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Original Research Article

OUR EXPERIENCE OF ABO-HEMOLYTIC DISEASE OF THE NEWBORN - CASE SERIES FROM A TERTIARY CARE HEALTH CENTER IN NORTH INDIA.

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ABSTRACT

Background: ABO-hemolytic disease of newborns (HDN) is a condition where there is an incompatibility between the blood types of the mother and fetus. It is the most common and leading cause of neonatal jaundice caused by maternal and fetal ABO incompatibility, mainly seen in neonates of blood group A or B born to mothers of blood group O. With the introduction of Rh immune globulin (RhIg), the incidence of Rh D allo-immunization has decreased over the last few decades. Consequently, hemolytic disease of newborns due to ABO incompatibility and other alloantibodies have now emerged as a significant cause of HDN. **Aim:** To assess the severity and clinical outcome of ABO HDN in our settings.

Material and Methods: Routine investigations were raised from the neonatology department after the delivery of the neonate for direct Coombs test (DCT), and ABO blood grouping was sent to the immuno-hematology laboratory of the Transfusion Medicine department. From May to July 2018, there were 4 cases of ABO–HDN diagnosed. In all cases, mothers were of blood group O, so their serum IgG titer was performed for anti-A and anti-B. The treatment and clinical outcomes of all four cases were analyzed.

Results: Forward grouping for all neonates was B+ve, except one A+ve. In all four cases, the Mother serum anti-A titer ranged from 1:512 to 1:1024; similar results were seen for anti-B. Titre was also performed on neonate eluate samples ranging from 1:128 to 1:1024 for anti-A and 1:512 to 1:1024 for anti-B.

Conclusion: ABO-HDN incidence is 15-17% in the Indian population. ABO-HDN should be suspected in neonates with blood type A or B born to mother blood type O with indirect Coombs test negative. Most of the cases of ABO-HDN had benign clinical outcomes without the need for exchange transfusion. The intervention of choice was phototherapy, which was also done in all cases except one.

Keywords: Hemolytic disease of the fetus/newborn, ABO-incompatibility, neonatal jaundice, alloantibodies, eluate, direct and indirect coombs test, phototherapy, exchange transfusion.

INTRODUCTION

The ABO-hemolytic disease of the newborn (HDN) leads to immune-mediated destruction of antibody-specific red cells in the fetus due to the transplacental transfer of maternal IgG immunoglobulin.[1] ABO incompatibility is the most common cause among all blood type incompatibility for hemolytic disease in newborns. After the introduction of prophylactic anti-D administration

there is a drastic reduction of Rh HDN, but ABO incompatibility has emerged as a significant cause of HDN.[2] ABO incompatibility in pregnancy means when the mother's antibodies react with the red blood cells of a newborn with a different blood type. Consequently, the red blood cells of the newborn are hemolysed, leading to fetal anemia and hyperbilirubinemia. Most commonly affected neonates are of blood group A, B, and AB born to mothers of O blood group or A blood group neonates born to A2B or B blood group mothers. [3] The severity of ABO-HDN is mild and self-limiting compared to Rh HDN, which is the most severe. Mild severity can be explained by a wider distribution of A and B antigens in the body other than red blood cells, compared to Rh antigens being localized to the red blood cell membrane only. But the subclass and quantity of maternal IgG anti-A or anti-B that cross the placenta affects the severity of ABO HDN as IgG1 and IgG3 have high red blood cell hemolytic potential than IgG2 and IgG4.[4] Therefore, in milder cases, most cases are managed with phototherapy alone, but a partial or exchange transfusion is needed in rare cases.[5-6]

MATERIAL AND METHODS

Our study group consists of four cases of neonates from the neonatology department, born to mothers with type O RhD-positive blood groups who underwent a regular prenatal examination in the General Hospital from May to July 2018 at Sanjay Gandhi Postgraduate Institute of Medical Science, Lucknow, Uttar Pradesh. Informed consent was taken from all the participants (pregnant women and the newborn's guardians). Three of four pregnant women were primigravidae and one multigravida, with no previous blood transfusion record and negative indirect Coombs test, i.e., excluding the possibility of other acquired antibodies. The diagnostic criteria of ABO-HDN are maternal and infant ABO blood group incompatibility, maternal indirect Coombs test negative, hyperbilirubinemia within 24 hours of birth, and positive direct Combs test in neonates.

