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TO STUDY THE INCIDENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN NEWBORNS IN A RURAL TERTIARY CARE HOSPITAL OF NORTH HARYANA

Paediatrics

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ABSTRACT

Introduction: 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 about 1 in 10 African American males in the United States. In India, there are 13 biochemical variants of G6PD being reported so far, out of which G6PD Mediterranean is most common in the caste groups. **Aim and Objectives:** To study the incidence of G6PD deficiency of newborns in a tertiary care hospital and early detection of G6PD deficiency and prevention of haemolytic triggers. **Material and methods :** The study was conducted in Department of Pediatrics at Maharishi Markandeshwar Institute of Medical Sciences and Research and its associated hospital, Mullana, Ambala on 200 neonates . In all neonates(inborn and outborn) in Dept. of Pediatrics at Maharishi Markandeshwar Institute of Medical Sciences and Research and its associated hospital, Mullana, Ambala blood samples were taken in a EDTA vial. Fresh whole blood was used as the enzyme activity decreases on storage at 2-8º C. Methaemoglobin reduction test was conducted in all the neonates and incidence was calculated. **Results** : In the present study 4.5% study subjects were Methaemoglobin reduction test positive i.e G6PD deficient. And there is statistically significant association of Methaemoglobin reduction test with the gender of study subjects with p value 0.028. however it was found non-significant with the gestational age, type of feeding and blood groups of study subjects. **Conclusion** : On the basis of our present study we can conclude that 4.5% study subjects were Methaemoglobin reduction test positive i.e G6PD deficient. And there is statistically significant association of methaemoglobin reduction test with the gender of study subjects with p value 0.028. however it was found non-significant with the gestational age, type of feeding and blood groups of study subjects

KEYWORDS

G6PD Deficiency, newborn, methemoglobin, anemia

INTRODUCTION :

G6PD gene is located on the long arm of the X chromosome (Xq28), and consists of 13 exons. G6PD locus is thought to be one of the most polymorphic loci among humans with almost 300 allelic variants reported. The G6PD enzyme monomer consists of 515 residues with over 59 kDa molecular weight. It was reported that the enzymatically active form of G6PD is either a dimer or a tetramer of a single polypeptide subunit according to cellular pH. (1)

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 In India, there are 13 biochemical variants of G6PD being reported so far, out of which G6PD Mediterranean is most common in the caste groups; whereas G6PD Orissa is most prevalent in the tribals of India. The 3rd most common variant seen in India is G6PD Kerala-Kalyan.(2). If this is the case, they might develop severe hemolysis causing severe neonatal hyperbilirubinemia, which could potentially lead to bilirubin-induced neurological dysfunction (3)

There are various methods available for detection of G6PD deficiency, but quantitative measurement of G6PD enzyme by measuring the reduction of NADP to NADPH using ultraviolet spectrophotometer is the basic diagnostic approach used commonly for detection of G6PD deficiency. Other than that, in a dye reduction test, the reduction of NADPH was linked to the reduction of the visible dye brilliant cresyl blue (4). Various other tests such as methylene blue, MTT tetra sodium, dichloro-indophenol or methemoglobin were also developed. In recent times, fluorescent spot test is more popular for rapid testing of deficiency in which reduction of NADPH is observed directly by virtue of its fluorescence, instead of linking the reduced pyridine nucleotide to a dye. These screening procedures are quite robust in the detection of the fully developed defect in males, but they fall short in the determination of female heterozygotes and in patients with relatively mild forms of G6PD deficiency.(5)

G6PD deficiency detection by neonatal screening is feasible and cost-effective. It also allows the early preventive measures against severe hemolysis, jaundice, kernicterus etc. to be implemented in neonatal life, as well as other preventive measures in later life. Some practical guidance to the family members of the G6PD deficient babies regarding the food items, herbs, chemicals and drugs to be avoided will be beneficial, if we diagnose the condition at the first opportunity.(6)

However, Depending on the prevalence of G6PD deficiency in a population, neonatal screening programs for G6PD deficiency should be established, aiming for early recognition and prevention of its complications

This study aimed to evaluate the success of our neonatal G6PD deficiency screening program, to assess the prevalence and gender distribution of G6PD deficiency in the North Haryana population, and to investigate the rate of G6PD-related neonatal morbidities.

METHODOLOGY

The cross sectional study was conducted in Department of Pediatrics at Maharishi Markandeshwar Institute of Medical Sciences and Research and its associated hospital, Mullana, Ambala. On A total of 200 neonates during dec 2021 to dec 2022 were included.

