Original Research Article

Reticulocytosis and direct coombs test as markers of haemolysis in neonatal hyperbilirubinemia requiring phototherapy

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Abstract

Background: Neonatal hyperbilirubinemia is seen in 60% full-term neonates in first week of life. Among these8-11 % require phototherapy. Causes of hyperbilirubinemia can be immune or non-immune causes. Reticulocytosis and Direct Coombs test positivity can be markers for haemolytic hyperbilirubinemia. **Objective:** To identify the incidence of reticulocytosis in immune and non-immune mediated haemolyticjaundice, to note whether thereis any significant difference in reticulocytosis between the two groups and to identify the association if any between reticulocytosis and Direct Coombs test. **Methods:** Aprospective study was done in a teaching institution including 100 term neonates requiring phototherapy. They were categorized into three groups as possible immune haemolytic(divided into Rh and ABO incompatibility) and possible non immune cause as per the maternal and baby blood groups. Reticulocyte count and Coombs test were done on starting phototherapy. Statistical analysis was done with SPSS version 20.0. Descriptive statistics, Chi-square test and Fisher's exact test were used for analysis. **Results:** Rh incompatibility group showed 100% positivity for both reticulocytosis and Direct Coombs test. In ABO incompatibility group reticulocytosis was 56.5% and Coombs positivity was 84.6%. 5% of those babies with Noincompatibility had reticulocytosis. Direct Coombs test was positive in 7.4% of babies in this group. There was a significant association between haemolytic cause of hyperbilirubinemia and reticulocytosis .No association seen between reticulocytosis and Coombs test positivity in ABO incompatibilitynor in No incompatibility group. **Conclusion:** Reticulocytosis, Coombs positivity are good markers to identify haemolysisas the major cause of hyperbilirubinemia

Key Words: Neonatal hyperbilirubinemia, Phototherapy, Reticulocytosis.

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INTRODUCTION

In the first week of life, the incidence of hyperbilirubinemia is approximately 60% full-term neonates.[1,2] 8-11 % of these babies require phototherapy[3]. Causes for jaundice can be immune haemolytic orvarious non-immune causes. The incidence of causes in a previous meta-analysis was ABO incompatibility (OR, 4.01; 95% CI:2.44-6.61), Rhesus hemolytic disease (OR, 20.63; 95% CI:3.95-107.65), G6PD deficiency (OR, 8.01; 95% CI:2.09-30.69), UGT1A1 polymorphisms (OR, 4.92; 95% CI:1.30-18.62), low gestational age (OR, 1.71; 95% CI:1.40-2.11), underweight/weight loss (OR, 6.26; 95% CI:1.23-31.86). ABO incompatibility (OR, 4.01; 95% CI:2.44-6.61).[4] It is difficult to find out in a baby with blood group incompatibility whether the major factor contributing for the hyperbilirubinemia is haemolysis or not. Haemolysis produces a good reticulocyte response. In immune haemolysis causing jaundice, the DCT is usually positive. Reticulocytosis has been defined as >7% at birth, >4% at day 3 of life and > 1% after 7 days of

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life.[5,6]Evaluating the incidence of reticulocytosis and positive Coombs test in immune haemolytic hyperbilirubinemia may help in assessing whether haemolysis is the major contributing factor for hyperbilirubinemia and also it will high light the need for searching for other causes

MATERIAL AND METHODS

A prospective study was done at a teaching hospitalbetween June 2017 and November 2017.Apparently healthy term neonates irrespective of their birth weight, who developed indirect hyperbilirubinemia and required phototherapy were enrolled in the study. Neonates who had prematurity, direct hyperbilirubinemia, haemangioma, and other congenital anomalies were excluded. The indication of phototherapy was determined based on the guidelines of American Academy of Pediatrics 2004.[7] After detailed history and examination, blood group of both mother and baby (venous sample) were noted. Babies were divided into three groups (1) those babies having possible Rh incompatibility (2) those babies having possible ABO incompatibility and (3) those without any incompatibility. Reticulocyte count and Direct Coombs test were done at the time of starting phototherapy for all the 100 babies. Data entry was made in the Microsoft Office Excel 2014. Statistical analysis was done with the help of SPSS trial version 20.0. Descriptive statistics, Chi-square test and Fisher's exact test were used for analysis of data. $p<0.05$ was considered as significant. Analysis was done to find out whether there is any significant difference in reticulocytosis among the three groups, to note the association between reticulocytosis and the etiology of jaundice and to note the association between reticulocytosis and Direct Coombs test in each group

