD activity levels with hyperbilirubinemia (TSB >5.0 mg/dl).

### Exclusion criteria:

1. Neonates with normal bilirubin level.

### Procedure methodology

The diagnosis of red cell enzyme, G6PD deficiency usually depends on the demonstration of decreased enzyme activity either through a quantitative assay or a screening test. G6PD activity was estimated by kinetic method done in Microlab 300 (MERCK) semiautoanalyzer. Normal G6PD activity of our laboratory is 6.4 – 18.7 U/g of Hb.

Total serum bilirubin (TSB) was estimated by modification of the Doumas reference method, which is a modification of diazo method by Jendrassik and Grof done in Dimensions RxL Max (Siemens) Autoanalyzer.

Hyperbilirubinemic neonates with TSB >5mg/dl were included in this study. Normal reference range of TSB of early neonates (Day 0 – Day 7) in our laboratory is upto 5.0 mg/dl. All samples were processed on the same day within two hours of collection.

### Statistical analysis

The independent-samples t-test was used to compare the means between two unrelated groups on the same continuous, dependent variables. Pearson's chi-square test was used to see if there was any association between categorical variables. Box plot was used to displaying variation in a set of data. Statistical analysis was performed with IBM SPSS Statistics version 21 software. P-value less than or equal to 0.05 was considered as statistical significance.

## III. Result

In the present study, out of 300 neonates with hyperbilirubinemia, 132 (44.0%) were G6PD deficient and among them 61% were male and 39% were female neonates. The male: female ratio is 1.6:1 in G6PD deficient group (Figure 1).

Table 1 and box plot (Fig. 2) shows comparison of mean total serum bilirubin (TSB) between G6PD deficient and G6PD non-deficient groups. In G6PD deficient neonates, mean total serum bilirubin level is more as compared to G6PD non-deficient group and is statistically significant ( $p < 0.05$ ). The mean TSB of G6PD deficient is  $(15.62 \pm 3.96)$  mg/dl and mean TSB of G6PD normal group is  $(13.46 \pm 3.55)$  mg/dl.

Figure 1. Shows gender wise distribution in G6PD deficient and G6PD non-deficient neonates.

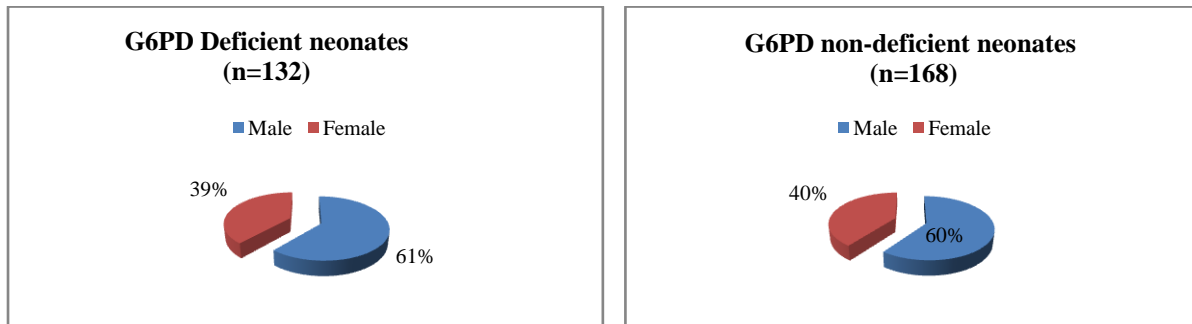
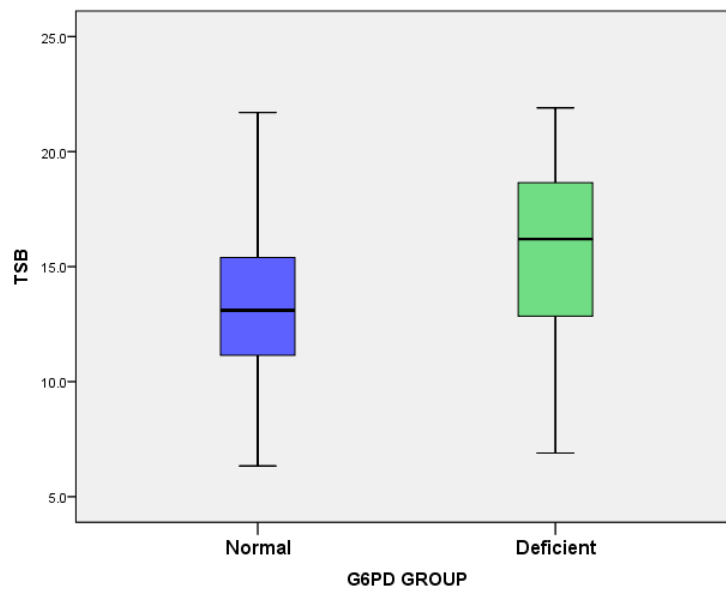


Table 1. Comparison of mean total serum bilirubin between G6PD deficient and G6PD non-deficient groups.

