Prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency among The Neonatal Population with Hyperbilirubinemia at Al-Galaa Teaching Maternity Hospital

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Abstract

Glucose-6-PD deficiency is a common clinically significant enzyme defect, causing severe indirect hyperbilirubinemia in neonates.

Patients and Methods: A total of 324 term neonates with hyperbilirubinemia were screened for G6PD-deficiency, from January to December 2010. The conversion of nicotinamide adenine dinucleotide phosphate to its reduced form in erythrocytes is the basis of diagnostic testing for the deficiency.

Results: The analysis of the results indicated that 10 (3.35%) neonates with indirect hyperbilirubinemia were G6PD-deficient. No statistically significant difference was detected between G6PD-deficient and non-G6PD deficiency groups in relation to the time of onset of jaundice, reticulocyte count, hematocrit level, serum bilirubin at admission and maximum serum bilirubin level. Phototherapy duration, duration of hospitalization and the need for exchange transfusion were higher in the G6PD-deficient group.

Conclusion: From this study we conclude that G6PD deficiency is a common enzyme defect in our neonatal population, which need early screening to provide the appropriate early management.

Key Words: G6PD – Hyperbilirubinemia – Neonate.

Introduction

GLUCOSE-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymatic disorder associated with high incidence of neonatal hyperbilirubinemia [1]. The G6PD enzyme catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate while concomitantly reducing the oxidized form of nicotinamide adenine dinucleotide phosphate (NADP+) to reduced nicotinamide adenine dinucleotide phosphate (NADPH), a required cofactor in many biosynthetic reactions, which maintains glutathione in its reduced form. Reduced glutathione acts as a scavenger for dangerous oxidative metabolites in the cell. With the help of the enzyme glutathione peroxidase, reduced glutathione also converts harmful hydrogen peroxide to water. Red blood cells rely heavily upon G6PD activity because it is the only source of NADPH that protects the cells against oxidative stresses. NADPH protects cells from oxidative damage. Because erythrocytes do not generate NADPH in any other way, they are more susceptible to other cells to destruction from oxidative stress. G6PD deficiency is an X-linked inherited disease that primarily affects males. The most common clinical feature of G6PD deficiency is lack of symptoms. However, in symptomatic neonates the presenting features are neonatal jaundice and/or acute hemolytic anemia. Jaundice usually appears by age 1-4 days, at the same time as or slightly earlier than physiological jaundice and later than in blood group allo-immunization. Acute hemolytic anemia results from stress factors such as oxidative drugs or chemicals, or infection. The prevalence of neonatal hyperbilirubinemia is twice that of the general population [2] in males who carry the defective gene and in homozygous females. It rarely occurs in heterozygous females [3,4]. Hemizygous males also suffer from the effects of deficiency. Homozygous females are found in populations in which the frequency of G6PD deficiency is quite high. Females who are either homozygous or who are phenotypically deficient heterozygous can demonstrate the manifestations of the disease. Infants with the severe variant of G6PD deficiency may develop hyperbilirubinemia sufficiently severe to cause kernicterus and death [5,6]. Where as in others this has not been observed [7]. This may reflect genetic mutations specific to different ethnic groups [8,9].
The mechanism by which G6PD deficiency causes neonatal hyperbilirubinemia is not completely understood. Although hemolysis may be observed in neonates who have G6PD deficiency and are jaundiced, other mechanisms appear to play a more important role in the development of hyperbilirubinemia. Hyperbilirubinemia is likely secondary to impairment of bilirubin conjugation and clearance by the liver leading to indirect hyperbilirubinemia. Infants with G6PD deficiency and a mutation of uridine diphosphoglucuronate glucuronosyltransferase-1 gene promoter (UDPGT-1) are particularly susceptible to hyperbilirubinemia secondary to decreased liver clearance of bilirubin.

G6PD deficiency should be considered in neonates who develop hyperbilirubinemia within the first 24 hours of life, a history of jaundice in a sibling, bilirubin levels greater than the 95th percentile, and in middle east and Asian males.

