



## **A STUDY OF DISTRIBUTION OF NEONATAL JAUNDICE DUE TO BLOOD GROUP INCOMPATIBILITY**

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### **ABSTRACT**

To study distribution of neonatal jaundice due to blood group incompatibility especially focusing on blood group irregular antibodies. Settings and design: 50 cases of neonatal pathological jaundice from Feb 2010 to Jan 2012 were studied prospectively which were further evaluated to find the distribution of ABO incompatibility, Rh incompatibility, rare group antibodies & to compare them to find out importance of all the three. ABO and Rh grouping, Antibody screening by Indirect Antihuman globulin test and Antibody identification tests were carried out on mother's blood sample. ABO and Rh grouping, Direct Anti – human globulin test, Hemoglobin, S. Bilirubin and reticulocyte count were done on neonate's blood sample. Additional investigations were done in selected cases. Statistical analysis used: Chi square test. The distribution of ABO and Rh incompatibility was equal (18%). Group O in neonates was seen with Rh incompatibility in 12% cases. Out of 9 ABO incompatible cases 5 cases suffered from hemolytic diseases of new born (56%). 22% positivity of DCT in Rh incompatible neonates as compared to 11% in ABO incompatible neonates. 100% positivity of DCT was seen in case of irregular antibodies. One case out of 9 Rh incompatible cases showed positive IAT (11%), none of the ABO incompatible cases showed positive IAT and one case of irregular antibody showed positive IAT (100%). Blood group incompatibility is an important cause of neonatal jaundice. This highlights the importance of routine antenatal antibody screening not just limiting to Rh negative females but in all pregnant females for ABO and irregular antibodies.

**Key words:** Neonatal jaundice, Blood group incompatibility, Irregular antibodies.

### **INTRODUCTION**

Although most jaundiced infants are otherwise healthy, they make us anxious because bilirubin is potentially toxic and if bilirubin level is very high it can lead to complications like kernicterus (Fig 1). Pathological unconjugated hyperbilirubinemia in a neonate can be due to fetomaternal blood group incompatibility, hemolytic anemia, G-6- PD deficiency, pyloric stenosis whereas conjugated bilirubin increases in conditions such as sepsis, biliary atresia, giant cell hepatitis, cystic fibrosis, galactosemia, tyrosinemia etc. The International Society of Blood Transfusion currently recognizes 30 blood group systems including: ABO system and Rh, MNS, Kell, Lewis etc. Thus in addition to ABO and Rh incompatibility many other irregular antibodies can be responsible for fetomaternal blood group incompatibility and produce hemolytic disease of

newborn. Aim of this study is to evaluate causes of neonatal jaundice and study the distribution of various fetomaternal blood group incompatibilities among them since there is an observed paucity of data on the prevalence of irregular antibodies in the Indian population, despite having one of the highest obstetric loads in the world

### **MATERIAL AND METHODS**

In our study 50 cases of neonatal pathological jaundice from Feb 2010 to Jan 2012 were studied prospectively which were further evaluated to find the distribution of ABO incompatibility, Rh incompatibility, rare group antibodies & to compare them to find out importance of all the three. All were neonates admitted in the neonatal intensive care unit of the hospital.

The data was collected through hospital records. The obstetric history of mother's was also collected through hospital records and interviews. ABO and Rh grouping, Antibody screening by Indirect Antihuman globulin test and Antibody identification tests were carried out on mother's blood sample. ABO and Rh grouping, Direct Anti – human globulin test, Hemoglobin, S. Bilirubin and reticulocyte count were done on neonate's blood sample. Additional investigations like newborn thyroid hormone levels, glucose–6–phosphate dehydrogenase levels etc. were done in selected cases.

## RESULTS

The most common blood group in mothers of jaundiced neonates was O (36%). Table 1 shows the distribution of blood group combinations of mother and neonate. It shows that the most frequent percentage combination of mother and neonate's blood group was B+ve mother with B+ve neonate. Group O in mothers was seen with ABO incompatibility in 18% cases. Group O in neonates was seen with Rh incompatibility in 12% cases. Table No 2 shows the distribution of cause of

pathological jaundice in neonates in the present study. The distribution of ABO and Rh incompatibility was equal (18%). Prematurity was present in 30% cases but among these it was present along with incompatibility in 6% cases and with sepsis in 10% cases. Out of 9 ABO incompatible cases 5 cases suffered from hemolytic diseases of new born (56%). Table 3 shows association bad obstetric history with blood group incompatibility and other causes of neonatal jaundice.