Group	Total Serum Bilirubin (mg/dl) (Mean ± SD)	p- value
G6PD-deficient neonates	15.62 ± 3.96	0.000*
G6PD- non-deficient neonates	13.46 ± 3.55	

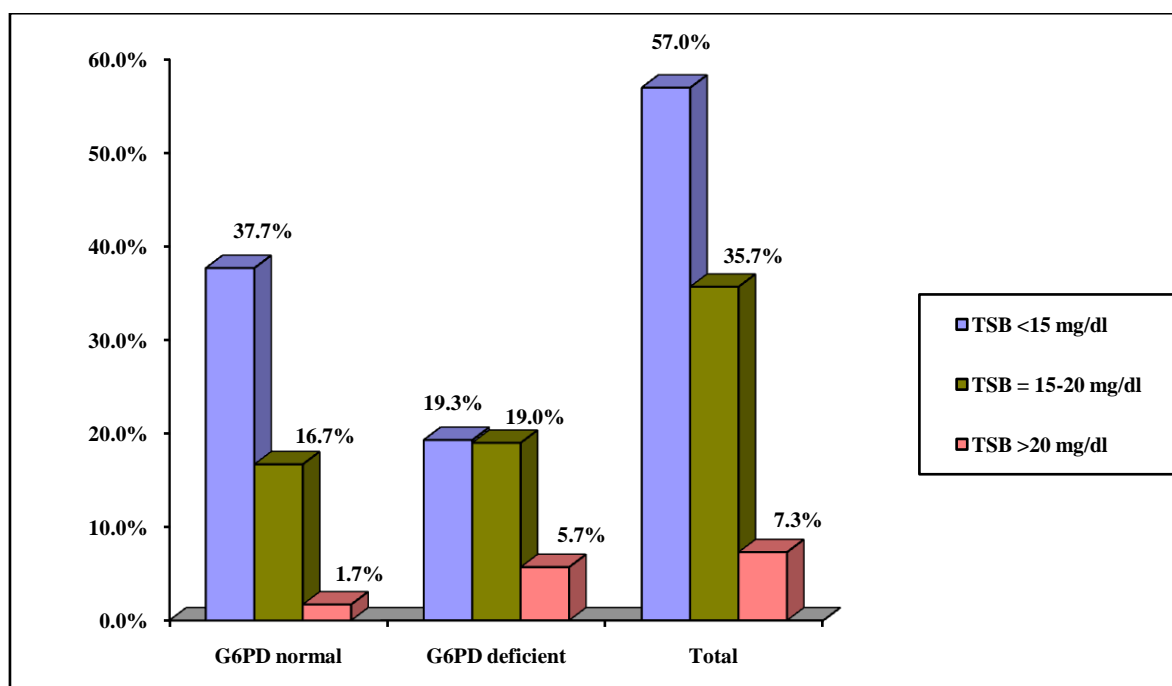
Figure 2. Box plot to compare the mean total serum bilirubin (TSB) levels between G6PD deficient neonates and G6PD normal neonates.



The subjects were again divided into 3 groups based on total serum bilirubin levels : group 1 with TSB level <15 mg/dl, group 2 with TSB level 15-20 mg/dl and group 3 with TSB > 20mg/dl. Out of the 300 icteric neonates, 171 (57.0%) had TSB <15mg/dl, 107 (35.7%) had TSB 15-20 mg/dl and 22 neonates (7.3%) had TSB > 20 mg/dl (Table 2).

In group 1, with TSB <15mg/dl G6PD deficient neonates were 19.3% and G6PD normal were 37.7%; in group 2 with TSB 15 – 20 mg/dl, 19.0% were G6PD deficient and 16.7% were G6PD normal and in group 3 with TSB >20mg/dl, G6PD deficient were 5.7% and G6PD normal were 1.7%. G6PD normal group showed mostly with total serum bilirubin levels less than 15 mg/dl (37.7%) whereas more of G6PD deficient group had TSB levels above 15mg/dl (Table 2).

