GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND ITS CORRELATION WITH SERUM BILIRUBIN IN NEONATES.

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ABSTRACT

G6PD deficiency is one of the most common inherited disorders of mankind, with more than 400 million people being affected worldwide. It is probably the most common inborn error of metabolism in human which in adults can cause chronic haemolytic anemia or drug induced or stress induced acute hemolysis, whereas in neonates it is one of the common cause of neonatal hyperbilirubinemia. **Purpose:** The aim of the study was to find out the extend of G6PD deficiency and to make a correlation between G6PD activity and serum bilirubin levels in neonates. **Patients and methods:** A total of 100 neonates of age 1-7 days were selected for the study in which 57 were males and 43 were females. Quantitative estimation of Total serum bilirubin, Direct serum bilirubin and Indirect serum bilirubin were done by end point method. G6PD activity was measured by kinetic (increasing) method. **Results:** Extend of G6PD deficiency was found to be 3% in which male female ratio was 2:1. 40% of the neonates were found to have hyperbilirubinemia and another 40% were normal. Only the direct serum bilirubin was statistically significant (p=0.013) with G6PD activity. Total serum bilirubin was statistically higher in G6PD deficient cases (mean= 9.90 ±6.57 mg/dl) than normal G6PD (5.65±3.56 mg/dl). Total and indirect serum bilirubin were found to be statistically significantly different between G6PD deficient and normal cases with p=0.015, 0.023 respectively. **Conclusion:** The prevalence of G6PD deficiency was found to be 3%, thus early detection of this enzymopathy regardless of sex and close surveillance of the newborns is important.
KEYWORDS: G6PD, hemolytic anemia, neonatal hyperbilirubinemia, serum bilirubin.

INTRODUCTION
Glucose 6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme critical in the redox metabolism of all aerobic cells. It oxidizes glucose-6-phosphate to 6-phosphogluconolactone, reducing NADP to NADPH (Fig.2.1).\cite{1} The HMP shunt is the only source of NADPH in the erythrocytes. Glutathione peroxidase (GSHPx) removes peroxide from the erythrocytes.\cite{2} Reduced glutathione (GSH) serves as a substrate for this enzyme.\cite{3} In G6PD deficient individuals, the NADPH production is diminished and detoxification of H$_2$O$_2$ is inhibited. Cellular damage results due to lipid peroxidation leading to erythrocyte membrane breakdown, and protein and DNA oxidation.\cite{4}

G6PD deficiency is probably the most common inborn error of metabolism in humans and was the first erythrocyte enzyme deficiency discovered.\cite{5} which in adults can cause chronic haemolytic anemia or drug induced or stress induced acute hemolysis, whereas in neonates it is one of the common etiological factor causing neonatal hyperbilirubinemia. Extensive work on the genetics, biochemistry and molecular pathology of the disorder has made it the best understood and the most thoroughly studied of the enzymopathies.\cite{6}

G6PD deficiency was discovered as a result of a series of investigations performed to understand why some persons were uniquely sensitive to the development of hemolytic anemia when they ingested the 8-aminoquinoline anti-malarial drug primaquine.\cite{3} It is an inherited X-linked recessive disorder with varied clinical presentations including neonatal jaundice, hemolysis, acute icterus after exposure to chemicals and drugs, anemia, acute jaundice following consumption of fava beans (favism), and also congenital chronic non-spherocytic hemolytic anemia.\cite{7} Of these manifestations, neonatal jaundice is the earliest one, and the most critical sign for early diagnosis of this genetic disorder.\cite{8}

Around 5% of neonates with G6PD deficiency will develop jaundice after the first 24 hours of life (in contrast to foetal erythroblastosis), and their serum indirect bilirubin reaches a peak at days 3 to 5, often more than 20 mg/dl. When jaundice becomes apparent from the end of first week, its peak may be delayed up to the 2nd week. Early diagnosis of deficiency of G6PD is quite important, because this disorder may cause severe hemolysis and anemia in the newborn, if undiagnosed.\cite{9} Neonatal jaundice is one of the most life and health threatening consequences of G6PD deficiency\cite{3} and if untreated may have irreversible neurological
sequelae.\textsuperscript{[10]} Jaundice develops later than in Rhesus alloimmunization, typically on the 2\textsuperscript{nd} and 3\textsuperscript{rd} day of life. In the majority of cases, anemia is not conspicuous suggesting that the underlying mechanism of hyperbilirubinemia is primarily hepatic in origin. A haemolytic component may predominate after neonatal exposure to oxidants, e.g. drugs.\textsuperscript{[10]}

Clinically, deficiency of G6PD affects as many as 400 million individuals worldwide. It is most commonly prevalent in African, South East Asian and Middle Eastern Populations. It affects 1 in 10 African American males in United States. In India, there are 13 biochemical variants of G6PD being reported so far, out of which G6PD Mediterranean is most common in caste groups; whereas G6PD Orissa is most prevalence in the tribal of India. The 3\textsuperscript{rd} most common variant seen in India is G6PD Kerala-Kalyan.\textsuperscript{[11]} The incidence of G6PD deficiency in Indian population varies from 0 to 27.02\%.\textsuperscript{[1]} At the continental level, highest prevalence of G6PD is predicted across sub-Saharan Africa, where prevalence drops below 5\% only on the edges of its distribution in eastern and southern Africa. In spite of being so common, the implications of G6PD-associated primaquine reactions are not currently of major concern due to the present status of malaria control across much of the continent.\textsuperscript{[12]}

The incidence of neonatal hyperbilirubinemia has repeatedly been shown to be several fold greater in G6PD-deficient populations than in the G6PD normal population.\textsuperscript{[13,14]} However, this incidence is not constant and may vary between population groups and from geographic area to geographic area.\textsuperscript{[15]} Two clinical manifestations are apparent: the first, severe jaundice resulting from acute hemolysis, akin to favism; the second, jaundice of more gradual onset.\textsuperscript{[16,17]}

