FREQUENCY OF GlUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN NEONATAL JAUNDICE IN DISTRICT SWAT, PAKISTAN

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ABSTRACT

Background: Hyperbilirubinemia is the most common cause of neonatal jaundice. Severe neonatal hyperbilirubinemia and kernicterus continue to be reported worldwide in otherwise healthy term infants. We conducted this study to estimate the incidence of severe neonatal hyperbilirubinemia and to determine underlying causes, improved knowledge of which would be valuable to help identify strategies for risk reduction.

Methods: 100 infants of both sexes, of the age between 1-28 days, with no infection and Rh incompatibility were included in the study. The activity of G6PD was determined by a dye-reduction method screening test devised by Sigma Diagnostics USA. Complete blood counts (CBC) with reticulocyte count, serum bilirubin (total and indirect) were performed and the results were recorded for further analyses.

Results: Out of the total 100 subjects, classified in two groups on the basis of presence of jaundice, 14 (14%) were G6PD deficient. The frequency of G6PD deficiency was markedly prominent, in neonates with jaundice.

Discussion: G6PD deficiency is one of the major causes of jaundice in neonates. The study area also have a considerable number of G6PD deficient individuals. This indicates a need for a more thorough assessment of newborn infants and consideration of strategies to identify at-risk newborns, such as pre-discharge measurement of serum bilirubin levels and G6PD deficiency screening.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) belongs to the Oxidoreductases class of enzymes. G6PD catalyses the first step in pentose phosphate pathway, also called hexose mono phosphate shunt (HMPS) by dehydrogenation of glucose 6 phosphate (G6P). This was first described by Warburg and Christian in 1931. In the red cells, this alternate anaerobic pathway for glucose metabolism is the only source for reduced NADP (NADPH- Nicotinamide Adenine dinucleotide Phosphate), which is required for methaemoglobin reductase activity and the maintenance of the level of reduced glutathione (GSH). NADPH and GSH in turn maintain an effective redox potential protecting cell membrane sulphhydryl groups, enzymes and haemoglobin against oxidative stress and injury. Under ordinary conditions a level of G6PD enzyme activity of 20% (or even less i.e. as low as 3%) is sufficient for normal red cell function.

G6PD deficiency is the most common red cell enzyme abnormality associated with haemolysis. The abnormality is affecting over 400 million people worldwide. It is also known to be associated with neonatal jaundice, kernicterus and even death. Being an X-linked condition, the prevalence of G6PD deficiency in any given population is determined by the number of deficient males. However, deficient females are also at risk of haemolysis and jaundice. The inheritance of G6PD deficiency shows a typical X-linked pattern with higher incidence in males than in females.

These may produce either qualitative and or quantitative abnormality of enzyme. World Health Organization (WHO) has classified different G6PD
variants according to the magnitude of the enzyme deficiency and the severity of haemolysis.

**G6PD Deficiency and Neonatal Jaundice (NJ)**

Jaundice is one of the most common problems during the neonatal period. About 60% of the full term and 80% of premature infants may develop unconjugated hyperbilirubinaemia during the first week of life. Out of the various causes of neonatal jaundice (unconjugated hyperbilirubinemia), G6PD deficiency is one of the commonest cause.

The pathogenesis of jaundice in G6PD deficient neonates remains unclear, because at least 50% of G6PD deficient babies do not develop Neonatal Jaundice. Alternative mechanisms for this neonatal hyperbilirubinemia, such as decreased hepatic bilirubin conjugation (inefficient bilirubin conjugation), have been proposed. This hypothesis has recently been confirmed by the demonstration of a decreased di-conjugated bilirubin fraction in many of the G6PD-deficient newborns, and lower serum bilirubin values observed in infants who received Phenobarbital either antenatally or postnatally due to induction of enzyme glucoronyl transferase. The present study was aimed to find out the frequency of G6PD deficiency in neonatal jaundice present in the study area and to compare the frequency of G6PD deficiency in neonates with and without jaundice.

**MATERIALS AND METHODS**

**Patients:**
A total of 100 newborns of the age of 1-28 days were included in the study. Out of the total 100 newborns, 50 with jaundice were placed in group A and the 50 without jaundice were placed in group B. Both male and female newborns were included in the study without racial discrimination. Patients with infection and known blood group incompatibilities were excluded from the study. The study was conducted at Saidu Group of Teaching Hospitals Saidu Sharif District Swat from January 2012 to June 2012.

**Sample Collection:**
2-3ml of venous blood samples were collected from the study subjects. In each case two smears were prepared for hematological examination. One ml of blood was placed in EDTA-disodium (Ethylene diamine tetra acetic acid) containing tube. 1-1.5ml of blood was allowed to clot in a plane glass tube for serum extraction.