Incluson Criteria

All neonates(inborn and outborn) in Dept. of Pediatrics at Maharishi Markandeshwar Institute of Medical Sciences and Research and its associated hospital, Mullana, Ambala.

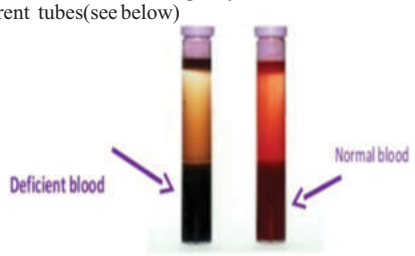
Exclusion Criteria

1. Neonates who have received blood transfusion recently.
2. Any newborn less than 24 hours of post natal life.
3. Neonates whose attendants refused to give consent.

Methodology :

- We used anticoagulated blood (EDTA vials) and tested the samples

within 1 hour of collection if left on the bench or within 6 hours if kept at 4ºC.
- We added 1 ml of blood to the tube containing 0.2ml of the combined reagent either freshly prepared or dried.
- Then the tube was closed with a stopper and the contents were gently mixed by inverting it 15 times.
- Control tubes were prepared by adding 1 ml of blood to a similar tube without reagents (normal reference tube) and to a tube containing 0.1ml of sodium nitrite-dextrose mixture without the methylene blue reagent('deficient' reference tube).
- Samples were incubated at 37ºC for 90 min.If the blood had been heparinized, incubation was continued for 3 hours.
- After the incubation, 0.1 ml volumes from the test sample, the normal reference tube and the deficient reference tube were pipetted into 10ml of water in separate , clear glass test tubes of identical diameter.
- The contents were mixed gently.The colours were compared in different tubes(see below)



RESULT

In the present study among the total, about 34 percent babies were female and rest 66 percent were male. In the present study 5 babies (about 2.5 percent) had ABO incompatibility.

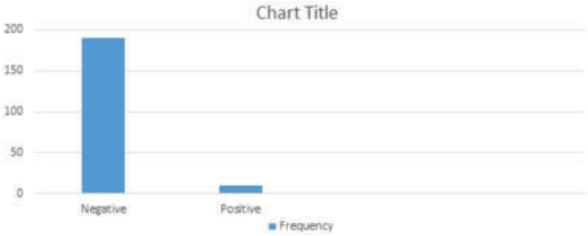


Fig 1: Table showing Rh incompatibility of the baby subjects

Fig 1 shows Rh incompatibility of the baby subjects where 10 babies (about 5 percent) had Rh incompatibility.

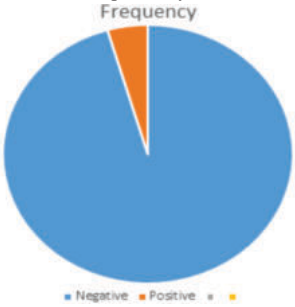


Fig 2: Table showing methaemoglobin reduction test for G6PD deficiency

Fig 2 shows the methaemoglobin reduction test for G6PD deficiency. Among them, 95.5 percent tested negative for G6PD deficiency and 4.5 percent were G6PD deficient.

Table 1: Association of Methemoglobin reduction test for G6PD deficiency by sex

Sex		Methaemoglobin Reduction Test For G6pd Deficiency		Total
		Negative	Positive	
Female	N	68	0	68
	%	35.6%	0%	35.0%
Male	N	123	9	132
	%	64.4%	100%	65.0%
Total	N	191	9	200
	%	100.0%	100.0%	100.0%
Chi Square (P Value)	4.85 (0.028)			

Table 1 shows the association of methaemoglobin reduction test for G6PD deficiency by sex. Among them, 9 males and 0 females have a positive result for methaemoglobin reduction test. The chi square test was done to find the result between two and the result is found to be significant with p value (0.028).

Table 2: Association of methemoglobin reduction test for G6PD deficiency by gestation of the babies

Term/Preterm		Methaemoglobin Reduction Test For G6pd Deficiency		Total
		Negative	Positive	
Preterm	N	48	3	51
	%	25.1%	33.3%	25.5%
Term	N	143	6	149
	%	74.9%	66.7%	74.5%
Total	N	191	9	200
	%	100.0%	100.0%	100.0%
Chi Square (P Value)	0.304 (0.581)			

Table 2 shows the association of methemoglobin reduction test for G6PD deficiency by term. Among them, three babies having preterm birth had positive result for G6PD deficiency. The chi square test was done to find the result between two and the result found to be non-significant difference among the two with p value (0.581).