G6PD deficiency can lead to an increased risk and earlier onset of hyperbilirubinemia, which may require phototherapy or exchange transfusion.

The conversion of nicotinamide adenine dinucleotide phosphate to its reduced form in erythrocytes is the basis of diagnostic testing for the deficiency.

**Aim of the study:**
To determine the prevalence of G6PD deficiency as a cause of neonatal hyperbilirubinemia (after excluding other haemolytic causes) in the studied population and to compare the clinical presentation and course of G6PD-deficient and non-G6PD deficiency patients.

**Patients and Methods**
A prospective case-control study of the neonates with hyperbilirubinemia, admitted to the special care baby unit of the neonatal department at Al-Galaa Teaching Maternity Hospital, Al-Galaa Teaching hospital is a hospital following the General Organization of Teaching Hospitals and Institutes in Cairo-Egypt, and receives around 22,000 deliveries per year. We studied all patients admitted from 1st of January to 31st of December 2010. All neonates admitted to the neonatal special care baby unit for hyperbilirubinemia or developing hyperbilirubinemia during their admission for other medical reasons were subjected to a jaundice work-up scheme according to the guidelines of the department for screening patients with hyperbilirubinemia including screening for free T4 and TSH, urine analysis, urinary reducing substance.

**These guidelines are as follows:** A complete blood picture, maternal and infant blood groups, peripheral blood smear for abnormal red cell morphology, a reticulocytic count, direct coomb's test, total and direct serum bilirubin (TSB, DSB) and G6PD deficiency test. We excluded all cases with non-haemolytic causes of hyperbilirubinemia. Neonatal hyperbilirubinemia due to haemolytic causes was diagnosed if the following criteria were found: Anemia (Hb <8gm/l), Haematocrit level (Hct <25%) reticulocytosis (retics count >5%), Coomb's test +ve. G6PD screening test was done for all indirect neonatal hyperbilirubinemia.

The following clinical data were obtained for each infant included in the study: Gestational age, birth weight, sex, mode of delivery, traumatic delivery, age at onset of hyperbilirubinemia, maximum TSB levels attained, treatment mode, duration of phototherapy, and the need for exchange transfusion, duration of hospital stay, TSB and Hb level at discharge. Our guidelines are to initiate phototherapy for neonates following the guidelines of the American Academy of Pediatrics (AAP) for neonatal hyperbilirubinemia. Again the decision for exchange transfusion follows the guidelines of the department depending on the age and clinical status and the guidelines of the AAP.

Blood transfusions were given to NB according to our protocol depending on haematocrit value and clinical condition of the baby. Phototherapy is given as single or double as required by the severity of hyperbilirubinemia.

The type of treatment and the length of stay in the hospital were recorded. Parents were informed if the result was positive to avoid risk factors of haemolysis and take precaution measures.

**Methods:**
A venous blood sample was collected under complete aseptic conditions and used for: Complete Blood Count (sysmex), reticulocyte count (using brilliant cresyl blue stain and retics % is calculated), blood grouping, and direct coomb’s test (direct antiglobulin test expressed as positive or negative). Total and direct bilirubin are measured using the Chembiotest Kit. The total bilirubin concentration is determined in the presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely coloured diazo dye (560-600nm). The intensity of the color of the dye formed is proportional to the concentration of total bilirubin in the
serum. Direct bilirubin is determined in absence of caffeine by the direct reaction with the diazotized sulphanilic acid to form red-coloured azobilirubin diazo dye. The color intensity measured at 546nm is proportional to the concentration of total bilirubin in the serum.