**MATERIAL AND METHODS**

Total 100 neonates of age group 1-7 days from the Department of Obstetrics and Gynaecology, and Paediatrics, M.M.I.M.S.R, Mullana, Ambala were taken for the study. Neonates with known causes of hyperbilirubinemia, and very sick babies were excluded. The study was conducted for the period of one year from Jan 2013 to Dec 2013. Quantitative estimation of Total serum bilirubin, Direct serum bilirubin and Indirect serum bilirubin were done by end point method.\textsuperscript{[19]} G6PD activity was measured by kinetic (increasing) method.\textsuperscript{[18]}

**RESULTS**

Among 100 neonates of age group 1-7 conducted for study, 57 were male and 43 were female. The extent of G6PD was 3\% in which male and female ratio was 2:1. Normal G6PD activity
was 4.6 to 15.0 U/gHb.\cite{18} Normal total serum bilirubin level was considered maximum 6mg/dl and 12mg/dl for term and pre-term infants respectively whereas minimum level was considered 1mg/dl and 2mg/dl.\cite{20} According to the total serum bilirubin levels, among the total 100 live neonates screened 40 were normal. Next 40 were with hyperbilirubinemia and 20 were with Hypobilirubinemia. G6PD activity was found to be negatively correlated with total serum bilirubin, direct serum bilirubin, indirect serum bilirubin and age whereas the birth weight was found to be positively correlated. Only direct serum bilirubin was statistically significant with regard to G6PD activity. (Fig 1).

In this study, total and indirect serum bilirubin were found to be statistically significantly different between G6PD deficient and normal cases with p=0.015, 0.023 respectively.

![Fig 1:-Correlation between G6PD activity and direct serum bilirubin of infants.](image-url)
DISCUSSION

This study was conducted to find out the extent of G6PD deficiency and to establish its relationship with serum bilirubin in neonates because it is one of the cause of neonatal jaundice. The frequency of this enzyme deficiency varies from 1.5%-51% in different parts of the globe.\[21\] The incidence of G6PD deficiency is 3% in the present study (male: female = 2:1).

In this study, 40% of neonates presented with neonatal jaundice while the other 40% were found to be normal. The mean of the total serum bilirubin was 5.86 ± 3.82 mg/dl. (fig 2) The mean peak total serum bilirubin level was 18.03 mg/dl in a study by Bora et al showed a higher maximum average total serum bilirubin level (20.2 mg/dl) in G6PD deficient babies as compared to G6PD normal babies (16.7 mg/dl).\[22\] Iranpour et al.\[23\] showed that mean bilirubin level in G6PD deficient group was 22.26± 8.36 mg/dl while studying 705 clinically icteric neonates. So, finding in the present study was with low mean of total serum bilirubin than the findings of the above studies.

In the present study, total serum bilirubin is statistically higher in G6PD deficient cases (mean= 9.90 ±6.57 mg/dl) than normal G6PD (5.65±3.56 mg/dl) cases (p< 0.05). The
supportive study conducted by Gendy et al, reported the same result. There was a significant
difference between G6PD deficient cases and G6PD normal cases as regards total serum
bilirubin (19.5 ± 3.3, 16.1 ± 4.1 mg/dl respectively) (p<0.05).[24]

No statistically significant difference between G6PD deficient and normal cases as regards
birth weight, total serum bilirubin, direct serum bilirubin and indirect serum bilirubin was
presented by Gendy et al.[24] In respect to this study, total and indirect serum bilirubin were
found to be statistically significantly different between G6PD deficient and normal cases with
p=0.015, 0.023 respectively in the present study which is supported by the study conducted
by Silao CLT et al (they reported no statistically significant difference between G6PD
deficient and normal cases as regards birth weight and direct serum bilirubin and they found
total and indirect serum bilirubin were found to be statistically significantly different between
G6PD deficient and normal cases with p=0.02, 0.01 respectively).[25] El-Menshay et al
reported that only total bilirubin level was found to be significantly different (p=0.001)
between G6PD deficient and normal cases, rest were not statistically significantly
different.[26]

The relationship between G6PD deficiency and hyperbilirubinemia in the newborn period is
well recognized, and our study proves it by showing the negative correlation between G6PD
activity and serum bilirubin levels. In addition to, the relationship between reticulocyte count,
haematocrit level and correlation between age onset of peak bilirubin should be established in
between G6PD-deficient and normal groups because age also plays an important role in
development of neonatal jaundice in both G6PD deficient and normal neonates as suggested
by Bushra et al.[27]

CONCLUSION

The prevalence of G6PD deficiency was found to be 3%, thus early detection of this
enzymopathy regardless of sex and close surveillance of the newborns is important. Early
detection reduces the risk of complications secondary to hyperbilirubinemia such as
kernicterus and hemolytic anemia. Implementation of newborn screening of neonates to
identify G6PD deficient individuals will avoid extension of hospital stay for affected
newborns through timely counselling of their parents and caregivers. Counselling should be
aimed at increasing awareness of hemolytic triggers. Further research focussed at estimation
of G6PD activity in the parents/siblings of the affected neonates with additional mutation
analysis will help to better explain inheritance patterns and is likely to improve the
management of affected neonates.

AUTHOR CONTRIBUTION
Bashu Dev Pardhe:- sample collection, analysis and calculation.
Dr. Rajesh Pandey:- Interpretation and manuscript preparation.
Dr. PD Sharma, Dr. Jasbir Singh, Mukund Joshi, Prem Paudyal :- manuscript evaluation.

DISCLOSURE
The author reports no conflicts of interest in this work.

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