Original Article

G6PD in Icteric Neonates

Glucose-6-Phosphate Dehydrogenase Deficiency in Icteric Neonates with indirect Hyperbilirubinemia

Nathumal Maheshwari[1], Ashok Kumar[1], Adnan Bashir[3], Shakeel Ahmed[4], Bilawal Hingorjo[5] and Anjum Rehman[2]

ABSTRACT

Objective: Determining the Glucose-6-phosphate dehydrogenase (G6PD) deficiency in icteric neonates with indirect hyperbilirubinemia reporting at a tertiary care hospital of Sindh.

Study Design: Cross Sectional study.

Place and Duration of Study: This study was conducted at the Department of Paediatrics, SMBB Medical College Layari General Hospital, Karachi, Sindh from January 2017 to May 2019.

Materials and Methods: A sample of 311 icteric neonates was selected by convenient probability sampling through inclusion and exclusion criteria. Complete blood counts and reticulocyte counts were analyzed on Sysmex KX-21 hematology analyzer. Serum bilirubin and glucose- 6-phosphate dehydrogenase were estimated by diazo method and color decolorization method (Sigma assay kit). Statistical analysis was performed at 95% confidence interval ($P \leq 0.05$) using SPSS software 21.0 (IBM, Inc USA).

Results: Of 311 sample size, 23 (7.39%) revealed G6PD deficiency and 288 (92.6%) neonates revealed normal G6PD concentrations. Age, Gestational age and hemoglobin were similar in both G6PD normal and deficient neonates. Anemia, reticulocyte counts, direct, indirect and total bilirubin revealed significant differences in G6PD deficient and normal neonates (P=0.0001).

Conclusion: The present study reports 7.39% frequency of Glucose-6-phosphate dehydrogenase deficiency in icteric neonates with indirect hyperbilirubinemia.

Key Words: Icteric Neonates, G6PD Deficiency, Indirect Hyperbilirubinemia.

Citation of article: Maheshwari N, Kumar A, Bashir A, Ahmed S, Hingorjo B, Rehman A. Glucose-6-Phosphate Dehydrogenase Deficiency in Icteric Neonates with indirect Hyperbilirubinemia. Med Forum 2020;31(8):58-62.

INTRODUCTION

Glucose-6-Phosphate-Dehydrogenase (G6PD) is the most important enzyme of hexose monophosphate (HMP) pathway of glucose metabolism. It catalyzes the first biochemical reaction of HMP glucose pathway; converting glucose 6 phosphates to 6-phosphogluconate.

[1.] Department of Paediatrics / Obstet & Gynae[2], Shaheed Muhtrama Benazir Bhutto Medical College, Layari General Hospital Karachi, Sindh.
[3.] Department of Paediatrics, Hamdard College of Medicine and Dentistry Hamdard University Hospital, Karachi.
[4.] NICH, JPMC, Sindh, Karachi.
[5.] Department of Paediatrics, Dow University of Health Sciences/ Civil Hospital Karachi.

Correspondence: Dr. Nathumal Maheshwari, Assistant Professor of Paediatrics / Obstet & Gynae2, Shaheed Muhtrama Benazir Bhutto Medical College, Layari General Hospital Karachi, Sindh.
Contact No: 03333622316
Email: drnathumal@gmail.com

Received: April, 2020
Accepted: May, 2020
Printed: August, 2020

G6PD deficiency is the most common enzymopathy that manifests clinically.[1,2] G6PD deficiency is a congenital inherited enzymopathy affecting many organs but the red blood cells (RBCs) are adversely affected at the most. HMP pathway generates NADPH from glucose that is essential for maintaining the RBC cell membrane integrity. NADPH is proton donor to the glutathione system of RBCs keeping it ready for scavenging the free radicals. Glutathione system maintains the vital functions of RBC by removing the oxygen free radicals that may alter cell structure.[1,2] Current estimates show the G6PD deficiency affects 400 million people over the Globe. G6PD deficiency is an X linked disorder.[3,4] G6PD deficiency was discovered 50 years age. G6PD deficiency is of clinical importance because of its severe effects on RBCs. One of the clinical manifestations of G6PD enzymopathy is the neonatal hyperbilirubinemia without hemolysis. Deficiency may be serious causing kernicterus leading to death. Neonatal infections are increased by G6PD deficiency.[4-6] G6PD deficiency was reported in 10% of black neonates in the United States without severe neonatal jaundice. Incidence of G6PD enzymopathy varies from country to country. Its incidence is reported as 40% in Nigeria, 18.4% in Saudi Arabia, 12.2% in India, 1.62% in Singapore, 1.57% in Jamaica, 3.5% in