**G6PD assay:** By the enzyme colorimetric UV method kit for quantitative in vitro determination of glucose-6-phosphate dehydrogenase activity in erythrocytes (by Biogamma). Principle of the test: The enzymatic activity of G6PD is used as a substrate for the Glucose-6-phosphate (G6P) in the presence of NADP+ is determined by the measurement of the rate of increased absorbance at 340nm, due to the reduction of NADP+ as indicated by the following reaction:

\[
\text{G6PD + NADP}^+ \rightarrow \text{G-6-P-DH} \rightarrow \text{gluconate-6-p} + \text{NADPH} + \text{H}^+
\]

**Preparation of the sample:** Fresh non-hemolytic plasma samples are used. A blood sample, 0.2ml is washed with 2ml aliquots of 0.9% NaCl solution, centrifugation after each wash for 10min at around 3000 rpm, and supernatants are discarded. The process is repeated 3 times. The washed centrifuged erythrocytes are suspended in 0.5ml of reagent 3 (digitonin 0.04mmol/l preservatives), and incubated for 15 minutes at 2-8 °C. Samples are then centrifuged for 5min at 3000 rpm and supernatants are used in the assay.

To calculate the enzymatic activity as mu/10⁹ erythrocytes, the calculated activity (mu/erythrocytes for ml of blood) for the RBCs/ml value. Reference value: 118-144µu/10³ erythrocytes.

**Statistical analysis:**

All the collected data were statistically analysed using SPSS (Statistical Package for Social Sciences) for Windows. For comparisons of data mean, standard deviation, Student t-test, Chi-square tests were used, p-values of less than 0.05 with confidence interval of 95% were considered to be statistically significant.

**Results**

A total of 1312 newborns were admitted to our neonatal intensive care unit at Al-Galaa Teaching Hospital during the study period (January to December 2010). The total number of cases with neonatal hyperbilirubinemia, backed-up by blood samples for serum bilirubin (direct and indirect), during hospital stay recorded during this period was 324 (24.7%) cases. The number of cases with neonatal indirect hyperbilirubinemia was reported to be 298 cases (92%). The number of cases with neonatal hyperbilirubinemia and haemolytic anemia due to G6PD deficiency was 10 (3.35%) cases.

Female neonates accounted for 146 (49%) of those with indirect hyperbilirubinemia and 152 (51%) were male neonates. The mean gestational age among indirect hyperbilirubinemia neonates was 38.2 (±2.45) weeks, 247 (82.88%) were full-term and 51 (17.12%) were preterm (≤36 weeks gestation). The mean age of onset of hyperbilirubinemia in studied neonates was 4.8 (±3.08) days. The mean bilirubin level at the time of diagnosis was 17.47 (±4.73) mg/dl, while the maximum serum bilirubin level attained was 20.32 (±4.81) mg/dl (Table 1).

During assessment of studied neonates to identify G6PD deficient cases, the etiologic cause of indirect hyperbilirubinemia was not diagnosed in 50.3% of cases, ABO incompatibility accounted for 31.2% of cases, while 8.05% were due to Rh incompatibility, 4.36% were due to combined Rh and ABO incompatibility, and 2.68% had jaundice related to a hypothyroid state (Table 2).

The prevalence of G6PD-deficient neonates among this group was 3.35%, sex-specific prevalence for boys was 80% and for girls was 20%. Male to female ratio in G6PD-deficient hyperbilirubinemic neonates was 4:1. Serum bilirubin levels at diagnosis among these babies ranged from 11-24mg/dL, with a mean of 17.07±6.64mg/dL. Hematocrit levels ranged from 25-54% with a mean of 44.78±5.18g/dL, and mean reticulocyte counts were 3.45±2.77%. The mean duration of phototherapy received was 4.11±2.88 days. Coomb’s test was positive in the hemolytic jaundice neonates not due to G6PD deficiency.

Mean serum bilirubin level at admission was 18.05±5.35 and 17.07±6.64mg/dl in G6PD normal and deficient groups, respectively (Table 3). Maximum serum bilirubin was 21.41±4.5 and 20.61±5.1mg/dl in G6PD normal and deficient groups, respectively. Both parameters showed statistically significant difference between G6PD-deficient and normal groups (p=0.001).

Sex distribution in G6PD-normal hyperbilirubinemic neonates was 65 females (47.45%) and 72 males (52.55%). The difference between sexes in relation to G6PD deficiency was statistically significant (p=0.013).

No statistical difference was detected between G6PD-deficient and non-G6PD groups in relation to the time of onset of jaundice, reticulocyte count,
hematocrit level, serum bilirubin at admission and maximum serum bilirubin level.