In all neonates, blood grouping (ABO/RhD) and direct Coombs test (DAT) were performed by column agglutination technique using a gel card of Bio-rad. Cold acid elution was performed in all four cases. Neonate RBCs were treated with DiaCidel solution (Bio-Rad), and Anti-A and Anti-B titers were performed on the eluate using the serial dilution technique via a test tube. All four maternal serum samples were treated with 0.01M Dithiothreitol (DTT) to destroy IgM antibodies, and IgG titer of Anti-A and Anti-B was performed.

RESULTS

All four women were found to be O+ve, with infants in the first three cases being B+ve and the fourth being A+ve. The total bilirubin in the first case was 12.3 mg/dl, the second was 19.13 mg/dl, the third was 13.12 mg/dl, and the fourth was 11.53 mg/dl. Direct Coombs' test strength on the gel card for first, second, third, and fourth neonates were 2+,3+, 2+, and 1+, respectively, as shown in Table 1. [Table 1] The IgG Anti-A vs. Anti-B, the titer of all fours mother's serum samples, were 1:512 vs1:512, 1:1024 vs. 1:1024, 1:1024 vs 1:1024 and1:1024 vs. 1:512 respectively, as shown in Figure 1.

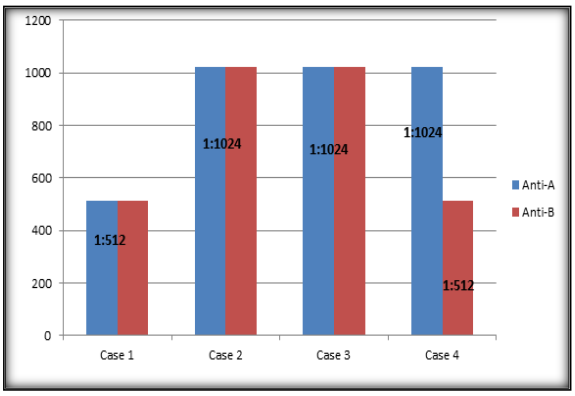

Figure 1: IgG Anti-A and Anti-B titer of four mother's samples case-wise

After cold acid elution, the eluate in the first three cases showed specificity to B cells, confirming the presence of maternal IgG type Anti-B, and the fourth eluate showed specificity to A cells, confirming the presence of IgG type Anti-A of maternal origin, as shown in. [Table 2]

Table 1: Case-wise mother and infant blood group with total bilirubin, direct coombs test results, and treatment

S.no	Case	Anti A	Anti-B	Anti-D	A/c	B/c	Interpretation (Mother blood group)	Infant blood group	Infant total bilirubin	DCT strength (Infant)	Treatment
1	Primigravidae(G1P0) 27weeks	-	-	+	+	+	O+ve	B+ve	12.3 mg/dl	2+	Phototherapy
2	Primigravidae(G1P0) 35weeks	-	-	+	+	+	O+ve	B+ve	19.13 mg/dl	3+	Partial exchange transfusion
3	Primigravidae(G1P0) 26 weeks	-	-	+	+	+	O+ve	B+ve	13.12 mg/dl	2+	Phototherapy
4	Pmultigravidae(G3P3) 22 weeks	-	-	+	+	+	O+ve	A+ve	11.53 mg/dl	1+	Phototherapy

G-Gravida, P-Para, A/c – A red blood cells, B/c –B red blood cells, DCT- direct combs test.

Table 2: Eluate showing specificity to different red cells in four cases

Case	A-cells	B-cells	Last wash
1-eluate	-	+	-
2-eluate	-	+	-
3-eluate	_	+	-
4-eluate	+	-	-

DISCUSSION

ABO incompatibility is the most common cause of hemolytic disease in the fetus and newborn; it leads to the destruction of fetal/newborn red blood cells by the IgG antibodies transferred from the mother during pregnancy and their clinical manifestation before or after birth.[7] ABO hemolytic disease of fetus /newborn mainly occurs in infants born to O blood group mothers with A or B blood group or A blood type newborns born to A2B or B blood group mothers. As implied, naturally occurring IgG type anti-A and anti-B are formed during fetal growth; therefore, the first pregnancy is affected compared to acquired Rh-D antibodies formed after sensitization, in which the second pregnancy is affected. Prevalence of ABO hemolytic disease of the fetus/newborn effect 15-20% of all pregnancies.[8] These naturally occurring IgG type Anti-A and Anti-B destroy fetal/ newborn red cells, resulting in fetal anemia/hyperbilirubinemia in 10% of the cases.