Malaysia and 0.1% in Japan and Europe, 10% in Iran and 14% in Bengal in neonatal hyperbilirubinemia without hemolysis.[1,3] Prevalence of G6PD deficiency is 32.5% in Africa and Arabian Peninsula. Prevalence of 62% is reported in Kurdish Jews. In Pakistan, the G6PD deficiency ranges between 3 to 6.9% similar to the Southern China and Russia.[1,3] The high and low prevalence of G6PD deficiency may be due to the consanguine marriages and homozygous female add more to enzymopathy. Other studies from Pakistan revealed 16%[6] and 30.1%[7]deficiency of G6PD in neonates with hyperbilirubinemia without hemolysis. Increasing neonatal hyperbilirubinemia in many geographical areas is linked with the co-occurrence of G6PD deficiency. However, currently, few empirical studies are reported from Pakistan, hence there is need of conducting more studies. The present prospective study was conducted to determine the frequency of Glucose-6-phosphate dehydrogenase (G6PD) deficiency in icteric neonates without hemolysis at a tertiary care hospital of Sindh.

MATERIALS AND METHODS

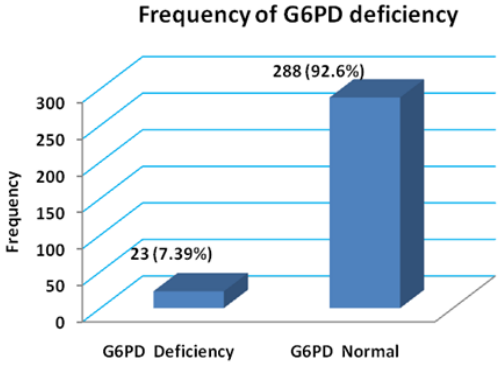
The present cross sectional study was conducted at the Neonatology unit, Department of Paediatrics, Shaheed Muhtrama Benazir Bhutto (SMBB) Medical College Layari General Hospital, Karachi Sindh. Research protocol was applied for ethical review committee approval. The study was conducted over duration of >2 years (January 2017 to May 2019). Sample size for the research protocol was calculated by 'sampling for proportions'. Sample size was calculated to be 311 (at 5% α-level of significance, 90% Power (test) at an expected % of G6PD deficiency in neonates as 16.2%.[1] A sample of 311 icteric neonates (hyperbilirubinemia) were selected by convenient probability sampling by the inclusion and exclusion criteria. A Performa was generated for the present research proposal for the collection of biodata, clinical presentation and laboratory findings of neonates. Jaundiced (icteric) neonates (neonatal unconjugated hyperbilirubinemia) were examined for the criteria of inclusion. Full term neonates, gestation age ≥38 weeks, neonatal age 2- 28 days, total bilirubin ≥12 mg/dl and predominantly unconjugated hyperbilirubinemia otherwise healthy, negative direct Coomb's test and C-reactive protein (CRP) within normal limits were included. Preterm neonates, term neonates (Total bilirubin <12 mg /dL), sepsis, septic neonates with renal failure or signs of disseminated intravascular clotting, toxoplasmosis, birth asphyxia, ABO and Rh incompatibility and direct hyperbilirubinemia were exclusion criteria. After clinical history, the neonates were examined by Pediatric consultant. The venesection was performed under strict aseptic measures and venous blood was collected in plain and EDTA bottles. 1 ml blood of EDTA tubes was processed for complete blood counts and reticulocyte counts on Sysmex KX-21 hematology analyzer. 2 ml blood was centrifuged to get sera for the detection of serum bilirubin and glucose- 6-phosphate dehydrogenase. Diazo method (Diazotized sulphanilic test) was used for the estimation of serum bilirubin. Commercial Sigma assay kits were used for G6PD estimation by the colour decolorization (qualitative) method. Study variables were saved in Microsoft Excel sheet. Data was analyzed by Statistical software (SPSS software 21.0, IBM, Inc USA). Quantitative variables were analyzed and interpreted by Student`s t-test and qualitative variables by Chi- square test. Statistical analysis was performed at 95% confidence interval ($P \leq 0.05$).