DISCUSSION

G6PD deficiency is one of the important causes of severe neonatal hyperbilirubinemia, which can lead to kernicterus. If detected early, children with this disorder can be reared with all the necessary precautions to lead a normal and healthy life. Hence, G6PD screening should be further reinforced for every newborn.

Sex

In the present study the sex distribution of the babies was considered in total and about 35 percent babies were female and the rest 65 percent were male. **Chandrashekhar C et al.**(7) Study shows out of 500 babies screened, 266(53.2%) were males and 234 (46.3%) were females. **Iranpour R et al.**(8) Study shows that in 2501 newborns, 1307 [52.3%] males and 1194 [47.7%] females were screened over a period of nearly three months. **Eghbalian F et al.** (9) A total of 1545 neonates out of which 782 males and 763 females were studied in the study. **Elella et al.**(10) study 2782 randomly selected samples out of which 1453 were males and 1339 were females.

Methaemoglobin Reduction Test

In the present study, among the study subjects, 95.5 percent had negative reduction test for G6PD deficiency, 3 percent had positive G6PD deficiency and the rest 1.5 percent had intermediate activity. **Hakim Y et al.** (11) study shows the screening results (qualitative methemoglobin reduction test) these results showed that 80 (94.1%) of the study sample showed normal screening test. **C E Amiwero et al.** (12) study showed that G6PD screening by methaemoglobin reduction method was carried out on all the subjects, was carried out in the first 15 subjects who were G6PD deficient by the qualitative screening and the first 15 subjects who were G6PD normal.

Methemoglobin reduction test for G6PD deficiency by sex

In the present study, among the study subject, a maximum of 4.5 percent had positive result and intermediate activity for methemoglobin reduction test and the result is found to be significantly higher in males. When the intermediate result was also considered deficient, the overall sensitivity of MRT was 44 of 49 (89.89). The sensitivity was somewhat higher in males (36 of 39. 92.3%6) than in females (8 of 10, 80%). Of 473 non-G-6-PD deficient samples, MRT correctly identified most (450) of the cases (95.19) representing a highly specific test. **Tilakarajan S et al.**(13) stated, in their study, that the methemoglobin reduction test was able to pick heterozygous females.

Their study tested subjects only with methemoglobin reduction test and suggested to analyzing its efficacy with a simultaneous enzymatic assay. **Oni G et al.**(14) 60% were male subjects and 40% were female subjects with frequency of G6PD deficient males 40 and that of females 35. **HaKim Y et al.** (11) results showed that 80 (94.1%) of the study sample showed normal screening test, 4 (4.7%) showed deficient male and only 1 (1.2%) showed female and the deficient male in this study reading (25.2 – 26.0 – 44.2 and 46.3) Mu/109 cell and 1 deficient female (66.6)Mu/109 cell by spectrophotometer. **MONDAL M et**

al.(15) study shows the total 176 neonates, 13.63% had G6PD deficiency, most are male. **Rajkhowa et al.**(16) study shows the mean (SD) of G6PD at birth was 12.56 (3.45) mU/g of Hb. Out of 1037 neonates, five were found to be G6PD deficient (value taken as less than 6.95 mU/g of Hb) and four of them were males and one was female.

Methemoglobin reduction test for G6PD deficiency by term of the babies

In the present study, among the study subject, 1 baby having preterm birth had positive result for G6PD deficiency and the result was found to be non-significant difference among the two. **Elella et al.** (10) Study shows neonates with G6PD deficiency (37.5% of G6PD-deficient group in comparison to no neonates in G6PD normal group) required exchange transfusion. Also, G6PD required more prolonged phototherapy in comparison to G6PD nondeficient neonates and they both contribute to a longer hospital stay.

Methemoglobin reduction test for G6PD deficiency by feeds of the babies

In the present study, out of 191 negative study subjects 29.31% had exclusive breast feeding, 35.6% had exclusive top feeding and 35.08% had mixed feeding whereas in positive study subjects 22.2% had exclusive breast feeding. 22.2% subjects had exclusive top feeding and 55.56% study subjects had mixed feeding, on comparing there is non-significant association with p value 0.45. **Mariolea Z et al.**(17) Study shows 19 (47- 5%) of the 40 G6PD females deficient by the evaluation method had normal values of enzyme activity.

Methemoglobin reduction test Cephalohematoma/Caput succedaneum of the babies

In the present study, among the study subject, none of the babies having Cephalohematoma/caput succedaneum were having intermediate activity and positive result for G6PD deficiency methemoglobin reduction test. But all the babies who do not have Cephalohematoma/caput succedaneum and classified in "no" category were having positive result for G6PD deficiency and the result was found to be non-significant. **Kumar P** and **Bisoi S et al.** (1) Studies shows the result in correlation with the present study. **Elella et al.** (10) study shows Cephalhematoma had not been observed in our patients, and this reflects the improvement in the obstetrical services including routine employment of episiotomy in primiparas women, avoidance of forceps delivery and vacuum extraction that are associated with increased incidences of cephalhematoma, better monitoring of parturition, and an early caesarean section for obstructed difficult deliveries.

