

# Neonatal Screening for Glucose-6-Phosphate Dehydrogenase Deficiency

Mritunjay Pao, Anjali Kulkarni, Vidya Gupta, Sushma Kaul and Saroja Balan

*Division of Neonatology, Apollo Center for Advanced Pediatrics, Indraprastha Apollo Hospital, New Delhi, India*

**Abstract. Objective:** This study was carried out to detect the incidence of erythrocytic Glucose-6-Phosphate dehydrogenase (G-6-PD) deficiency, to compare the incidence of hyperbilirubinemia in G-6-PD deficient neonates as compared to G-6-PD normal neonates and to assess the usefulness of neonatal screening for G-6-PD deficiency. **Method:** In a retrospective hospital based study 2,479 male and female neonates consecutively born at Indraprastha Apollo hospital between July 1998 to June 2003 who were screened for G-6-PD levels were evaluated for the incidence of G-6-PD deficiency. **Results :** Incidence of G-6-PD deficiency was found to be 2.0%. Incidence in males was 2.83% and female was 1.05%. The incidence of hyperbilirubinemia was found to be 32% in G-6-PD deficient neonates which was significantly higher than the incidence of hyperbilirubinemia in neonates with normal G-6-PD, which was 12.3% ( $P < 0.001$ ). **Conclusion :** Our data suggests that neonatal screening for G-6-PD deficiency is a useful test for preventing and early treatment of complications associated with it. [Indian J Pediatr 2005; 72 (10) : 835-837] E-mail : kulkarnianjali@hotmail.com

**Key words :** Glucose-6-phosphate dehydrogenase deficiency; Screening

Glucose-6-phosphate dehydrogenase deficiency is the most common enzyme deficiency involving more than 400 million people worldwide.<sup>1</sup> G-6-PD deficiency was first reported in India in 1961.<sup>2</sup> The exact incidence in India is not known; several studies have reported very high incidence 2-27.9%.<sup>3-6</sup> G-6PD catalyzes the first step in the hexose monophosphate pathway of glucose metabolism, and it produces NADPH which keeps glutathione in its reduced form. Glutathione protects red blood cells (RBCs) from oxidative damage. G-6-PD deficiency is clinically manifested as acute hemolytic anemia, chronic non-spherocytic hemolytic anemia and neonatal hyperbilirubinemia, which is of hemolytic origin by agents causing destruction of the red blood cells.<sup>7</sup> Many authors have reported significantly higher levels of bilirubin in G-6-PD deficient neonates compared to G-6-PD normal neonates even without any evidence of hemolysis.<sup>8-10</sup> The present study was carried out (i) to detect the incidence of erythrocytic G-6-PD deficiency, (ii) to compare the incidence of hyperbilirubinemia in G-6-PD deficient neonates as compared to G-6-PD normal neonates and (iii) to assess the usefulness of neonatal screening for G-6-PD deficiency.

## MATERIAL AND METHOD

In a hospital-based retrospective study, all male and female neonates consecutively born at Indraprastha Apollo Hospital from July 1998 to June 2003 were

included. Their inpatient hospital records were reviewed to determine the incidence of erythrocytic G-6-PD deficiency. In order to eliminate other factors causing neonatal hyperbilirubinemia, the authors included only healthy term babies and neonates with other factors such as polycythemia, sepsis, ABO and Rh incompatibility, infant of diabetic mother, gastrointestinal obstruction and cephalhematoma were excluded from the study.

Both qualitative and quantitative studies were done from blood samples taken from cord blood for confirmation (kit: Sigma Diagnostics, U.S.A.). G-6-PD deficiency was defined as a level less than 100 U/Trillion RBCs. Hyperbilirubinemia was defined peak serum bilirubin levels <sup>3</sup> 15mg/dl. Serum bilirubin levels were obtained when infants were visually icteric and phototherapy was started when indicated (12 mg/dl at 24-48hours old, >14 mg/dl at 48-72 hours, >15mg/dl at 72-120hours, and >17 mg/dl at >120hours old).

## RESULTS

**Incidence of G-6-PD deficiency:** A total of 2,479 neonates were included in this study. There were 1,343 males and 1,136 females. 50 neonates were found to be G-6-PD deficient, incidence being 2.0%. The difference in the incidence of G-6-PD deficiency in males 2.83% (n=38) and females 1.05% (n=12) was significant ( $p < 0.002$ ). The mean level of G-6-PD was  $62.84 \pm 22.55$  U/Trillion RBCs in the G-6-PD deficient neonates, whereas the mean level of G-6-PD in 100 neonates with normal level was  $364 \pm 134.42$  U/Trillion RBCs.

**Correspondence and Reprint requests :** Dr. Anjali Kulkarni, Senior Consultant, Neonatology, C-1/4, Tilak Lane, New Delhi 110001

**Incidence of hyperbilirubinemia in G-6-PD deficient neonates:** Out of the 50 G-6-PD deficient neonates in this study, 16 had hyperbilirubinemia, an incidence of 32%. Of these 16 neonates, three neonates required exchange transfusion. None of the neonates with hyperbilirubinemia had any other factors that could have caused hyperbilirubinemia such as polycythemia, sepsis, in infant of diabetic mother, ABO incompatibility, cephalhematoma etc.

**Incidence of hyperbilirubinemia in male was significantly higher than female neonates with G-6-PD deficiency:** Of a total of 38 male infants with G-6-PD deficiency, 14 had hyperbilirubinemia, an incidence 36.8%. Of 12 female neonates with G-6-PD deficiency only 2 had hyperbilirubinemia, an incidence of 16.6% ( $p < 0.001$ ).