RESULTS

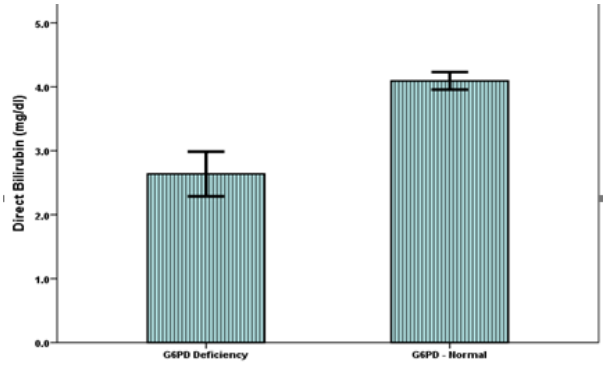
Of 311 sample size, 23 (7.39%) revealed G6PD deficiency and 288 (92.6%) neonates revealed normal G6PD concentrations (Table 1, Graph-1).

Table No.1: Demographic features, blood and biochemical findings

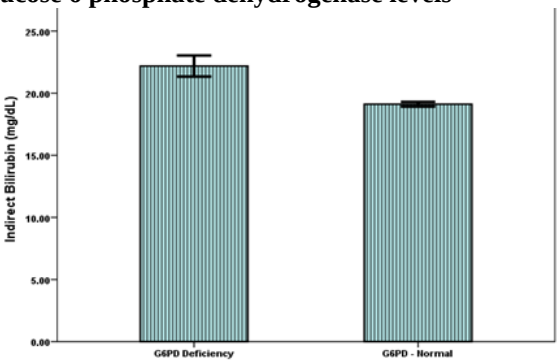
Parameter	G6PD Deficiency	G6PD Normal	P-value
Age (days)	5.61±2.10	5.86±2.12	0.57
Gestational age (weeks)	38.73±0.41	38.7±0.39	0.79
Hemoglobin	16.97±1.65	17.47±1.54	0.14
Reticulocyte counts	2.43±0.89	4.06±1.17	0.0001
Direct Bilirubin (mg/dL)	2.63±0.80	4.09±1.18	0.0001
Indirect Bilirubin (mg/dL)	22.17±1.95	19.11±1.59	0.0001
Total Bilirubin (mg/dL)	23.97±2.37	19.23±1.63	0.0001
G6PD Deficiency	23 (7.39%)	288(92.6%)	0.0001
Anemia	7 (30.4%)	57(19.79%)	0.0001
Total	23	288	311



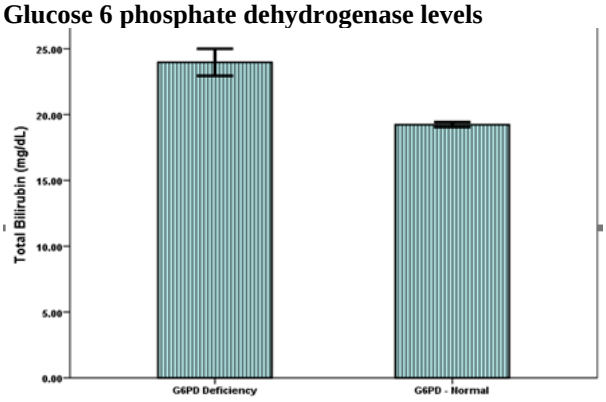
Graph No.1: Bar graph showing frequency of normal and deficiency of Glucose 6 phosphate dehydrogenase



Graph No.2: Bar graph showing serum direct bilirubin in neonates with normal and deficient Glucose 6 phosphate dehydrogenase levels



Graph No.3: Bar graph showing serum indirect bilirubin in neonates with normal and deficient Glucose 6 phosphate dehydrogenase levels



Graph No.4: Bar graph showing serum total bilirubin in neonates with normal and deficient Glucose 6 phosphate dehydrogenase levels

Of 311 sample; 215 (69%) were male and 96 (31%) were female. Male to female ratio was 2.29:1. Of 23 (7.39%) G6PD deficient neonates; 17 (73.9%) were male and 6 (26.08%) (P=0.0001). Age of G6PD normal and deficient neonates was 5.61±2.10 days and 5.86±2.12 days respectively (P=0.57). Gestational age was 38.73±0.41 weeks in G6PD deficient and 38.7±0.39 weeks G6PD normal neonates (P=0.79). Hemoglobin 16.97±1.65 and 17.47±1.54 g/dl in G6PD deficient and normal neonates respectively (P=0.14). Anemia was noted in 7 of 23 (30.4%) in G6PD deficient and 57 of 288 (19.79%) of normal G6PD neonates (P=0.0001). Reticulocyte counts, direct (Bar graph-2), indirect (Bar graph- 3) and total bilirubin (Bar graph- 4) showed statistically significant different in G6PD deficient and normal neonates (P=0.0001).