For diagnosis of ABO- Hemolytic disease of the fetus and newborn following points should be considered - first affected pregnancy, A and B blood type newborn born to blood type O mother, bad obstetric history, previous fetal loss, neonatal hyperbilirubinemia within the first 24 hours of birth, positive direct Coombs test in neonates, and negative maternal indirect coombs test.[9] A negative direct Coombs test should not exclude hemolytic disease in newborns; one must also focus on laboratory and clinical aspects of newborns and exclude other causes of hyperbilirubinemia like G6PD deficiency and Rh isoimmunization. However, this disease has mild clinical presentation, which can be attributed to various factors like-expression of A and B antigens in organs other than red blood cells, leaving fewer antibodies to associate with red blood cells, low antigenic density at birth, and the presence of A and B substances in plasma.[10] Therefore, maximum cases are treated with phototherapy and, in rare circumstances, cause fetal hydrops, which need exchange transfusion. In our case series, three cases were treated with new modalities, like improved phototherapy, except one that required a partial exchange transfusion. Bilirubin levels should be checked periodically after birth to prevent neurological manifestations like kernicterus, as fatal cases of ABO–HDN were reported.[11]

In the study looking at pre-term infants, ABO-incompatibility causes more problems in babies born in the third trimesters compared to the second and first trimesters, indicating the timing of maternal alloantibodies production, as seen in our second case.[12] Maternal antibodies titer also affects the severity of ABO hemolytic disease as concordant with our second case; discordant with the third case may be explained by IgG subtypes linked with the severity of the disease, which testing is lacking in our case series. In medical literature, several studies indicate a correlation between the strength of DCT and the severity of ABO incompatibility in newborns. Stronger positive DCT indicates more red cell hemolysis, as concordant with case 2, which had 19.13mg/dl of bilirubin and needed a partial exchange transfusion.[13-14] Elution testing should be followed after a positive or negative direct Coombs test in neonates, and a positive or negative indirect Coombs test in mothers helps detect the antibodies on the surface of the red blood cells of neonates and assists in identifying specific antibodies causing hemolysis.[15]

Much medical literature has reported the incidence of spontaneous abortion in type O mothers with ABO incompatibility, indicating antigen variation between husband and mother on the red cell membrane leads to the production of antibodies against the fetus and newborn which necessitates the need for maternal anti-A and anti-B titer in their first pregnancy with negative indirect Coombs test.A study done in 2009 in India showed the maximum fetal loss in couples of blood type O women married to A and B blood type men. [16-17] Medical literature showed that total fetal loss was higher in ABO-incompatible than in compatible mating.[18]

CONCLUSION

ABO-incompatibility between mother and newborn is the most common and leading cause of hemolytic disease in the newborn, the second being Rh incompatibility, followed by other minor antigen incompatibilities. With the introduction of Rh immune globulin (RhIg), the incidence of Rh D alloimmunization has decreased over the last few decades. Consequently, hemolytic disease of newborns due to ABO incompatibility and other alloantibodies have now emerged as a significant cause of HDN. Though ABO HDN is typically less severe than Rh hemolytic disease of newborns (Rh-HDN), it can still cause jaundice, anemia, and other health issues in newborns. Therefore, in pregnant female mothers, early detection of maternal antibody production via antibody screening followed by its titer; in neonates, direct Coombs test and periodic monitoring of bilirubin levels is necessary, and timely intervention with phototherapy, intravenous immunoglobins and

exchange transfusion are crucial in reducing the risk of complications.

Lastly, Transfusion Medicine specialists and technicians should be vigilant when encountering ABO incompatibility between mother and fetus or mother and father for better patient care. We studied four cases, which is a limitation of our study, so we recommend a large population-based prospective follow-up study.

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