**Incidence of hyperbilirubinemia in neonates with normal G-6-PD levels:** The incidence of hyperbilirubinemia in G-6-PD normal neonates in the same study period was found to be 12.3%. The incidence of hyperbilirubinemia in G-6-PD deficient neonates (32%) was found to be significantly higher as compared to the incidence of hyperbilirubinemia in G-6-PD normal neonates (12.3%) ( $P < 0.001$ ).

**Mean maximum serum bilirubin level:** The mean maximum serum bilirubin level in the G-6-PD deficient group was 17.8 mg/dl. In neonates with normal G-6-PD levels the mean maximum bilirubin level was 14.6 mg/dl.

**Lowest level of hemoglobin:** The lowest level of hemoglobin in neonates with G-6-PD deficiency was 7.0 gm/dl in and a reticulocyte count of upto 8%.

**Mean duration of phototherapy:** The mean duration of phototherapy in the G-6-PD deficient and neonates with normal G-6-PD levels was  $3.8 \pm 1.3$  days and  $2.3 \pm 1.6$  respectively ( $p < 0.001$ ).

## DISCUSSION

In this retrospective study the incidence of G-6-PD deficiency was high (2.0%). Several Indian authors<sup>3-6</sup> have reported very high incidence in different Indian communities. It was found that the incidence of hyperbilirubinemia was significantly higher in G-6-PD deficient neonates (32%) as compared to G-6-PD normal neonates (12.3%), this finding being comparable to other studies.<sup>11-14</sup> All infants admitted in the nursery were at a minimum risk of exposure to agents causing hemolysis as all staff caring for the neonates routinely wash hands and all parents were required to wash hands properly before handling their babies. In the present study, presence of other factors such as polycythemia, sepsis, ABO and Rh incompatibility, infant of diabetic mother, gastrointestinal obstruction and cephalhematoma which could lead to neonatal hyperbilirubinemia were looked for and

excluded. Many researchers have reported that neonates with very low levels of G-6-PD had higher levels of hyperbilirubinemia.<sup>11,15</sup>

The gene for G-6-PD deficiency is located in the terminal region of the long arm of the X-chromosome at position q28. Most of the mutations affecting this gene are single base mutations. It is an X-linked condition which usually manifests itself in males carrying the mutant gene. The phenotype in females may be normal homozygote, G-6-PD deficient homozygote or heterozygous.<sup>16,17</sup> In females the condition manifests when there are two defective copies of the gene in the genome i.e., homozygous. Random X-chromosome inactivation may result in two RBC populations in female heterozygotes, one population consists of RBCs with normal G-6-PD activity and the other population with G-6-PD deficient cells.<sup>18</sup> X-inactivation may be nonrandom or one or the other clone may be selected preferentially, there may be varying phenotypes and the RBCs of the heterozygous females may exhibit normal, intermediate or grossly deficient G-6-PD activity.<sup>19</sup>

An estimated 390,000 babies with G-6-PD deficiency are estimated to be born each year in India.<sup>20</sup> According to reports from India the various genetic mutations are G-6-PD Mediterranean (563 C→T), which is the most common, followed by G-6-PD Kerala-Kalyan (949 G→A) and G-6-PD Orissa (131 C→G). Mutations are mostly seen in exon 6 and 7, which is in close proximity to the G-6-PD binding site.<sup>21</sup> G-6-PD Mediterranean was found to have significantly lower red cell enzyme activity and more severe clinical manifestations than the other two. G-6-PD Chatham (1003 G→A) with undetected red cell enzyme activity and G-6-PD Insuli (989 G→A) with normal G-6-PD activity are very rare in the Indian population.<sup>22</sup> G-6-PD Mediterranean is the most severe variety, association with drug induced hemolytic anemia, and chronic nonspherocytic hemolytic anemia have been reported from India.<sup>23</sup> G-6-PD Mediterranean mutation is found in Vatalia Prajapatis of North India<sup>24</sup> and Parsis, G-6-PD Kerala-Kalyan mutation has been reported from Maharashtra, Kerala, Punjab and people originating from Andhra Pradesh and Tamil Nadu.<sup>21</sup> G-6-PD Orissa mutations have been reported in Tribals of central, Eastern and Southern India.<sup>25</sup>

A point mutation at the 1376 base pair of G-6-PD cDNA accounts for more than half of G6PD deficient population in Taiwan.<sup>26</sup> Naphthalene mothballs were a major cause of neonatal hyperbilirubinemia and acute hemolytic anemia in G-6-PD deficient patients in Taiwan. Following the introduction of neonatal population screening programmes and major health awareness campaigns by the Government, there was a drastic decrease in the incidence of neonatal hyperbilirubinemia and acute hemolytic anemia in G-6-PD deficient patients in Taiwan and Singapore.<sup>27</sup> Severe neonatal hyperbilirubinemia resulting in extreme cases to Kernicterus and death is a potentially serious

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complication of G-6-PD deficiency.<sup>28-29</sup> One study from India reported G-6-PD deficiency to be the commonest cause of neonatal hyperbilirubinemia in very low birth weight neonates.<sup>30</sup> Studies from India have reported drug-induced hemolysis in G-6-PD deficient patients severe enough to cause renal shut down and renal failure.<sup>31,32</sup> Most G-6-PD deficient people go through life without ever being aware of the fact they have this genetic trait,<sup>33</sup> although being at a risk for serious complications. G-6-PD deficiency is one of the disorders, which deserves to be screened in the newborn period in India.<sup>34</sup> As the test itself is not costly, the authors recommend that neonatal screening for G-6-PD deficiency should be routinely done, so that complications like acute hemolysis and hyperbilirubinemia can be managed early and kernicterus eliminated.

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