DISCUSSION

The present study is the first research study being reported from our tertiary care hospital reporting on frequency of Glucose 6 Phosphate Dehydrogenase (G6PD) in icteric neonates with indirect hyperbilirubinemia. The findings are of utmost clinical importance for better management of neonatal hyperbilirubinemia. We think G6PD assay should be performed for all those presenting with icterus and neonatal hyperbilirubinemia. The present study found frequency of 7.39% G6PD deficiency in icteric neonates with indirect hyperbilirubinemia. G6PD deficiency predominated in the male neonates. The findings are in agreement with previous studies.[1,8,10-14] G6PD deficiency is an X- linked recessive disease. Its deficiency causes neonatal jaundice, chronic hemolytic anemia, increased infections, favism and drug-induced hemolysis.[9] Of 311 sample; 215 (69%) were male and 96 (31%) were female. Male to female ratio was 2.29:1. Of 23 (7.39%) G6PD deficient neonates; 17 (73.9%) were male and 6 (26.08%) (P=0.0001). These findings are also in keeping with previous studies.[1,8,11-14] Munir et al[8] conducted study at the Children Hospital, PIMS, Islamabad, analyzed 160 icteric neonates and reported 6.7% frequency of G6PD deficiency and male neonates predominated. The findings of above study in line with the present study. Siddiqui et al[10] reported 5.4% frequency of G6PD deficiency that is inconsistent with present study, reason is clear that the sample size of above study was small compared to the present study. Alvi et al[11] reported 10% G6PD deficiency in neonates with indirect hyperbilirubinemia. Frequency of 10% G6PD deficiency is higher than the present study. However, another previous study[12] reported 8.2% of neonates were G6PD deficient that approximates to our present observations of 7.39% G6PD deficiency in icteric neonates. Another previous study[13] reported 8.2% of neonates were G6PD deficient. Above findings is in keeping with the present study. A previous study[14] showed 12% of adults patients having G6PD enzyme deficiency. In present study, significant differences were observed in reticulocyte counts, direct, indirect and total bilirubin in G6PD deficient neonates. The findings are in agreement with previous studies.[1,15-17] However, Munir et al[8] reported no difference in reticulocyte counts and hemoglobin in G6PD-deficient neonates compared to G6PD normal neonates. This is inconsistent to present and other previous studies.[15-17] We observed hyperbilirubinemia and icterus in >90% of neonates that is supported by a previous studies[11,12] that observed similar 80%- 90% neonates revealed

jaundice within first 7 days of life respectively. Daliri et al[18] analyzed 284 neonates and reported 12.1% frequency of G6PD deficiency of sample. A recent study[19] published results of screening of 5652 neonates, and reported 12.4% prevalence of G6PD deficiency. Male predominated for the G6PD deficiency. The findings of above study are in support to observations of the present study. A study[1] from Pakistan analyzed 400 male children. They reported overall prevalence of 10% G6PD deficiency the findings are in keeping with the present study. Another recent study[20] analyzed G6PD in 100 neonates with icterus and reported 6% frequency of G6PD deficiency. Jan et al[21] analyzed 1695 neonates with jaundice and found 152(9%) deficiency of G6PD. They further added the most of the neonates presented with icterus in the first 4 days of life, this is in agreement with the present study. Evidence based findings of present prospective in light of published literature from Pakistan points towards the need of screening of glucose-6-phosphate dehydrogenase for the icteric neonates with indirect hyperbilirubinemia. This will help prevent the hemolytic crises, hyperbilirubinemia and associated complications like kernicterus in neonates. Timely measures to combat the morbidities of G6PD deficiency will help better management and outcome of neonates. One of the limitations of present study is small sample size hence results cannot be generalized to other settings. But the strength of study lays in its prospective study design and inclusion criteria of research protocol.

CONCLUSION

The present study reports 7.39% frequency of Glucose-6-phosphate dehydrogenase deficiency in icteric neonates with indirect hyperbilirubinemia. We recommend performing G6PD assay for all those icteric neonates presenting with indirect hyperbilirubinemia to prevent hemolytic crisis, kernicterus and other complications. Timely measures to combat the morbidities of G6PD deficiency will help better management and outcome of suffering neonates.