**Table 2. Graphical presentation to show 3 groups of neonates based on TSB levels (TSB <15mg/dl; TSB 15-20 mg/dl and TSB >20 mg/dl) in G6PD normal and G6PD deficient groups.**



There was statistical significant difference of prevalence of G6PD deficiency between group 1 and group 2 (19.3% vs. 19.0%) ( $p=0.001$ ), group 1 and group 3 (19.3% vs. 5.7%) ( $p=0.000$ ) and group 2 vs. group 3 (19.0% vs. 5.7%) ( $p=0.038$ ) (Table 3).

**Table 3. Prevalence of G6PD deficiency in 3 groups based on TSB levels.**

Group	All	G6PD deficiency
TSB <15 mg/dl	171 (57.0%)	58 (19.3%)
TSB = 15-20 mg/dl	107 (35.7%)	57 (19.0%)
TSB >20 mg/dl	22 (7.3%)	17 (5.7%)

A statistical significant association was observed between the 3 groups of hyperbilirubinemic neonates with G6PD non-deficient and G6PD deficient groups. Major 113 (66.1%) G6PD non deficient neonates had TSB levels <15 mg/dl whereas maximum G6PD deficient neonates belong to TSB levels >15 mg/dl, (53.3% with TSB 15-20mg/dl and 77.3% with TSB >20 mg/dl) (Table 4).

**Table 4. Association between 3 groups of hyperbilirubinemic neonates (TSB <15 mg/dl, TSB 15 – 20 mg/dl and TSB >20 mg/dl) with G6PD non-deficient and G6PD deficient groups.**

Group	G6PD normal	G6PD deficiency	p value
TSB <15 mg/dl (n=171)	113 (66.1%)	58 (33.9%)	0.000*
TSB = 15-20 mg/dl (n=107)	50 (46.7%)	57 (53.3%)	
TSB >20 mg/dl (n=22)	5 (22.7%)	17 (77.3%)	

Table 5 shows comparison of mean TSB level in G6PD normal and G6PD deficient groups on the basis of age (in days) of neonates. There is a significant difference in mean TSB level of the neonates with age (in days) Day 2, Day 3, Day 4, Day 5 and Day 7 in G6PD normal and G6PD deficient groups. Except neonates with age Day 2, mean TSB level of G6PD deficient neonates are more than G6PD normal group (i.e., neonates with age Day 3, Day 4, Day 5 and Day7).

**Table 5. Comparison of mean TSB level in G6PD normal and G6PD deficient groups on the basis of age (in days) of neonates.**

	Age in days	G6PD normal neonates		G6PD deficient neonates		p value
		N	Mean ± SD	N	Mean ± SD	
TSB	Day 1	1	-	1	-	-
	Day 2	13	15.45 ± 4.04	13	12.03 ± 3.17	0.024*
	Day 3	54	13.36 ± 3.98	37	16.18 ± 3.87	0.001*
	Day 4	44	12.45 ± 3.25	29	16.64 ± 3.17	0.000*
	Day 5	25	13.91 ± 2.32	17	15.84 ± 3.62	0.042*
	Day 6	16	13.81 ± 4.01	18	14.41 ± 3.92	0.664
	Day 7	15	13.54 ± 2.81	17	17.12 ± 3.88	0.006*

#### IV. Discussion

In India, 13 biochemical variants of G6PD have been reported so far, out of which G6PD Mediterranean is most common in the caste groups; whereas G6PD Orissa is most prevalent in the tribals of India, followed by G6PD Kerala-Kalyan, the 3<sup>rd</sup> most common variant.<sup>3</sup>

G6PD deficiency was present in all the tribal groups studied from North-East India. A very high frequency was observed in Angami Nagas (27.0%) from Nagaland followed by Rabhas (15.8%) and Mikirs (15.6%) from Assam. Mizo population of Mizoram showed 17.5%, out of 490 study subjects to be G6PD deficient.<sup>8,9</sup> In the present study, out of 300 icteric neonates, 44.0% were G6PD deficient. The male to female ratio of G6PD deficient neonates was 1.6:1. Similar study from other parts of India showed male: female ratio of G6PD deficient to be 1:1 in West Bengal; 3:1 in Vataliya community in Western India.<sup>10,11</sup>

In this study, the subjects were divided into 3 groups based on total serum bilirubin level (TSB): group 1 with TSB <15mg/dl, group 2 with TSB 15-20mg/dl and group 3 with TSB >20mg/dl. Out of 300 hyperbilirubinemic neonates, 57.0% had TSB <15mg/dl, 35.7% had TSB 15-20 mg/dl and 7.3% had TSB > 20 mg/dl. There was statistical significant difference of prevalence of G6PD deficiency between group 1 and group 2 (19.3% vs. 19.0%) (p=0.001), group 1 and group 3 (19.3% vs. 5.7%) (p=0.000) and group 2 vs. group 3 (19.0% vs. 5.7%) (p=0.038). Major (37.7%) G6PD non deficient neonates had TSB <15 mg/dl whereas maximum G6PD deficient neonates belong to TSB levels > 15 mg/dl.