CONCLUSIONS

This study aimed to determine the Incidence of G6PD deficiency in a tertiary care centre of Ambala Punjab, On the basis of our present study we can conclude that 4.5% study subjects were methaemoglobin reduction test positive i.e G6PD deficient. And there is statistically significant association of methaemoglobin reduction test with the gender of study subjects with p value 0.028. however it was found non-significant with the gestational age, type of feeding and blood groups of the study subjects. In the present study 2.5% study subjects had ABO incompatibility whereas 5% babies had RH incompatibility.

REFERENCES

1. Kumar P, Yadav U, Rai V. Prevalence of glucose-6-phosphate dehydrogenase deficiency in India: an updated meta-analysis. Egypt J Med Hum Genet. 2016;17(3):295–302.
2. Mohanty MD, Dash DK, Rath B. New born screening for glucose-6-phosphate dehydrogenase deficiency in Orissa, IMS and SUM hospital: a quantitative assay. Experiment. 2014;22:1525–30.
3. Kaplan M, Hammerman C. Severe neonatal hyperbilirubinemia: a potential complication of glucose-6-phosphate dehydrogenase deficiency. Clin Perinatol. 1998;25(3):575–90.
4. BEUTLER E, Halasz A. A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. Blood. 1966;28(4):553–62.
5. Shah II, Jarullah J, Jarullah B. Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in India: a systematic review. Adv Biosci Biotechnol. 2018;9(09):481.
6. Kirkman HN, Gaetani GD, Clemons EH, Mareni C. Red cell NADP+ and NADPH in glucose-6-phosphate dehydrogenase deficiency. J Clin Invest. 1975;55(4):875–8.
7. Chandrasekhar C, Garg A, Goyal MB, Kumar S, Ramji S, Kapoor S. Newborn screening for G6PD deficiency: A 2-year data from North India. Indian J Public Health. 2015;59(2):145.
8. Iranpour R, Akbar MR, Haghshenas I. Glucose-6-phosphate dehydrogenase deficiency in neonates. Indian J Pediatr. 2003;70(11):855–7.
9. Eghbalian F, Aqaie R, Jiriaee N, Jenabi E. Prevalence of glucose-6-phosphate dehydrogenase deficiency among neonates with hyperbillirubinemia in the West of Iran. Adv Hum Biol. 2021;11(4):81.
10. Elella SA, Tawfik M, Barseem N, Moustafa W. Prevalence of glucose-6-phosphate dehydrogenase deficiency in neonates in Egypt. Ann Saudi Med. 2017;37(5):362–5.
11. Hakim Y, Abdelbagi A, Mahgoub R, Mustafa J. Screening For Glucose 6-Phaosphate Dehydrogenase Among Neonates At Wad Medani Obstetrics And Gynecology Teaching Hospital, Gezira State-Sudan (2015–2016). J Med Dent Sci Res. 2018;5(5):18–26.
12. Amiwero CE, Olatunji PO. Re-evaluation of methaemoglobin reduction as a screening procedure for glucose-6-phosphate dehydrogenase (G6PD). Afr J Med Med Sci. 2007;36(2):177–81.
13. Suvitha T, Niveditha SK. G6PD screening in neonatal hyperbilirubinemia. Indian J Neonatal Med Res. 2014;3:1–6.
14. Oni GA, Johnson RAE, Oguntibeju OO. Detecting patients with glucose-6-phosphate dehydrogenase deficiency. Journal, Indian Acad Clin Med. 2005;
15. Mondal M, Datta AK, Mandal S, Das PK. Study of Glucose-6-phosphate dehydrogenase deficiency in Neonatal Jaundice. IOSR J Pharm Biol Sci. 2012;1(5):30–6.
16. Rajkhowa P, Nath C, Dutta A, Misurya I, Sharma N, Barman B, et al. Study of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency and Genotype Polymorphism of G6PD B and G6PD (A+/A-) in Patients Treated for Plasmodium vivax Malaria in a Tertiary Care Hospital in North East India. Cureus. 2020;12(11).
17. Zannos-Mariolea L, Kattamis C. Glucose-6-phosphate dehydrogenase deficiency in Greece. Blood. 1961;18(1):34–47.