Following investigations were carried out on each sample:
1. Complete blood count (CBC) with peripheral smear and reticulocyte count
2. Examination for Heinz bodies
3. Serum Bilirubin (total and direct)
4. G6PD screening Test

CBCs including red cell indices (RBC count, Hct, MCV, MCH and MCHC) were performed by automated haematology analyzer. Control samples were also processed from time to time as part of Quality control programme.

**DLC (Differential Leukocytes Count)**

Already prepared and fixed peripheral blood smears from each sample, were stained with Giemsa stain. The stained smears were examined under the microscope for DLC (Differential leukocytes count) and RBC morphology following standard protocol.

**Reticulocyte Count:**
Reticulocyte count was performed on peripheral blood following incubation with New methylene blue (1g of New methylene blue was dissolved in 100ml of iso-osmotic phosphate buffer pH 7.4).

**Heinz Bodies:**
The samples were examined for Heinz bodies (denatured haemoglobin) by staining with methyl violet (0.5g of methyl violet in 100ml of 9g/L of NaCl).

**Serum bilirubin:**
Total serum bilirubin and direct serum bilirubin were estimated by using Randox kit No. BR 411 based on Jendrassik Grof method on Microlab 300 chemistry analyzer. Indirect serum bilirubin was calculated by subtracting direct fraction from total bilirubin.
**G6PD Screening Test**

G6PD screening test was performed on each sample using Sigma Diagnostics Kit based on visual qualitative color reduction method following standard protocol (Spain Diagnostics 21, Quai Du Clos Des, Spain)\(^7\).

Coefficient of correlation of different variables was done on SPSS software. The regression coefficient was worked out and represented graphically as regression line.

**RESULTS**

A total of 100 neonates (age 1-28 days), were classified in two groups as A and B, of 50 subjects each. Group A comprised of neonates with jaundice and Group B neonates without jaundice. Maximum number of patients in group A was brought to the out door patient department for treatment of jaundice. While most of those in group B were brought to the hospital for vaccination or routine check up.

In Group A there were 39 males (78%) and 11 females (22%). While there were 37 (74%) males and 13 (24%) females in group B. The total number of male and female subjects included in the study were 76 (76%) and 24 (24%) respectively.

The mean haemoglobin level in group A & B were 15.83±0.15 & 16.29±0.12 g/dL with levels of more than /3.5 g/dL in 80% & 90% of the neonates in both the groups respectively. The haemoglobin level difference between group A & B was significant (p<0.01). The mean white blood cell counts in group A & B were 11.96±0.31 & 12.14±0.30 x10\(^3\)/l respectively with more than 85% having a count in the range of 10-14 x10\(^3\) in both groups. The mean platelet count in group A & B were 289.84±11.86 & 290.14±11.65 x10\(^3\)/l respectively with more than 80% neonates falling in the range of 250-350 x10\(^3\)/l in both groups. No significant difference was seen between WBC count and platelets count of group A & B. The red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in both group A & B were falling in the normal range for more than 85% of the neonates. The remaining 15% had mild to moderate degree of low or high counts for the above parameters (table 1). The mean reticulocyte counts in group A & B were 4.30±0.18 & 3.84±0.13 % respectively. The difference between the reticulocyte counts was significant (p<0.05). Six neonates in group A and two neonates in group B had reticulocytes count of more than 6 % (6.5-8%). Out of these eight neonates, four from group A and one from group B had G6PD deficiency. No Heinz bodies were found in all of the neonates with normal G6PD enzyme activity and most of the G6PD deficient neonates, except in three cases who had high reticulocyte as well. Out of these three neonates, two were from group A and one from group B. The mean serum bilirubin total, bilirubin direct and indirect levels in group A were 13.50±0.46, 2.81±1.29 & 12.10±0.40 mg/dL respectively. While the mean serum bilirubin total, direct and indirect levels in group B were 1.89±0.5, 0.68±0.03 & 1.21±0.04 mg/dL respectively. The difference between serum bilirubin total and indirect of group A & B was significant (p<0.01). Ten neonates in group A had a serum bilirubin total and indirect level of >15.0 mg/dL and >14.0 mg/dL respectively (table 1).

G6PD deficient subjects in group A and B were 9 (18%) and 5 (10%) respectively. Out of which 14% were male and 4% were female were in group A and 8% male and 2% female in group B. The mean gestational age of neonates in group A & B were 37.5±0.10 & 37.8±0.12 weeks respectively. The history for consanguinity was present in 8 out of 9 and 4 out of 5 G6PD deficient neonates in group A & B respectively. The reticulocyte count was 2 to 6.0% in four and one G6PD deficient neonates in group A & B respectively. While Heinz bodies were found in two and one G6PD deficient neonates in group A & B respectively (table 2). Five out of nine G6PD deficient neonates had serum bilirubin total and indirect level of 7.0 to 22.0 mg/dL and 6 to 20.0 mg/dL respectively.