It is clear from this table that no significant association was observed between bad obstetric history and blood group incompatibility i.e.  $\chi^2 = 2.138$ ,  $P > .05$  22%. Positivity of DCT in Rh incompatible neonates as compared to 11% in ABO incompatible neonates. 100% positivity of DCT was seen in case of irregular antibodies. One case out of 9 Rh incompatible cases showed positive IAT (11%), none of the ABO incompatible cases showed positive IAT and one case of irregular antibody showed positive IAT (100%). Highest number of exchange transfusions were seen in ABO Incompatible neonates (8%). The maximum total serum bilirubin concentration was 29.8 mg/dl in our study.

**Table 1. Distribution of blood group combinations of mother and neonate among all cases in percentages.**

Mother's blood group		Neonate's blood group							
		A		B		O		AB	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
A	+ve	8	0	4	0	2	0	4	0
	-ve	4	0	0	0	6	0	0	0
B	+ve	2	0	14	0	8	0	0	0
	-ve	0	0	2	0	0	0	0	0
O	+ve	10	0	8	2	8	0	0	0
	-ve	0	0	2	0	6	0	0	0
AB	+ve	4	0	4	0	0	0	0	0
	-ve	0	0	0	0	0	0	0	0

**Table 2. Distribution of causes of pathological jaundice in neonates.**

Causes of pathological jaundice	Number of cases (n=50)
ABO incompatibility	9 (18%)
Rh incompatibility	9 (18%)
Irregular antibodies	1 (2%)
Prematurity	15 (30%)
Septicemia	15 (30%)
Cirrhosis	1 (2%)
Cephalhematoma	1 (2%)
Idiopathic	4 (8%)

**Table 3. Association of bad obstetric history with blood group incompatibility and other causes of neonatal jaundice**

Obstetric history	Causes		Total
	Blood group incompatibility	Other causes	
Normal Obstetric history	11 (22%)	24 (48%)	35 (70%)
History of abortion/ leaking PV	8 (16%)	7 (14%)	15 (30%)
Total	19 (38%)	31 (62%)	50 (100%)

$$\chi^2 = 2.138 \quad \text{d.f}=1 \quad P > .05$$

**Figure 1.**



## DISCUSSION

If the mother's blood type does not match the fetal blood type (baby is type B or A and mother is type O), then the mother's immune system may create antibodies against the fetus's blood type, which then can travel back across the placenta to the fetus [1]. After the baby is born, some of the baby's red blood cells (RBCs) may be coated with the maternal antibodies, leading to destruction of some of the RBCs (hemolysis, also referred to as hemolytic disease of the newborn) by the baby's immune system [2]. ABO incompatibility occurs in approximately 15% of all pregnancies, but hemolytic disease of the newborn develops in only 4% [3].

In our present series of 50 cases of neonatal jaundice, in 46 cases we could find a definite etiology for jaundice. Out of these 19(38%) had blood group incompatibility, 15 (30%) had sepsis, 22(44%) were premature and 1 case each of cirrhosis and intracranial hematoma. In four of the cases no definite cause could be found and they were labeled idiopathic, though prolonged labour was associated in 2 of these cases. Among the 19 cases of blood group incompatibility, 9(18%) were due to ABO incompatibility, 9(18%) were due to Rh incompatibility and one case due to anti c (irregular antibody).

In our study it was found that ABO incompatible mother and their children were 9 out of 50 cases (18%) but hemolytic disease due to ABO incompatibility, developed in 5 cases i.e. 56% of the cases and Rh incompatibility is seen in 18% cases. A similar study was done by S.C. Gupte and H.M. Bhatia at Wadia Maternity Hospital and Seth Medical College, Bombay [4]. During February 1969 to November 1970 they studied 1000 infants, among them 38.3% of infants had ABO incompatibility in comparison to expected incidence of 26.4 % in normal population. In a study by Ozolek JA et

al showed that in USA 6.9% of all births are of neonates with maternal-fetal ABO incompatibility and ABO-HDN is now the single most common cause of neonatal jaundice [5].

