Original Article

Frequency of G6PD Deficiency and Coombs Test Positivity in Newborn Presenting with Hyperbilirubinema

Muhammad Qasim Khan[1], Subhan-ud-Din[2], Akhtar Ali Shah[1] and Muhammad Fazil[1]

ABSTRACT

Objective: To study the prevalence of G6PD deficiency and coombs test positivity in newborn presenting with hyperbilirubinema.

Study Design: Descriptive study.

Place and Duration of Study: This study was conducted at the Paediatric Department of Bacha Khan Medical College & Mardan Medical Complex Teaching Hospital Mardan from January 2017 to July 2018.

Materials and Methods: A total of hundred newborn with hyperbilirubinama and fifty healthy newborns as a control group were included in the study. All the newborns were subjected to G6PD test, coombs test, Retic count, full blood count, blood groups and total, direct, indirect bilirubin level.

Results: A total of 9% Newborn with hyperbilirubinema showed G6PD Deficiency. Mean bilirubin level was 25±2.562 mg/dl, significantly elevated as compared to control group. P<0.00236. Similarly 10% newborn showed coombs test positive and these were Rh incompatible. Mean bilirubin level was 26±2.156 mg/dl, significantly higher as compared to control group. P<0.000326. 80% Newborn had physiological jaundice. Mean bilirubin level were 13±2.562 mg/dl, significantly higher than control group.P< 0.00422.

Conclusion: The study concluded that Newborn presenting with hyperbilirubinamais significantly associated with high prevalence of G6PD deficiency and positive coombs test which indicates Rh-incompatibility. Therefore all the Pediatrician should have priority to screen all newborn presenting with hyperbilirubinamafor G6PD deficiency and coombs test. As these two conditions are very common in Pakistan this will early identify newborn with hyperbilirubinemia leading to serious complication like kernicterus.

Key Words: Hyperbilirubinama, G6PD, Coombs Test,kernicterus

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INTRODUCTION

Neonatal hyperbilirubinemia is a common problem among neonates and in majority of neonates it is reported[1]. Neonatal hyperbilirubinemia is usually reported in 60% of full term and 80% in preterm babies in the 1st week of life[2]. Although jaundice is mostly physiological phenomenon in neonates but in 10-12% of cases they need admission[3]. Neonatal hyperbilirubinemia is the yellowish discoloration of skin and white part of eyes due to high bilirubin level. Other symptoms include lethergy or poor feeding. In majority of the cases there is no specific cause and jaundice is mainly physiological and this usually last for one week. But in some cases this may result from some pathological factors and includes red cell breakdown, liver disease, infection, hypothyroidism, G6PD deficiency, autoimmune hemolytic anemia, ABO&Rh incompatibility, unsuccessful breast feedings, earlier gestational age and low birthright[4,5]. Marked hyperbilirubinemia is associated with significant complication like kernicterus which is serious neurological disease and death[6]. However quick and accurate treatment reduce the risk of neonatal kernicterus[7]. In severe cases hyperbilirubinemia causes complication like Kernicterus, cereberal palsy and death. Therefore determining the etiology of jaundice can lead to timely prevention and treatment[8]. It is important that all the neonates with hyperbilirubinemia should be properly screened to identify the etiology. The American Academy of Pediatrics recommends. neonatal blood groups, coombs test, complete blood count, smear, G6PD level, direct and indirect bilirubin level and combination of universal screening as most effective method for identifying infants at risk of hyperbilirubinemia[9-5].

The aim of the study is to know the prevalence of G6PD deficiency, coombs test positivity, Rh and ABO incompatibility, one of the most important causes of neonatal hyperbilirubinemia by applying G6PD deficiency test, coombs test and blood group of baby and mother in neonates presenting with hyperbilirubinemia. This study will identify neonates

[1.] Department of Pediatrics, Bacha Khan Medical College Mardan.

[2.] Department of. Pathology, Gajju Khan Medical College Swabi.

Correspondence: Dr. Muhammad Qasim Khan, Assistant Professor of Pediatrics, Bacha Khan Medical College Mardan.
Contact No: 0333-9205167
Email: qasimkhan.dr.02@gmail.com

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with the common etiology in Pakistan and will further guide the paediatrician to know the common causes of hyperbilirubinemia and will provide timely treatment and reduce mortility and morbidity of neonates from hyperbilirubinemia.

MATERIALS AND METHODS

The study was conducted in the Paediatric and Pathology Department of Bacha Khan Medical College and Mardan Medical Complex Teaching Hospital Mardan, for one year from December 2017 to September 2018.