**DISCUSSION**

The total male and female neonates included in the study were 76 (76%) and 24 (24%) respectively. The higher number of males in the study may be
because of the Pakhtoon traditions, where male are given more importance as compared to females and thus many female infants may not had been brought to the hospital or the difference may be real. More female neonates were included in study by Kapian.

The present study showed that the maximum number of cases in group A were from 2-7 days with peak at day 3 followed by day 4, whereas, the maximum number of cases in group B were from 2-7 days with peak at day 3 and 4 followed by day 2. The maximum number of cases in group A presented with jaundice at the ages of 2-7 days. Rehman et al. has also reported maximum number of cases in the same age group of neonates. In both group A & B, the ages of the mothers of the neonates were ranging from 17-34 years with peak at 25 years. In group A & B the total number of primary gravida mothers were 14 & 17 respectively. In group A & B 30 & 22 mothers had 1st cousin marriages respectively. This high consanguinity has also been reported in Pathan families where marriages within the families are very common. A positive history of thalassemia was present in 8% and 6% of the neonate’s families in group A & B respectively. This is slightly higher than the study of other co-workers.

The mean gestational ages in group A & B is 37.62±0.12 and 38.06±0.12 weeks respectively, which is slightly lower than the study of Kaplan et al. The probable reason is the number of subjects and difference in genetic makeup. The difference between the mean gestational ages of group A&B is significant (p<0.01) and which is due to the fact of lower the gestational age of neonates more is the possibility of NNJ. The differential leucocytes count was normal as probably no associated illness was present in the neonates examined in the present study.

The serum bilirubin total and indirect levels seen in group A were in the range of 5.80-24.54mg/dL and 4.10-21.44mg/dL respectively. Five neonates with G6PD deficiency had a serum bilirubin total & indirect levels of 2.15mg/dL and 2.14mg/dL respectively. The difference between serum bilirubin total and indirect levels in group A & B was significant (p<0.01). The apparent reason for low levels of bilirubin in group B was exclusion of neonates with NNJ.

Frequency of G6PD deficiency has become an important variable, making a big proportion of the jaundiced neonates. The frequency of G6PD deficiency in the present study is 14% which is highly significant (p<0.0001) and correlates with other such studies carried out nationally as well as internationally. Qamer and co-workers have reported 29.3% & 12% by Gandapur and coworkers. The variations in G6PD deficiency may be due genetic make up of ethnic groups, frequencies of carrier individuals, sample size, socio-cultural differences and the methodology used for the detection of G6PD deficiency. In our study substantial sample size was used. Comparison of the frequency of G6PD deficiency in group A & B favours the role of G6PD deficiency in NNJ. Other such studies favour its role in NNJ. G6PD deficiency has been observed both in jaundiced and nonjaundiced neonates; therefore G6PD deficiency screening should be done to avoid associated complications.

CONCLUSION

The frequency of G6PD deficiency is significantly high both in jaundiced and non-jaundiced neonates, so G6PD deficiency screening is recommended in all neonates of the study area.
1. G6PD deficiency is one of the common etiological factors in neonatal jaundice.
2. First cousins marriages should be discouraged.

RECOMMENDATIONS

Severe neonatal hyperbilirubinemia continues to occur frequently. G6PD deficiency is also one of the major causes. The frequency of G6PD deficiency is significantly high in jaundiced neonates of the study area. This indicates a need for a more thorough assessment of newborn infants and consideration of strategies to identify at-risk newborns, such as pre-discharge measurement of serum bilirubin levels and G6PD deficiency screening. Increase in sample size and the study of genetic mutations in G6PD deficiency.
in the Study area are recommended.

**Table-1**: Summary of abnormal findings in G6PD deficient and G6PD normal neonates

<table>
<thead>
<tr>
<th>Group</th>
<th>G6PD deficient neonates (14)</th>
<th>G6PD normal neonates (16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gest. Age (mos)</td>
<td>1st cousin marriage</td>
</tr>
<tr>
<td></td>
<td>follow up</td>
<td></td>
</tr>
<tr>
<td>Group A (50)</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Group B (50)</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*p value is < 0.01

Mean±SEM1 gestational age, 1st cousin marriage, reticulocyte count, Heinz bodies serum bilirubin total & indirect in G6PD deficient and G6PD normal neonates of group A & B. Figure in parenthesis indicate the number of neonates in each group.

**Table -2**: Frequency of G6PD deficiency with gender distribution in neonates of group A & B

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender</th>
<th>G6PD Deficient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (50)</td>
<td>Male</td>
<td>7</td>
<td>14*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>4**</td>
</tr>
<tr>
<td>Group B (50)</td>
<td>Male</td>
<td>4</td>
<td>8***</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1</td>
<td>2****</td>
</tr>
</tbody>
</table>

*p<0.0001, **p<0.001, ***p<0.0001, ****p<0.001

Figure in parenthesis indicate the number of neonates in each group.

**REFERENCES**


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