Neonatal Screening for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Eastern India

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ABSTRACT

Introduction: The overall magnitude of the frequency of G6PD deficiency ranges between 0 to 10 percent in the Indian population. This prospective study was planned to estimate the prevalence of G6PD deficiency in newborn population born in a tertiary care centre in Eastern India.

Methods: This prospective observational study was undertaken among all consecutively delivered neonates born in a tertiary care teaching hospital of Eastern India, between Apr 2016 and Oct 2017. Prematurity less than 32 weeks and perinatal asphyxia requiring extensive resuscitation were excluded from the study. Umbilical cord blood samples were collected in EDTA containers, drawn from the placental side of the umbilical cord incised while severing it at the time of birth. The G6PD levels were estimated quantitatively based on ultraviolet (UV) method by quantitative spectrophotometric assay using ILab 650 fully automated analyzer. All babies wherein the cord blood G6PD levels was less than 6.95 mU/g of Hb was taken as deficient.

Results: Mean (SD) of G6PD at birth was 12.56 (3.45) mU/g of Hb. Out of 1037 neonates, five were found to be G6PD deficient. There was increased incidence of neonatal jaundice requiring phototherapy in G6PD deficient neonates, and it was statistically significant.

Conclusion: The prevalence of G6PD deficiency was 0.48% in term and late preterm neonates as assessed quantitatively in cord blood.

Keywords: Eastern India; G6PD deficiency; Neonatal screening

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INTRODUCTION
Glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most common of all clinically significant enzyme defects in humans, causes a hereditary predisposition to hemolysis.¹ This X-linked inherited disorder may cause neonatal hyperbilirubinemia and acute hemolytic crisis. Though most affected individuals remain asymptomatic, exposure to oxidative stresses such as ingestion of fava beans, certain drugs (aspirin, chloramphenicol, chloroquine, primaquine, sulphanilamide and chemicals like naphthalene, henna) or infection (viral or bacterial), can elicit acute hemolysis.² In neonates, G6PD deficiency can cause severe hyperbilirubinemia and even possible kernicterus in view of limited capacity of the neonatal liver to metabolize and eliminate bilirubin, and the limited capacity of the RBCs of the neonate to withstand oxidative stress.³ According to World Health Organization, the prevalence of G6PD deficiency in India ranges between 0 – 10%.⁴ A recent meta-analysis of 72 studies with a study population of 38565 and 2623 G6PD deficient subjects confirmed the overall magnitude of the frequency of G6PD deficiency to be 8.5% in the Indian population.⁵ However, the prevalence of G6PD deficiency is not uniform throughout the country. The frequency of G6PD deficient allele has been found to be comparatively higher in North and West India, and uniformly low in South India, except in Andhra Pradesh and Tamil Nadu.⁶ In a recent study from Eastern India, in a small sample of newborn population, the prevalence was found to be 14.68%, much higher than the national average.⁷ As there is paucity of literature from eastern part of the country and reportedly higher prevalence than the rest of India, we planned this prospective study to estimate the prevalence of G6PD Deficiency in newborn population born in a tertiary care center in Eastern India.

METHODS
Our study was a descriptive observational type with a longitudinal design undertaken among all consecutively delivered newborns born in a tertiary care teaching hospital of Eastern India, between Apr 2016 and Oct 2017. Prematurity less than 32 weeks and perinatal asphyxia requiring extensive resuscitation were excluded from the study. Informed written consent was obtained from one of the parents and the study was approved by the Institute Ethics Committee.

The mother’s age, parity, comorbid conditions like diabetes, PIH, hypothyroidism, any previous history of child / themselves with G6PD deficiency, unexplained anemia, jaundice requiring exchange transfusion was recorded. The type of medications given to the mother till birth of the baby was recorded. At birth, the baby’s weight, gender, APGAR score, congenital abnormalities were noted. Umbilical cord blood samples were collected in EDTA containers, drawn from placental side of the umbilical cord incised while severing it at the time of birth. The estimation of G6PD levels was quantitative based on ultraviolet (UV) method. G6PD level was estimated within 48 hours by quantitative spectrophotometric assay using ILab 650 fully automated analyzer. 0.2 ml of blood was washed with 2 ml aliquots of 0.9% NaCl. It was centrifuged after each wash for 10 min at around 3000 rpm. It was repeated thrice and centrifuged erythrocytes were suspended in 0.5 ml solution of Digitonin and let stand for 15 mins at + 4 °C and centrifuged again. The supernatant was used within 2 hrs. The supernatant was analyzed by ILab 650 fully automated analyzer. G6PD activity was obtained as mU/g Hb. All babies wherein the cord blood G6PD levels was less than 6.95 mU/g of Hb was taken as deficient.

Anticipated frequency of G6PD to be 8.5%,⁵ with absolute precision of 1.5%, and power of 95%, the sample size was calculated to be 935. All the data was entered into Microsoft Excel 2012 spreadsheet and analyzed. Descriptive statistics of the various clinical and laboratory parameters and measures of central tendency using the mean and median with standard deviation have been performed. Chi square test and student ‘t’ test were used to test the nominal significance at the p value < 0.05 level.

RESULTS
A total of 1085 newborns were delivered in the study period. Twenty-two neonates were excluded from the study because of prematurity (less than 32 wks), eight had birth asphyxia and eighteen neonates could not be recruited as the sampling of
blood was missed. Finally, a total of 1037 newborns participated in the study, with mean gestation of 38.2 weeks, and mean birth weight of 2.93 kg. The demographic variables of participating neonates are depicted in table 1.