Jaundiced neonates with gestational age less than 28 weeks; jaundiced neonates with weight of less than 500 gm; and babies whose mothers were hepatitis A IgM positive, HBsAg (hepatitis B surface antigen) positive, and anti-HCV (hepatitis C antibody) positive were excluded from the study.

After getting approval from the hospital ethical committee to conduct the study, data was collected of all those neonates who met inclusion criteria presenting through Out-patient department (OPD) or accident/emergency department and admitted in Paediatric and Neonatology department of Mardan Medical Complex Teaching Hospital Mardan.An informed consent was taken from parents or relatives of the neonates with hyperbilirubinemia for further evaluation. The required investigation done (as below) in the pathology department of the hospital, after taking history and doing physical examinations. Alls the information and other data like name ,age, sex, address, date of admission, and date of discharge were entered into a parforma.

A total of 100 newborns presenting with hyperbilirubinemia, both male and female were included in the study and 50 newborns were taken as healthy individual as a control group. Patients were divided into three groups: Group A included newborn with hyperbilirubinemias with G6PD deficient group. Group B included newborns with hyperbilirubinemia with coombs positive group and group C included newborn with hyperbilirubinemia with physiological jaundice. All newborns with hyperbilirubinemia were subjected to parameters like total bilirubin, direct and indirect bilirubin level, G6PD test, coombs test, Retic count, special smear and full blood count. For this purpose samples were collected in EDTA and Gel tubes and from these samples the above investigations were performed.

G6PD tests were performed according to standard procedure by reduction test, the principle of which is : G6PD is released from lysed erythrocytes and catalyses the conversion of Glucose-6 Phosphate to 6 Phospho-gluconate with conversion of NADP to NADPH and this NADPH can be detected by dye reduction test according to standard procedure. Sample when did not change colour after 60 minutes declared G6PD deficient and sample which changed colour within 50-55 minutes were declared sufficient in G6PD.

Coombs tests were performed on all newborns with hyperbilirubinemia according to procedures as: Newborn EDTA blood taken and wash 3 times with saline. Then 5% solution prepared by adding 95drops of saline with 5 drops of washed red bloob cells of neoborn, then took two drops of this washed red blood cells and mixed with two drops of coombs reagent and then centrifuged for 15 seconds, then put one drop on slide and put a cover slip on it and examined under Microscope for agglutination and declared coombs test positive when agglutination seen.

Retic count also performed on all newborns with hyperbilirubinemia according to standard procedure as take equal quantity of EDTA blood and Retic reagent in a test tube and incubate at 37C^{o}for 10-15 minutes then prepared slide and examined for Retic counts.

Bilirub in in also performed on the sample of newborn presenting with hyperbilirubinemia (on Kit Randox) according to standard procedure. Take sample of newborn serum, then add R1 reagent 200 UI in a test tube. Then add R2 50 UI, then add R3 1ml and incubat at 37C^{o} then after 10 minutes add 1ml R4 then analyze by Microlab 300 chemical analyzer (Merck).

Full blood counts were also performed on all these newborn for determination of Hb, TLC and Platelets by Hematology analyzer (sysmex x100 Japan). All data were subjected to statistical analysis by using Chi-square test and T-Test. Level of significance was set at p value<0.005.

RESULTS

A total of 100 newborns with hyperbilirubinemiawere included in the study. They were both males and females. All these newborns were admitted in nursery unit. In all these newborns G6PD test, Coombs test, Total bilirubin, Direct and Indirect Bilirubin, Retic counts, complete blood counts, Neonatal and Maternal blood groups with Rh antigen were performed in this study. All newborns with hyperbilirubinemia were divided into Group A, which included G6PD deficient newborns; Group B in which Coombs positive newborns were included; Group C included newborns with physiologicalhyperbilirubinemia and 50 newborns were included as healthy newborns. In this study newborns with Hyperbilirubinemia,9% of newborns were G6PD deficient with mean bilirubin levels were 25±2.625 mg/dl.

Direct bilirubin level were 2±0.56 mg/dl and indirect bilirubin levels were 22±1.25mg/dl,which were significantly higher than the control group. P<0.00256.

Similarly 11% newborns, Rh incompatibility were detected in which babies were Rh positive and Mothers were Rh negative. All these newborns were presented with hyperbilirubinemia. Mean total bilirubin level, were 26±2.156 mg/dl; direct bilirubin levels were

2±0.56 mg/dl and indirect bilirubin levels were 23±1.25 mg/dl in all these newborn. Hyperbilirubinemiawere significantly higher as compared to control group P<0.00325.

