Quantitative Assay of Glucose-6-Phosphate Dehydrogenase in Normal Non-Icteric Early Neonates: A Hospital-Based Study

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ABSTRACT

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) a housekeeping enzyme found in the mitochondria of all cells of the body plays a crucial role in preventing cells from oxidative damage by reactive oxygen species (ROS). As Red Blood Cell (RBC) lacks a subcellular structure like mitochondria, it is most vulnerable due to the lack of G6PD enzyme resulting in mild hemolytic jaundice to fetal death. The Prevalence of neonatal jaundice is most common but due to a lack of newborn screening for the G6PD test, neonates are misdiagnosed and the whole treatment becomes worthless. The main aim of this study was to evaluate the level of G6PD in normal early neonates.

Methods: This was a cross-sectional study conducted on 43 normal early neonates of the Indian sub-population from January to June 2019 at the Laboratory Department, Padmashree institute of clinical research, Bangalore, India. Blood parameters like G6PD levels, direct and total bilirubin, and hemoglobin levels of the normal neonates were measured. This study was approved by the ethical board of the hospital.

Results: The bilirubin level (direct and total), and hemoglobin level were found within the established normal reference interval. The observed G6PD levels in normal healthy full-term neonates against the available various reference intervals were established. In this study the cut-off value of G6PD enzyme activity was determined as 8.05 ± 1.2 U/g Hb and this in-house obtained reference interval level was significantly (*p< 0.05) lower than that of other reference intervals such as Kit insert Ref. Interval (9.55 ± 4.6 U/g Hb) and Ref. interval (12.5 ± 2.3 U/g Hb) as inferred by other studies.

Conclusions: In conclusion, we found evidence for a newly established reference interval for normal neonates between the ages of 1 to 11 days and the reference value is remarkably lower than the available reference interval. This established reference interval can be used as an appropriate diagnostic reference for clinical decision-making than using it as a reference interval.

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INTRODUCTION

Among various cytoplasmic enzymes, Glucose-6-phosphate dehydrogenase (G6PD) has a crucial role in preventing cells from oxidative damage by generating reactive oxygen species (ROS).1 NADPH is produced in the pentose phosphate pathway by the catalytic action of G6PD and it is responsible for the maintenance of a reducing environment in cells including RBCs which are vulnerable to oxidative damage. The gene coding for G6PD is located on X-chromosome, band Xq28. The 18 kb long G6PD gene has 13 exons; the protein-coding region contains 12 segments ranging in size from 12 to 236 bp.2 

G6PD deficiency is the most common x-linked heritable enzyme abnormality affecting around 400 million people in the world3 and there are as many as 160 G6PD mutations in the gene.4,5 G6PD-deficiency can lead to hemolytic anaemia which gives rise to neonatal jaundice and life-threatening reactions during the first week of life. It is an important cause of pathological hyperbilirubinemia, jaundice, and kernicterus.5

Newborn babies with G6PD deficiency appear normal at birth. Exposures to infection with microorganisms, medications (primaquine, sulfa drugs), and intake of food (fava beans, legumes) can trigger the destruction of red blood cells leading to hemolysis.6 Most cells use alternative metabolic pathways to produce NADPH except RBCs which solely depend on G6PD to produce NADPH. Thus, G6PD deficiency results in the destruction of RBC whenever there is oxidative stress and causes hemolytic anaemia. When RBCs are breaking down quicker than they are synthesized for replacement, it leads to a rise in heart rate, feeling of excessive tiredness, and shortness of breath. When neonatal jaundice takes place due to hemolysis eventually it can lead to serious conditions like damage to neurons or even death. Neonatal indirect hyperbilirubinemia (NIH) is a frequently observed problem among infants affecting 80% of preterm newborns and 60% of full-term, especially in the first three days of life. Phototherapy should be given to infants who showed prolonged neonatal jaundice occurring due to G6PD deficiency.7 Though infants with G6PD deficiency may show severe complications in the newborn period, they generally experience normal physiological growth and development. Hence, the main objective of this study was to evaluate the level of G6PD in normal early neonates.

METHODS

This was a cross-sectional study conducted from January to June 2019 at the Laboratory Department, Padmashree Institute of Clinical Research, Bangalore, India. All of the healthy full-term delivered new-born less than 28 days during the study period (n=43) were included in this study.

Premature birth, parents having auto-immune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), etc., and parents having a clinical history of G6PD deficiency, and other genetic diseases were excluded from this study. Similarly, neonates suspected of drug-induced hemolytic jaundice, age above 1 month, and neonates with hyperbilirubinemia (Total bilirubin > 12mg/dl) were excluded from the study. Written informed consent was obtained from the parent or guardian. The study proposal was approved by the Institutional Review board PICR, Bangalore with the reference number PICR_23-2-2019.

Specimen handling and analysis: The EDTA blood specimens received in the laboratory were processed for the analysis of routine clinical pathology parameters sought by the treating clinicians. The samples were brought to the clinical chemistry laboratory of the Padmashree institute of clinical research and stored at 2 – 8 °C. Researches has shown that the preserved whole blood G6PD enzyme stability is 5 to 7 days.4,9,11,12 Hence, in the interest of avoiding analytical bias, the assay was conducted on the same day.

ASSAY PROTOCOLS

The analytes of interest in EDTA blood were assayed by the following methods.

1. Whole blood Hemoglobin:
   Method: Semi-Conductor laser scatter combined with chemical dye method (cyanide-free spectrophotometric method) was used.
   Instrument & reagents: Mindray BC 5380 and reagents used as provided by the manufacturer. Manufactured by Mindray Medical International Co. Ltd.
   QC material: 3 levels of QC material of Bio-Rad (low, normal, and high) per day.
   Detection limit: 4-18 g/dl.