Twenty-eight neonates whose cord blood sample could not be analyzed, had normal values of G6PD on estimation from venous blood during initial hospitalization at birth. Mean (SD) of G6PD at birth was 12.56 (3.45) mU/g of Hb. Out of 1037 neonates, five were found to be G6PD deficient (value taken as less than 6.95 mU/g of Hb). Four of them were males and one was female. There was increased incidence of neonatal jaundice requiring phototherapy in G6PD deficient neonates, and it was statistically significant (Table 2).

**DISCUSSION**

G6PD is an enzyme that protects erythrocytes which are susceptible to oxidative stress against oxidative injury by providing reducing power in the form of nicotinamide adenine dinucleotide phosphate (NADPH). The G6PD gene is located on the X chromosome. Males are hemizygous and females can be homozygous or heterozygous. G6PD is usually measured by enzyme activity in lysate from whole red blood cells with either quantitative or qualitative assays. In India, there are 13 reported biochemical variants, out of which G6PD Mediterranean is the commonest in the caste groups; whereas G6PD Orissa is most prevalent among the tribals of India.

In our study, we found the prevalence of G6PD deficiency, by quantitative estimation, to be 0.48% in neonates, far less than that of the national average of 8.5%. The study from Kolkata which reported a much higher prevalence (14.68%) of G6PD deficiency, had studied G6PD status by semi quantitative assay in cord blood. On the other hand, we used quantitative assays which is presumed to be more accurate.

Our study shows lesser prevalence than the findings of mean G6PD deficiency of 0.048 (min 0.017 to max 0.140) in West Bengal described by Bhasin as well. In fact, our findings match with the regional distribution of G6PD deficiency in southern India like prevalence of 0% in Karnataka, 0.03% in Andhra Pradesh, and 0.07% in Tamil Nadu, and contradict the frequency reported by Bhasin who found G6PD deficiency to be higher in East India zone (0.067 from 24 studies) as compared to other zones (Central India from three studies - 0.061; North India from 34 studies - 0.058; West India from 110 studies - 0.032 and South India from 53 studies - 0.032). Recent reports like a study from

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**Table 1. Baseline characteristics**

| Variable                  | Results (n = 1037) (%) |
|---------------------------|------------------------|
| Gestation (weeks)         |                        |
| 32 - 37                   | 162 (15.6)             |
| > 37                      | 875 (84.4)             |
| Birth weight (gm)         |                        |
| ≥ 2500                    | 888 (85.6)             |
| < 2500                    | 149 (14.3)             |
| Male gender               | 502 (48.4)             |
| Maternal comorbidities    |                        |
| Pregnancy induced hypertension | 59 (5.6)   |
| Gestational diabetes      | 166 (16)               |
| Hypothyroidism            | 65 (6.2)               |

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**Table 2. Descriptive statistics of G6PD deficiency**

| Parameter                 | No of Patients N = 1037 (%) | G6PD values | P Value |
|---------------------------|-----------------------------|-------------|---------|
|                           |                             | G6P < 6.95  | G6PD > 6.95 |        |
| Sample type               |                             | 5           | 1004      | 0.02    |
| Umbilical                 | 1009 (97.3)                 |             |          |
| Venous                    | 28 (2.64)                   | 0           | 28       |
| Gender                    |                             | 4           | 498      | 0.30    |
| Male                      | 502 (48.4)                  |             |          |
| Female                    | 535 (51.6)                  | 1           | 534      |
| Birth Weight              |                             | 2           | 164      | 0.91    |
| 1.5 - 2.49 kgs            | 166 (16)                    |             |          |
| ≥ 2.5 kgs                 | 871 (84)                    | 3           | 868      |
| Hyperbilirubinemia        |                             | 2           | 153      | 0.002   |
| Required phototherapy     | 155 (15)                    |             |          |
| No phototherapy           | 882 (85)                    | 3           | 879      |
South India also, revealed no case of G6PD deficiency when they studied 500 neonates screened for G6PD by Fluorescent spot test method.12

It is thought that as the gene of G6PD Mediterranean allele passed through the North and then spread throughout the adjoining areas by several contacts between the Mediterranean people and Indians, it is not unusual that the distribution of G6PD deficient allele among Indian population, would be comparatively higher in the North and West zone.6 Regarding the eastern zone, the deficiency of G6PD has been least studied in this part of the country. Hence our findings are notable and also reassuring about the expected rarity of G6PD deficiency in eastern India like the southern parts of India.

The strength of our study is that it was a prospective study. We used quantitative assays and hence our findings reflect the prevalence of G6PD deficiency more accurately. The limitation of our study is that the sample size was small. We did not test for genetic testing like DNA sequencing, or flow cytometry for intracellular G6PD activity.

CONCLUSIONS

The prevalence of G6PD deficiency was 0.48% in term and late preterm neonates as assessed quantitatively at birth. These neonates with G6PD deficiency should be watched for neonatal hyperbilirubinemia and avoidance of the mentioned food items, herbs, chemicals and drugs causing hemolysis should be ensured. Larger study in future with a large sample size along with confirmation through genetic testing is warranted for more accurate estimation of G6PD deficiency in the population.

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