Author's Contribution:

Concept & Design of Study:	Nathumal Maheshwari
Drafting:	Ashok Kumar, Adnan Bashir
Data Analysis:	Shakeel Ahmed, Bilawal Hingorjo, Anjum Rehman
Revisiting Critically:	Nathumal Maheshwari, Ashok Kumar
Final Approval of version:	Nathumal Maheshwari

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Mohtasimbillah, Ahmad S, Ahmad I, Khan H, Gul H, Taj AS. Prevalence of G6PD deficiency in School going enrolled children in District Mardan. J Med Sci 2017; 25: (3) 308-312.
2. Beutler E. G6PD deficiency. Blood 1994; 84: 3613-36.
3. Sadiq MA, Muqeem A, Bilal A, Hashim R. Frequency of glucose-6-phosphate dehydrogenase (G6PD) deficiency among the asymptomatic healthy individuals. Pak J Pathol 2018: 29(1): 1-3.
4. Rosalind EH, Frederic BP, Anand PP, Oscar AN, Peter WG. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A Geostatistical Model-based Map 2012;9(11):1-15
5. Rai V, Kumar P. Epidemiological study of glucose-6-phosphate dehydrogenase deficiency in scheduled caste population of India. J Anthropol 2012: Article ID 984180.
6. Hussain M, Irshad M, Kalim M, Ali L, Ali L. Glucose-6-Phosphate dehydrogenase deficiency in jaundiced neonates. J Postgrad Med Inst 2010; 24(2): 122-6.
7. Rehman HK, Roghani MT. Erythrocyte glucose 6-phosphate dehydrogenase deficiency and neonatal jaundice. J Pak Med Assoc 1995; 45:259—264.
8. Munir SS, Tahir NB, Qazi TU. Frequency of G6PD deficiency in neonatal hyperbilirubinemia. Gomal J Med Sci 2014; 12:93-6
9. Srivatsa A, Bharti B, Shighi SC. Does nimesulide induce hemolysis in glucose-6-phosphate dehydrogenase deficiency? Acta Paediatr 2003;92: 637–8.
10. Siddiqi AA. Incidence and aetiology of neonatal jaundice in AGA and SGA babies. [Dissertation]. Karachi: College of Physians and Surgeons of Pak 1998.
11. Alvi MY, Laeeq A, Khan MA, Iqbal MA. Glucose-6-phosphate dehydrogenase (G6PD) deficiency associated with neonatal jaundice. Pak Paed J 2006; 30: 28-33.
12. Rehman H, Khan MA, Hameed A. Erythrocyte G6PD deficiency and neonatal jaundice. Pak Paed J 1995; 19: 259-60.
13. Imran M, Mohammad E. Neonatal jaundice due to G-6PD deficiency. Pak Paed J 1984; 8:126-8.
14. Ishaq M, Ishaq T, Subhan M, Afridi J. The frequency and age presentation of Glucose-6-phosphate dehydrogenase (G6PD deficiency) in 200 patients of hemolytic anemia. J Postgrad Med Inst 2006; 20:170-3.
15. Sinha R, Sachendra B, Syed VS, Nair L, John BM. To study the prevalence of glucose 6 phosphate dehydrogenase (G6PD) deficiency in neonates with neonatal hyperbilirubinemia and to compare the

course of the neonatal jaundice in deficient versus non deficient neonates. J Clin Neonatol 2017;6: 71-4.

16. Ali N, Anwar M, Ayyub M, Bhatti F, Nadeem M, Nadeem A. Frequency of glucose-6-phosphate dehydrogenase deficiency in some ethnic groups of Pakistan. J Coll Physicians Surg Pak 2005; 15(3):137-41.
17. Khattak MI, Ishaq T, Subhan M, Afridi J. The frequency and age presentation of G6PD deficiency in 800 patients of hemolytic anemia. J Postgrad Med Inst 2006; 20: 170-3.
18. Daliri S, Asadollahi K, Musavi MH, Karimi A, Khademi GA, Azizi M, Abangah G. The relationship between neonatal factors and involving with glucose-6-pho sphate dehydrogenase deficiency (G6PD) and patients' outcome in Fars Province. J Bas Res Med Sci 2018; 5(1):15-21.
19. Sharma S, Sharma M. To determine the prevalence of glucose-6-phosphate dehydrogenase deficiency using a novel water soluble tetrazolium-8 formazan method' for neonatal screening in the region of Himachal Pradesh, India. Adv Hum Biol 2019; 9:37-41.
20. Mazari N, Habiba U, Akhter N.Glucose-6-phosphate dehydrogenase deficiency in jaundiced neonates and its relationship with severity of hyperbilirubinemia. 10th World Hematology and Oncology Congress October 22-23, 2018 Warsaw, Poland. J Blood Disord Treat 2018; 1:1.
21. Jan AZ, Zahid SB, Ahmad S. Frequency of G6PD deficiency and its severity in neonatal jaundice in Rehman Medical Institute, Peshawar. Khyber Med Univ J 2013; 5(1): 36-9.