Table No.1: Frequency of G6PD deficiency, Positive Coombs and physiological jaundice in Newborn with hyperbilirubinemia

	Frequency of Parameters in Newborns with Hyperbilirubinemia	%age
Group A	Newborns with G6PD deficiency	9%
Group B	Newborns with Rh. Incompatibility	11%
Group C	Newborns with Physiological Jaundice.	80%

Table No.2: Mean Value of Total Bilirubin Direct and Indirect bilirubin levels in Newborns presenting with hyperbilirubinemia

	Newborn with G6PD Defici-ency Group A	Newborns with Rh income-patibility Group B	Newborns with Physio-logical Jaundice Group C	Control Group
Total Bilirubin	25± 2.562 mg/dl	26± 2.156 mg/dl	13±2.562	1.5±0.32
Direct Bilirubin	2±0.56 mg/dl	2±0.56 mg/dl	2±0.56	0.5±0.12
Indirect Bilirubin	22±1.25 mg/dl	23±1.25 mg/dl	11±1.25	1±0.32

Similarly in rest of the newborns with hyperbilirubinemia no abnormality were detected and they were classified as physiological jaundice. Mean bilirubin levels were 13 ± 2.562 mg/dl. Retic counts were also performed. Retic count were comparatively higher than the control group.

DISCUSSION

The prevalence of hyperbilirubinemia is twice that of general population in males who carry the defective gene and in homozygous female. It rarely occurs in heterozygous females[10]. Hyperbilirubinemia is secondary to impairment of bilirubin conjugation and clearance by the liver leading to indirect hyperbilirubinemia[11]. Neonatal hyperbilirubinemia is not harmful and most neonates get better without treatment within one to two weeks. A very high bilirubin level lead to kernicterus and may lead to serious complications like cereberal palsy, deafness or other form of brain damage[12]. A lot of risk factors are responsible for hyperbilirubinemia of which G6PD deficiency, autoimmune hemolytic anemia and blood group incompatibility studied and evaluated in these newborn.

In the present study 9% newborn with hyperbilirubisnemia G6PD deficiency were detected. A similar study has been conducted by Leong and reported that newborn Hyperbilirubinemia is associated with significant prevalence of G6PD deficiency[13]. Isa et all also reported in their study that G6PD deficiency is very common in neonatal hyperbilirubinemia and 42% of the newborn were detected G6PD deficient[14-45]. G6PD deficiency is a X-linked disorder with male predominance and about 400 different types of G6PD with distinctive biochemical characteristic and about 100 various mutations have been identified[16]. G6PD deficiency is common worldwide and is more common in Mediterranean area, Middle East, India, China, Africa[17]. G6PD deficiency is a an independent risk factor for hyperbilirubinemia with bilirubin level more than 18mg/dl[18]. The exact mechanism in which G6PD deficiency lead to hemolysis is not clear, but is suggested that G6PD converts NADP to its reduced form NADH; and reduced NADPH protects RBC from oxidative damage i.e acute hemolysis occurs when RBC exposes to oxidative stressors like infections, oxidative drugs, fava beans etc[19]. Neonatal occurrence of Autoimmune hemolytic anemia is very rare. Its annual incidence is one case in 80,000 live births annually. Motta et al reported one case of Autoimmune hemolytic anemia in newborn infant[20]. ABO incompatibility is also a common condition in newborn and causes minimal hemolysis. It may cause raised level of bilirubin and anemia but is less severe than Rh hemolytic disease[21].

In the present study 11% showed coombs positive in neonates with hyperbilirubinemia. Various studies have been conducted and showed significant prevalence of Rh incompatisbility with positive csoombs in neonates. Patel et all also reported in their study that newborn presenting with hyperbilirubinemia is associated with significant prevalence of Rh incompatibility[22]. Similarly McIntosh N et all also reported that Rh incompatibility positively associated with newborn presenting with hyperbilirubinemia[23].

CONCLUSION

The present study concluded that newborn presenting with hyperbilirubinemia is significantly associated with high prevalence of G6PD deficiency and Rh incompatibility. Therefore all thepaediatrician should strictly screen all newborn presenting with hyperbilirubinemia for G6PD deficiency and Rh incompatibility. As these two risk factors are very common in our population / newborn presenting with hyperbilirubinemia; and these two investigation(G6PD test & coombs test) will timely prevent further attack of jaundice and reduce its complication like Kernicterus and further reduce mortility and morbidity from hyperbilirubinemia.