In our study incompatibility rate found in group O mother with group A neonates was 44% and incompatibility in group O mothers with group B neonates was 56%. This is comparable to a similar study done by R. Renuka Nair et al in 1980 [6]. The study showed that incompatible neonates of group O mothers were more affected than A & B group because group O individuals produce more IgG antibodies. Antibodies formed by group O individuals may be stronger as compared to other groups. This is also cited by Dean L. Bethesda in his book on red cell antigens, 2005[7]. The anti -A and anti-B formed in group O individuals tend to be of the IgG type (and therefore can cross the placenta), whereas the anti-A and anti-B found in the serum of group B individuals, respectively, tends to be of the IgM type. A comparable study was done by Ziprin JH et al in 2005[8]. They reported two cases of fetal hydrops secondary to ABO incompatibility. The first was a twin pregnancy and second was a preterm baby. Both cases had severe anemia and both revealed elevated titres of IgG anti-B antibodies.

In our study there was an equal distribution of ABO incompatibility and Rh incompatibility (18%). This may be due to lack of proper antenatal screening and awareness regarding IgG administration among the pregnant females in our region. The decreased incidence of Rh incompatibility worldwide may be due to the better antenatal screening and administration of Rh Ig in today's time. More Rh negative mothers are identified during routine screening nowadays, and severe hemolytic disease is prevented. Many of the red blood cell (RBC)

alloantibodies of the Rh system have been associated with HDN; however, the severity of the disease is usually the greatest with anti-D. Prevention of Rh HDN became feasible in the late 1960s after pioneering research by Finn Clarke and Freda; there was a dramatic decline in Rh HDN [9]. Since then, other Rh and non Rh red cell antibodies have become relatively more important and are now responsible for the greater proportion of HDN cases.

The increased incidence of ABO incompatibility is revealed by a study done by Dr. Hassan et.al, Iran [10]. This descriptive analytic study has been done from October 2002 to September 2009 on 1568 newborns over a period of seven years. 795 neonates were included in their study. (237 infants with hemolytic jaundice and 558 infants with idiopathic jaundice). 17% of newborns with ABO incompatibility, (7%) of newborns with Rh disease, (6%) of newborns with G6PD deficiency, and (2%) newborns with minor blood group incompatibility developed hyperbilirubinemia. Among the newborns affected with kernicterus 12 cases were placed in group with ABO hemolytic disease (9%), 3 cases were in Rh isoimmunization group (5.5%), 4 cases were in G6PD deficiency group (8.9%) and 9 cases were idiopathic. Therefore this study showed that ABO incompatibility was the most common cause of hemolytic jaundice among neonates in north east of Iran which is comparable to our study. In the study done at Iran, Only one third of newborns with ABO incompatibility developed severe hemolytic disease of newborn. In our study ABO incompatibility was seen in 9 cases though severe hemolytic disease requiring exchange transfusion occurred in 4 (44%). A study by Meharban Singh showed that ABO incompatibility exists in 25% of cases but HDN develops in 1:10 of such offsprings [3]. In our study the incidence of ABO hemolytic disease is much more. This may be because the number of cases in our study is 50 only which is very small to represent whole population.

In our study out of 50 cases one case showed the presence of irregular antibodies (anti-c) i.e (2%). A similar case of hemolytic disease of the newborn due to rhesus isoimmunization (anti-c) has been reported by Z. Felc at Celje, Slovenia in November 2001 [11] and ShilpaSingla et al at AIIMS New Delhi in 2010[12]. Within the rhesus blood group system the most immunogenic antigens after D are c and E. These antibodies are found most usually in women who are Rh (D) positive and lack the c and E antigens. In the combined study by John T Queenan et al [13] of 8 years, 1.62% incidence of irregular antibodies was reported. Polesky [14] reported a 2.44% incidence of irregular antibodies in a 6 year study of 43,000 obstetric patients.

Our study showed that 11% infants with ABO incompatibility had positive DCT and 22% infants with Rh incompatibility had positive DCT. This is similar to a study done by E.L. Romano et.al in 1973 [15]. They found that the minimum number of IgG anti-A (or anti-

B) molecules detectable on A or B red cells by the antiglobulin reaction was found to be the same- that is, about 150 molecules per red cell-with newborn as with adult cells. Furthermore, the ratio of anti-IgG bound to IgG anti-A(or anti-B) molecules was the same whether the anti-A(or anti-B) molecules were present on newborn or on adult cells and was similar to that found for anti-IgG bound to IgG anti-Rh. In 15 infants (11 group A, 4 group B) with hemolytic disease of the newborn due to ABO incompatibility the amount of anti-A or anti-B on the red cells ranged from 0.25 to 3.5 ug antibody per ml red cells, corresponding to, 320 antibody molecules per cell; only five infants had more than 0.55 ug antibody per ml red cells. These amounts are far smaller than those found in most moderate or severe cases of Rh-hemolytic disease. It is concluded that the weak direct antiglobulin reactions observed in ABO -haemolytic disease as observed in our study are due simply to the fact that the number of anti-A or anti-B molecules on the infant's red cells is at the lower limit of sensitivity of the test. Since ABO -haemolytic disease can be quite a severe process it seems probable that IgG anti-A and anti-B molecules are more effective than anti-Rh molecules in bringing about red cell destruction [15].