2. Bilirubin Total & Direct
   Method: Diazo method
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The full-term normal neonate’s total bilirubin level was $10.0 \pm 1.21 \text{mg/dL}$ against the PDC reference interval of $11.7 \pm 5.8 \text{mg/dL}$. The measured total bilirubin among normal neonates is once again underscored to evolve our own established reference interval for normal healthy neonates of Indian sub-population. (Figure 3)

Whole blood G6PDH levels

In the present study we observed our in-house obtained reference interval of G6PDH levels in normal healthy neonates against the available various reference intervals as $8.05 \pm 1.2 \text{U/g Hb}$ which is comparable with Kit insert Ref. interval ($9.55 \pm 4.6 \text{U/g Hb}$) and Review article Ref. interval ($12.5 \pm 2.3 \text{U/g Hb}$). (Figure 4)

Figure 4: G6PDH level of normal neonates compared to various reference intervals available from Kit Ref interval, Literature Ref interval. (Expressed as Mean ± SD)

DISCUSSION

Hemoglobin is one of the most often sought-after investigations to confirm or rule out the suspected nutritional or anemic status in various disorders. The measured total bilirubin among normal neonates has been underscored by the established reference interval for normal healthy neonates of the Indian sub-population. In the present study, the full-term early neonate’s total bilirubin level was $10.0 \pm 1.21 \text{mg/dL}$ against the PDC reference interval of $11.7 \pm 5.8 \text{mg/dL}$. These findings are similar to the research conducted by Gómez-Manzo et al.¹ Kaplan et al.⁵ and Kosha et al.⁷ Similarly, in a present study the Hb level in normal neonates was $17.7 \pm 2.5 \text{g/dL}$, and the measured Hb level in established reference interval group did not show any significant ($p>0.05$) difference than the normal reference interval which was in accordance to other studies.²,⁴,⁷

Though the observed values in normal early neonates did not show any statistically significant difference ($p>0.05$) against the PDC reference interval, the available reference interval from literature ³,⁸ had a larger reference interval when compared to the present study outcome. In this regard, one resolved question underscores that the obtained narrow range augmented the diagnostic sensitivity to apprehend the pathophysiological process, clinically relevant, and also important to monitor the disease progression. Interestingly, the established reference interval level was significantly (*$p<0.05$) lower than that of other reference intervals.⁵,⁶ The study outcome underscores the heterogeneity of the clinical materials with respect to a number of factors like nature, age, gender, and race.

Globally more than 400 million people are suspected to be suffering from G6PD deficiency.⁸ The overall G6PD allele frequency is supposed to be around 8% and Asia comprises a major number of the G6PD deficient population because of its high population density.⁷ One of the studies of WHO⁹ showed the prevalence of G6PD deficiency in India ranging (from 0-to 10%) which is found to be population-specific and is more prevalent among tribals while compared to the caste population.¹¹

The measurement of G6PD in neonates is important in the sense that in India 390,000 children are born annually with G6PD deficiency causing significant mortality and morbidity in early childhood¹². The Prevalence of G6PD deficiency area-wise was found to be ranging from 0%-30.70% in eastern India, 0%-27.9% in western India, similarly it ranges from 0%-23.21% in Northern India to 0%-18% in Southern India.¹³ There are various methods to determine an individual’s G6PD status. Some methods such as genetics test are more advantageous for population studies whereas others are more useful for managing cases and decision making e.g., tests that measure enzyme activity.¹⁴ Neonatal screening for G6PD deficiency is very essential and established in many countries. There are tests like routine semiquantitative fluorescent spot test which could analyze all cases with severe G6PD deficiency but it may miss with partial deficiency. Quantification of G6PD activity using UV kinetic method can analyze and detect even the cases with partial deficiency and gives the enzyme level with high accuracy.¹⁵ Present study has also followed the same UV kinetic method to evaluate G6PD.

The present study aims to obtain the G6PD enzyme levels for neonates. Determination of enzyme levels in neonates is very important since infants are at a higher risk of complications of severe neonatal hyperbilirubinemia like Kernicterus during this period. Wen-chien yang et al. determined the reference level of G6PD activity for 7-90 days, old infants, without G6PD mutations.
as 13.6±3.7U/g Hemoglobin which is close to our finding where we obtained as 8.05 ± 1.2 U/g Hb for neonates.

It is evident that the usage of narrow reference interval G6PDH would be a better way to increase the diagnostic sensitivity of the assay which otherwise would have been considered the critical values as normal reference interval. In the present study the G6PD level was 34% lower than the literature reference interval. Hence, the diagnostic sensitivity of the assay is statistically significantly increased and the study outcome underscores that the established reference interval certainly has a significant racial difference in the G6PD level.

In this study, we tried to determine the cut off value of G6PD enzyme activity in neonates and our findings of 8.05 ± 1.2 U/g is also similar to the study done by Tang et al.17 in 1992 where the mean ± standard deviation reference levels were 8.10 ± 2.04 U/g hemoglobin (Hb) for neonates.

Though the very high number of samples and categorization of genotype groups are needed to determine reference levels, this study is equally helpful as it gives the presumptive idea of cut-off value of G6PD in neonates which ultimately helps in disease diagnosis in the clinical setting.

Limitation: The reference interval on the referred sample size (n=43) can be adopted as the reference interval of normal neonates of the Indian sub-population. However, the study may be extended to evolve a consensus pan India reference interval by increasing the sample size to the compliance of rigorous statistical evaluation.

CONCLUSIONS

In conclusion, we found evidence for a newly established reference interval for normal neonates between the ages of 1 to 11 days and the determined reference value is remarkably lower than the available reference interval. This established reference interval can be used as an appropriate diagnostic reference for clinical decision-making than using it as a reference interval.

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REFERENCE


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