Duration of phototherapy treatment, duration of hospitalization and the need for exchange transfusion were higher in G6PD-deficient group.

Table (1): Demographic features and laboratory values of studied neonates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (gms)</td>
<td>2973±529.91</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.2±2.45</td>
</tr>
<tr>
<td>Onset of jaundice (days)</td>
<td>4.8±3.08</td>
</tr>
<tr>
<td>Bilirubin at diagnosis (mg/dl)</td>
<td>17.47±4.73</td>
</tr>
<tr>
<td>Maximum bilirubin level attained (mg/dl)</td>
<td>20.32±4.81</td>
</tr>
<tr>
<td>Phototherapy</td>
<td>3.4±1.2</td>
</tr>
<tr>
<td>Duration of jaundice (days)</td>
<td>5.7±1.6</td>
</tr>
</tbody>
</table>

Table (2): Etiologic distribution of indirect hyperbilirubinemia.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO incompatibility</td>
<td>93</td>
<td>31.2</td>
</tr>
<tr>
<td>Rh incompatibility</td>
<td>24</td>
<td>8.05</td>
</tr>
<tr>
<td>Rh &amp; ABO incompatibility</td>
<td>13</td>
<td>4.36</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>8</td>
<td>2.68</td>
</tr>
<tr>
<td>G6PD deficiency</td>
<td>10</td>
<td>3.35</td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>150</td>
<td>50.3</td>
</tr>
<tr>
<td>(other causes including physiologic jaundice)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Comparison between causes of indirect hyperbilirubinemia.

<table>
<thead>
<tr>
<th></th>
<th>Non Hemolytic</th>
<th>Hemolytic</th>
<th>Hemolytic</th>
<th>Hemolytic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=151</td>
<td>G6PD N=55</td>
<td>G6PD N=10</td>
<td>G6PD N=10</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male: Female (Ratio &amp; Number)</td>
<td>1.01:1</td>
<td>1.11:1</td>
<td>4:1</td>
<td></td>
</tr>
<tr>
<td>Onset of jaundice (day)</td>
<td>5.32±2.9</td>
<td>2.15±1.4</td>
<td>4.57±3.2</td>
<td></td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td>2.05±1.32</td>
<td>3.21±3.03</td>
<td>3.45±2.77</td>
<td></td>
</tr>
<tr>
<td>Coomb’s test</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.23±4.62</td>
<td>42.44±5.33</td>
<td>44.78±5.18</td>
<td></td>
</tr>
<tr>
<td>Bilirubin at admission (mg/dl)</td>
<td>15.32±5.6</td>
<td>18.05±5.35</td>
<td>17.07±6.64</td>
<td></td>
</tr>
<tr>
<td>Maximum bilirubin attained (mg/dl)</td>
<td>18.09±4.3</td>
<td>21.41±4.5</td>
<td>20.61±5.1</td>
<td></td>
</tr>
<tr>
<td>Duration of Phototherapy (days)</td>
<td>2.97±1.68</td>
<td>3.75±2.01</td>
<td>4.11±2.88</td>
<td></td>
</tr>
<tr>
<td>Duration of Hospitalization (days)</td>
<td>4.67±2.26</td>
<td>5.8±1.92</td>
<td>6.48±1.03</td>
<td></td>
</tr>
<tr>
<td>Need for exchange Transfusion (Number/%)</td>
<td>0 (0%)</td>
<td>3 (2.2%)</td>
<td>1 (10%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. (1): Distribution of indirect hyperbilirubinemia.