RESULTS

Of the total 100 neonates under study, there were 48 females and 52 males. 27 neonates were having weight< 2,500 gm. The birth weight of the study group ranges from 1.9 kg to 4 kg with a mean birth weight of 2.83 and a standard deviation of 0.477 Proportion of babies with Rh incompatibility and ABO incompatibility was 8out of 100(8%),23 out of 100(23%) respectively .69% had other causes of jaundice including G 6PD deficiency and Minor blood group incompatibility. The percentage of babies with reticulocytosis and positive Coombs test was 25% and 32% respectively in neonates requiring phototherapy. The percentage of babies showing reticulocytosis was100% in Rh incompatibility in contrast to-56.5% in ABO incompatibility. 5% of those babies without blood group incompatibility also had reticulocytosis Coombs test positivity was seen in 100% cases in Rh incompatibility.

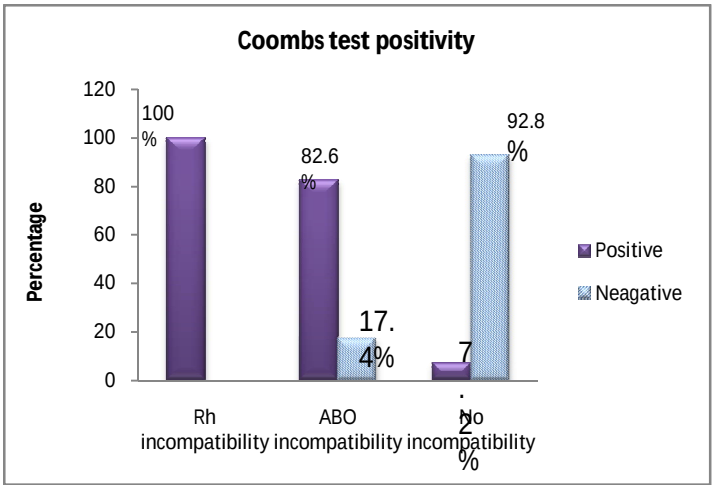

Figure 1: Graph showing Coombs test positivity(Figure 1)

Table 1: Association between hemolytic hyperbilirubinemia and reticulocytosis

Immune hyperbilirubinemia	Reticulocytosis		Total	p value
	Reticulocytosis, N (%)	No reticulocytosis N (%)		
Yes	21 (67.7)	10 (32.3)	31	0.000*
No	4 (5.8)	65 (94.2)	69	
Total	**25**	**75**	**100**	

Table 2: There was significant association between reticulocytosis and immune hyperbilirubinemia. ($p<0.05$) Association between ABO incompatibility and reticulocytosis

Incompatibility	Reticulocytosis		Total	p value
	Reticulocytosis, N (%)	No reticulocytosis N (%)		
ABO incompatibility	13 (56.5)	10 (43.5)	23	0.000*
No incompatibility	4 (5.8)	65 (94.2)	69	
Total	**17**	**75**	**92**	

There is an association between ABO incompatibility and reticulocytosis ($p<0.05$) There was no association between reticulocytosis and Coombs test positivity in ABO incompatibility ($p>0.05$) nor in No incompatibility group Table3,4

Table 3: Association between Reticulocytosis and Coombs test positivity in ABO incompitibility

Reticulocytosis	Coombs test		Total	p value
	Positive, N (%)	Negative, N (%)		
Reticulocytosis	12 (92.3)	1 (7.7)	13	0.281
No reticulocytosis	7 (70)	3 (30)	10	
Total	**19**	**4**	**23**	

Table 4: Association between Reticulocytosis and Coombs test positivity in No incompatibility

Reticulocytosis	Coombs test		Total	p value
	Positive, N (%)	Negative, N (%)		
Reticulocytosis	0 (0)	4 (100)	4	1
No reticulocytosis	5 (7.7)	60 (92.3)	65	
Total	**5**	**64**	**69**	