Author's Contribution:

Concept & Design of Study:	Muhammad Qasim Khan
Drafting:	Subhan-ud-Din
Data Analysis:	Akhtar Ali Shah, Muhammad Fazil
Revisiting Critically:	Muhammad Qasim Khan, Subhan-ud-Din
Final Approval of version:	Muhammad Qasim Khan

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

REFERENCES

1. Tikmani SS, Warraich HJ, Abbassi F, Rizvi A, Darmstadt GL, Zaidi AK. Incidence of Neonatal Hyperbilirubinemia: a population based prospective study in Pakistan. Trop Med Int Health 2010;15(5): 502-507.
2. Koosha A, Rafizadeh B. Evatuation of neonatal indirect hyperbilirubinemia at ZanJan Province of Iran. Prevalence of Glucose-6-Phrsphate Dehydrogenase deficienc, Singapore Med J 2007; 48:424 – 428.
3. Stoll BJ, Klliegman RM. Jaundice and hyperbilirubinemia in the newborn. In: Behrman RE, Kliegman RM, and Jenson HB, editors. Nelson Textbook of Pediatrics. 17th ed. Philadelphia: Saunders;2004.p. 562-96
4. Maiseps MJ, Bhatani VK, Bogen D, Newman TB, Stark AR, whatchko JF. Hyperbilirubinemia in the newborn infant > 35 week of gestation an update with clarifications. Pediatrics 2009;124:1193-1198.
5. Karen E, Muchowski MD. Evaluation and Treatment of Neonatal Hyperbilirubinemia. Am J Physician 2014;89: 873-878.
6. Weng YH, Chu YW. Clinical characteristics of G6PD deficiency in infants with Marked Hyperbilirubinemia. J Pediatr Hematoloncol 2010; 32:11-14.
7. Watchko JF. Hyperbilirubinemia and bilirubin Toxicity in the late preterm infant. Clin Perinatol 2006;33:839 – 52.
8. Halmak LP, Stevenson DK. Neonatal jaundice and liver disease. Neonat perinatal Medicine. 7thed. Missori: St Louis Mosby; 2002.p.1309-52.
9. Maisels MJ, Deridder JM, King EA, Balasubramaniam M. Routine transcuteneous bilirubin measurements combined with Clinical risk factors improve the prediction of subsequent hyperbilirubinemia. J Perinatol 2009; 29:612-617.
10. Kaplan M, Hammerman C, Verman HJ, Steven DK, Boulter E. Acute hemolysis and severe hyperbilirubinemia in Glucose-6 Phasphate dehydrogenase deficient hetrozygotes. J Pediatr 2001;139:137 – 40.
11. Beulter E. G6PD deficiency. Blood 1994;84:3613 –36.
12. Muchowski KE. Evaluation and treatment of neonatal Hyperbillirubinemia.Am Fam Physician 2014;89:873 – 878.
13. Leong A. Is there a need for neonatal screening of G6PD deficiency in Canada. Megill 2007;10: 31-34.
14. Goyal M, Garg A, Goyal MB, Kumar S, Ramjii S, Kapoor K. Newborn screening for G6PD deficiency- A 2 year data from North India. Ind J Pub Health 2015;59:145-148.
15. Chunyan FU, Luos, Li, Xieb, yang Q, et al. Newborn screening of G6PD deficiency in Guangxi China. Determination of Optimal cut off value to identify hetrozygotes female neonates: Scientic Reports 2018. Artict number 833.
16. Salvati AM, Maffi D, Caparari P, Pasquino MT, Cafrio MP, Trzia A. G6PD Deficiency and hereditary hemolytic Anemia. Ann1st super Sanita 1999;35:193-203.
17. Al Arrayed S. Frequency of G6PD deficiency among Bahraini students: a ten years study. Bah Med Bull 2010;32:18-12.
18. Agrawal SK, Kumar P, Rathi R, Sharma N, DAS R. Prasad R et al. UGT1A1 gene polymorphisms in North Indian neonates presenting with un-conjugated hyperbiliribenemia. Pediatr Res 2009; 65:675-680.
19. Frank JE, Maj MC. Diagnosis and management of G6PD deficiency Am Fam Physician 2005;72: 1277-82.
20. Motta M, Carvazza A, Miglion C. Chorico G. Autoimmune hemolytic anemia in newborn. Arch Dis Child fetal Neonatal 2003;88:341-342.
21. Drabik-crary K, Reddy VV, Benjamin WH, Boctor FN. Severe hemolytic disease of the newborn in a group B African-American infant delivered by a group O mother. Ann Clin Lab Sci 2006;36: 205-7
22. Patel A, et al. Int J Reprod Contracept Obstet Gynecol 2017; 6(4):1368-137.
23. Mclntosh N, Stenson B. The newborn. In: Mclntosh N, Helms PJ, Symth Rl, editors. Forfar & Arneil's textbook of pediatrics. 6th ed. Edinburgh: Churchill Livingstone; 2003.p.177-391.