**Discussion**

Glucose-6-phosphate dehydrogenase deficiency, the most common enzyme deficiency worldwide, causes a spectrum of disease including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. Persons with this condition also may be asymptomatic. This X-linked inherited disorder most commonly affects African, Asian, Mediterranean, or Middle-Eastern populations. Approximately 400 million people are affected worldwide. Homozygotes and heterozygotes can be symptomatic, although the disease typically is more severe in persons who are homozygous for the deficiency. Neonatal jaundice is the most common clinical manifestation of G6PD deficiency. It has been reported that one-third of children with G6PD deficiency develop neonatal jaundice [3,4]. In our study, 3.35% of neonatal jaundice cases were due to G6PD deficiency all of them responded to treatment with phototherapy except one in whom serum bilirubin rise required further treatment with an exchange transfusion. Tanphaichitr et al. [20] reported that 49% of G6PD patients developed neonatal jaundice, of which 28.82% were physiological and 20% were pathological.

The prevalence of G6PD in neonates with indirect hyperbilirubinemia varies worldwide according to ethnic variations. Our finding that 3.35% of the cases of neonatal indirect hyperbilirubinemia were the result of G6PD deficiency was higher than that reported from Europe and Far east, but far much lower than that reported in Saudi Arabia [21], Nigeria [22] and in American Blacks [23] (18.4, 40 and 14% respectively) which shows a comparatively high prevalence. Studies from other parts of the world report low prevalence rates, e.g. in Spain [24], France [25] and Singapore [26] (1.57, 2.1 and 1.62% respectively) In other countries, it ranges from 0.09% in Italy 13 to 2.1% in Iran [27]. Different subtypes of the disease could explain the different presentations in different geographical locations worldwide [3,12].
The G6PD deficiency is an x-linked recessive disease, the frequency of affected males is expected to be equal to the number of heterozygote females, but our study failed to produce this result. In our study we detected a male: female ratio of 4:1. Similar ratio was reported in a study from Iran [27]. So the number of girls with G6PD deficiency should not be underestimated. No correlation was observed between the gestational age and G6PD deficiency. It is reported that more neonates with G6PD deficiency had serious hyperbilirubinemia than term neonates. Near-term neonates are also susceptible because of the delayed conjugation and difficult feeding.

Hyperbilirubinemia in G6PD-deficient neonates is thought to be secondary to reduced hepatic conjugation and excretion of bilirubin, rather than increased bilirubin production resulting from hemolysis [28]. In our study we found no difference in reticulocyte count and hematocrit level between G6PD-deficient and G6PD-normal cases.

In the G6PD-deficient group both serum bilirubin at admission and maximum serum bilirubin levels were not higher when compared to G6PD-normal group. The difference was not statistically significant. In certain populations, hyperbilirubinemia secondary to G6PD deficiency results in an increased rate of kernicterus and death, [26,27] whereas in other populations this has not been observed [18]. This may reflect genetic mutations specific to different ethnic groups [18,19]. In a study from Oman, 71% of kernicterus patients were reported to be due to G6PD deficiency [29]. In our study we reported no cases of kernicterus in neonates with hyperbilirubinemia whether G6PD-deficient or not. However, one case developed clinically significant rise in serum bilirubin requiring additional exchange transfusion.

The age of onset of jaundice was 4.57 ± 3.2 days in G6PD-deficient neonates. This was not significantly different from the finding in G6PD normal neonates. This finding should be underlined to state the importance of early screening programmes for G6PD-deficiency, which could cause severe neonatal morbidity. The World Health Organization recommends screening all newborns in populations with a prevalence of 3 to 5 percent or more in males [30]. The early characterization of G6PD activity provides an aetiological diagnosis for neonatal jaundice, as well as the opportunity to give the newborn’s family information concerning haemolytic crisis prevention.

**Conclusion**

The analysis of the results indicated that 10 neonates (3.35%) with indirect hyperbilirubinemia were G6PD-deficient. No statistically significant difference was detected between G6PD-deficient and non-G6PD deficient groups in relation to the time of onset of jaundice, reticulocyte count, hematocrit level, serum bilirubin at admission, maximum serum bilirubin level. However, phototherapy duration, duration of hospitalization and the need for exchange transfusion were higher in G6PD-deficient group.

From this study we conclude that G6PD deficiency is a common enzyme defect in our neonatal population causing severe indirect hyperbilirubinemia requiring treatment. Early neonatal screening programmes should be instituted to anticipate and institute early treatment.

**References**