DISCUSSION

Neonatal hyperbilirubinemia is one of the major reasons for prolonged hospital stay and if it is left untreated can cause morbidity. A recent report by Bhutani *et al* [8] at least 481000 term/near-term neonates are affected by hyperbilirubinemia each year, with 114 000 dying and an additional 63 000 surviving with kernicterus. The main modality of treatment is Double surface phototherapy .The degree of hyperbilirubinemia ,gestational age of the baby and the cause of hyperbilirubinemia decides the degree of morbidity .The sustainable development goals (SDGs) which is being implemented now , focuses on persons with disability. It is essential that we manage hyperbilirubinemia as one key component of optimising neurodevelopmental outcome. Without any risk factors (normal birth weight infant delivered vaginally at 39 to 41 weeks' gestation by a non-Asian, non-obese -, multiparous mother) the rate of non-hemolytic neonatal hyperbilirubinemia was 0.7%.[9] According to a recent study the contribution of immune haemolytic anemia as a cause is significant hyperbilirubinemiais 24.3% and 1% for ABO incompatibility and Rh incompatibility respectively.[10] In a study in Gujarat [11] out of 63, ABO incompatibility was15% , Rh incompatibility 8%. Nearly 1/3rd (32.9%) babies were ABO incompatible and 4.1% babies were Rh incompatible in a study in NEpal.[12]Our study had 23% of babies with Rh incompatibility and 5% with ABO incompatibility which is at par with previous studies. When there is more than one cause for hyperbilirubinemia in a neonate with blood group incompatibility, it is difficult to establish the major cause.Reticulocytosis and direct Coombs test positivitycan be taken as evidence of haemolysis, and incompatibility playing themajor part for the underlying the disease**.** According to a previous study[13]highest level of reticulocytes was noted inimmune haemolytic as well as in G-6PDDeficiency. In another study [14] 33.5% (67) of the 200 babies studied had reticulocytosis (haemolytic cause for jaundice), 32 of these 67 (47.76%) babies had ABO and Rh incompatibility. In contrast a study in Tehran showed no difference in reticulocytosis in immune and G6PD deficiency (other causes of NON immune hyperbilirubinemia was excluded in the study).[15]Reticulocytosis was seen in 25% of babies requiring phototherapy giving a clue that haemolysis may not be the sole reason for jaundice in term neonates.100% babies in Rh incompatibility, 56.5% in ABO incompatibility and 5.8% in other causes of hyperbilirubinemia showed reticulocytosis. There was a significant association between ABO incompatibility and reticulocytosis ($p<0.05$) and also when all cases of incompatibility (both ABO and Rh together)the association was significant. This association thus gives a clear suggestion that reticulocytosis if present can be taken as a marker for haemolysis otherwise other causes of jaundice is to be considered even if there is blood group incompatibility. The Coombs test has been shown to have a positive predictive value of 12%–53% and a sensitivity of 15%–64% for the subsequent development of hyperbilirubinemia.[16] Coombs test readings were associated with a higher incidence of hyperbilirubinemia as per study by Kaplan etal.[17] Our study showed Coombs positivity of 100% in Rh incompatibility,82.6% positivity in ABO incompatibility and 7.2% in no incompatibility group.But to our surprise there was no association between reticulocytosis and Coombs positivity in either ABO incompatibility group or when all immune hyperbilirubinemia causes were taken together. The RH group alone was not analyzed for associations as it showed 100% positivity for both reticulocytosis and Coombs test. The reason for Coombs positivity in No incompatibility group may be inclusion of minor incompatibility and other RH factors incompatibility like Rh E. In these cases further analysis for minor blood groups is necessary in clinical practice

Our study suggests that Coombs test and reticulocytosis can be used as separate tools for identifying babies with immune causes as major contributing factor for hyperbilirubinemia. This information will aid in selecting cases for immunoglobulin infusion which is another effective modality of treatment for hyperbilirubinemia but is very expensive.

LIMITATIONS

In our study No incompatibility group was not further subdivided according to etiology. A differentiation between haemolysis due to abnormalities in RBCs and other causes like dehydration, breast milk jaundice etc was not done in this group. More over this group may include minor blood group incompatibilities which were not addressed.

CONCLUSION

Reticulocytosis and Coombs positivity are good markers of haemolysisin neonatal hyperbilirubinemia. It would be wise if these tests are included in routine investigation panel for neonatal hyperbilirubinemia as this will help to pin point the etiology to a certain extent and also to guide regarding other modalities of treatment especially use of immunoglobulin.

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