In our study 30% cases had neonatal sepsis, 18% cases had Rh-HDN, 18% had ABO-HDN, 2% had anti-c hemolytic disease and another 44% were pre-term. Prematurity and LBW were increasingly seen in neonates suffering with sepsis in our study. Sixteen percent neonates had culture proved sepsis. TORCH infection, Coagulase -ve Staph aureus and Enterobacteriaceae were the common pathogens in our study.

In our study history of multiple abortions was seen in 3 out of 9 cases with Rh incompatibility (33%). This is due to maternal sensitization to fetal blood group antigens which cause hemolytic disease in the subsequent pregnancies. Presence of bad obstetric history was increasingly seen with neonatal hyperbilirubinemia in a study done by Kapoor et.al at Lucknow, from January 1992 to march 1993. Bad obstetric was present in 30% cases in our study. Though we could not find a significant association of bad obstetric history with blood group incompatibility, this may be because our sample size is not big enough [16].

In our study there were 22 (44%) cases of premature neonates with hyperbilirubinemia. Occurrence of prematurity in jaundiced neonates is also supported by a study done by Agarwal et al at Meerut in 2006 [17]. They found that newborns of mothers with short gestation period were found to have high serum bilirubin levels. It was also reported that the total serum bilirubin binding capacity and molar binding ratio of bilirubin to albumin are low in preterm babies and thereby a greater risk of developing kernicterus. It may also be due to greater immaturity of liver and less active hepatic enzymes in the preterm neonate. Prematurity (33.3%) and neonatal

septicemia (25.9%) were the most common causes of pathological jaundice in a study by Kaini et al in Nepal also showing 11% ABO and 7.4% Rh incompatibility [18]. These results are comparable to our study. The reason for high occurrence of ABO incompatibility in our study is that we have evaluated jaundiced infants as compared to other publications who evaluated all newborns. 18% of newborns in our study underwent exchange transfusions. Of the 9 newborns who underwent exchange transfusion, two (4%) required more than one exchange transfusion. The most common causes of exchange transfusion overall were ABO incompatibility (8%). A similar study showing increased numbers of exchange transfusions done in ABO incompatibility was done by Badiie Z [19].

Also Dikshit and Gupta [20] and Sanpavat [21] reported that ABO hemolytic disease of the newborn is the most common cause of exchange transfusion in term neonates (35.9% and 21.3% respectively). Exchange blood transfusion remains the gold standard for effective treatment of neonatal hyperbilirubinemia. Although reports show a progressive decline over the years in the number of neonates needing exchange transfusions, it is still required in upto 7% of neonates admitted to nurseries. This reduction in the number of exchange transfusions may be due to the development of anti-Rh globulin for Rh-negative mothers and the widespread use of phototherapy for neonatal jaundice. The maximum total serum bilirubin concentration was 29.8 mg/dl in our study.

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## CONCLUSION

There was an equal distribution of ABO and Rh incompatibility causing neonatal jaundice in our study (18%), but immune hydrops due to irregular antibodies (2%) also form an important cause of neonatal morbidity and mortality. This highlights the importance of routine antenatal antibody screening not just limiting to Rh negative females but in all pregnant females for ABO and irregular antibodies. There is a need to impose properly formulated protocols to screen pregnant women with unfavourable obstetric history of late trimester mishaps and pregnancies with fetal hydrops. Blood bank guidelines for the screening of maternal serum antibodies and facilities have to be updated to decrease the occurrence of preventable perinatal morbidity and mortality.

All pregnant women should have samples taken early in pregnancy, ideally at 10–16 weeks gestation, for ABO and Rh typing and for screening for the presence of irregular antibodies. When an antibody screen is positive further tests should be carried out to determine the antibody specificity and significance. Incidence of Rh incompatibility can be reduced by routine antenatal screening and anti D usage, but usage of coomb test and antibody identification in antenatal period for other blood group incompatibility may decrease severe jaundice and its complications in many neonates.

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## CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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