Bisoi S et al studied 109 newborns, out of which 14.68% were G6PD deficient and among them 23.8% of G6PD deficient and 12.5% of normal-G6PD had TSB>15mg/dl, and this was statistically insignificant. Kaplan et al reported that the TSB levels in G6PD deficient neonates are higher within 3 hours of life compared to their healthy counterparts [2.9±0.7 mg/dl vs 2.6±0.6 mg/dl (p<0.05)].<sup>10,12</sup>

Pao M, et al in their study of 2479 neonates of both sex, found the incidence of hyperbilirubinemia to be 32% in G6PD deficient neonates which was significantly higher than the incidence of hyperbilirubinemia in neonates with normal G6PD, which was 12.3% (p<0.001).<sup>13</sup>

In this study mean TSB in G6PD deficient neonates is significantly more as compared to normal G6PD neonates. In comparison of mean TSB level in G6PD normal and G6PD deficient groups on the basis of age (in days) of neonates, there was significant difference in mean TSB level of the neonates with age (in days) Day 2, Day 3, Day 4, Day 5 and Day 7 in G6PD normal and G6PD deficient groups. Except neonates with age Day 2, mean TSB level of G6PD deficient neonates are more than G6PD normal group.

G6PD deficiency is an example of balanced polymorphism, in which high rate of mortality caused by this disorder is offset by the protection that it offers against *Plasmodium falciparum* malaria. Alleles of the G6PD gene that encode a deficient enzyme attain high frequencies in areas where malaria is or has been endemic. Malaria is endemic in most North Eastern states of India with *P. falciparum* being the predominant parasite. It is believed that this disorder is selected due to malarial endemicity in many regions of the country. A correlation was found between high prevalence of malaria due to *P. falciparum* and incidence of G6PD deficiency.<sup>(14-19)</sup>

Neonatal jaundice is one of the most common diagnoses in the neonatal period; it is estimated to occur in 60% of term newborns in the first week of life. In rare instances, the total serum bilirubin (TSB) reaches levels that can cause kernicterus. Pranoy Dey et al found G6PD as the most common cause (23%) followed by ABO incompatibility (20%), Rh Incompatibility (16%), preterm (14%), exaggerated physiological (12%), sepsis (10%), miscellaneous (7%) requiring exchange transfusion in neonatal hyperbilirubinemia. For preventing the

kernicterus and other complications of hyperbilirubinemia, jaundice should be managed by phototherapy or exchange transfusion.<sup>20</sup>

Many countries of South East Asia (e.g. Malaysia, Singapore, Taiwan, Hong Kong and the Philippines) the Middle East and Europe (where the incidence of G6PD deficiency is high) have been successfully running a neonatal screening program since 1965. Data from Singapore reveal that with the preventive measures, the incidence of kernicterus has dropped dramatically, and in the last 20 years there has been only one reported case of kernicterus in newborns. Greece, where such a program is in operation since 1977, has reported a fourfold reduction in the hospital admission of patients for the treatment of hemolytic crisis.<sup>21,22</sup>

### Limitation of the Study

Our study has a few limitations. Firstly the sample size is small. Secondly it has innate problems of being a retrospective study. Third no proper control group was selected to compare the results; no neonates with normal TSB level were included. Fourth, added parameters like hematology parameters and history of phototherapy and exchange transfusion would have given more information.

### V. Conclusion

In G6PD deficient neonates, significant hyperbilirubinemia (TSB > 15 mg/dl) is observed and this may be a risk factor for neurological complications like kernicterus. Many parts of Northeastern states of India are malaria endemic and G6PD deficiency shows high frequency in malaria endemic region. Therefore, large scale prospective cohort studies are needed to implement a screening control program for G6PD deficiency at national level. Neonatal screening for G6PD deficiency is a need in order to early diagnose and reduce its acute and fatal complications.

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