International Journal of Scientific Study

Original Article

Hemolytic Disease of the Newborn: A study of 50 cases

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Abstract

Background: Hemolytic disease of the Newborn (HDN) is characterized by the presence of IgG antibodies in maternal circulation, which causes hemolysis in the fetus by crossing the placenta and sensitizing red cells for destruction by macrophages in the fetal spleen with consequent hyperbilirubinemia.

Aim: The present study was carried out to evaluate the importance of various etiologies of Hemolytic Disease of Newborn in our hospital, to study the effect of sex, birth weight, gravidity of the mother and blood group in the outcome of disease and also to study the efficacy of Direct Antiglobulin test on predicting the outcome of alloimmune HDN.

Methods: Infants with indirect hyperbilirubinemia were taken as subjects and were compared with a control group of healthy infants. Patients were divided into two groups. Patients with indirect bilirubin less than 12 mg/dl and having mild disease were classified into Group A and patients having indirect bilirubin more than 12 mg/dl were labeled as Group B.

Result: Out of the 50 patients studied, 23 belonged to group A and remaining 27 to group B. Group C (control group) comprised of 50 healthy infants. ABO incompatibility was the leading cause of hemolysis (in 48%) followed by Rh incompatibility (in 22%), septicemia in 26% and G6PD deficiency in 4%.

Conclusions: In our study, we concluded that alloimmune hemolytic anemia due to ABO incompatibility is the most common cause of HDN. Gender of the baby and gravidity of the mother does not affect the outcome of disease process. However HDN due to Rh antibodies is uncommon in primigravida. Direct Antiglobulin test of baby has a strong predictive value determining the outcome of alloimmune hemolytic disease of newborn but it does not predict the severity of disease.

Key Words: Hemolytic Disease of newborn (HDN), Direct Antiglobulin Test, ABO Incompatibility, Rh Incompatibility

Introduction:

Hemolysis due to alloimmune antibodies is seen with acute and delayed RBC transfusion reactions, following stem cell transplantation where there is an antigenic blood type difference between the donor and stem cell recipient, and during the neonatal period as a result of differences in maternal and fetal RBC antigens[1], The spectrum of clinical problems in hemolysis occurring in the fetus ranges from minimal hyperbilirubinemia to severe anemia with hydrops fetalis and/or kernicterus. HDN is characterized by hemolysis as a consequence of maternal sensitization to fetal RBC antigens inherited from the father resulting in the presence of IgG antibodies in maternal circulation which causes hemolysis in the fetus by crossing the placenta[2]. Early detection and treatment of neonatal hyperbilirubinemia is important in prevention of bilirubin-induced

encephalopathy[3]. It is classified as RhD HDN, ABO HDN and HDN due to other blood group antibodies (non-ABO, non-RhD) according to the specificity of causative IgG antibodies. RhD incompatibility is still one of the most common cause of HDN, although other RBC incompatibilities are increasing in incidence4. The role of Rh (D) antibody in classic erythroblastosis fetalis was first elucidated by Levine and Katzin in 1941[5]. In this study, we studied 50 cases of Hemolytic Disease of Newborn for their etiology. We also studied the effect of gender of the baby, birth weight, blood group and gravidity of mother on outcome of disease. We also performed Indirect Antiglobulin test on mothers' sera and Direct Antiglobulin test on babies' RBCs for predicting the outcome of alloimmune hemolytic disease of newborn.

Materials and Methods:

In this study, infants with indirect hyperbilirubinemia were taken as subjects. These were divided into two groups. Patients with indirect bilirubin less than 12 mg/dl and having mild disease were classified into Group A and patients having indirect bilirubin more than 12 mg/dl were labeled as Group B. The control group was chosen from normal infants born in our institute who did not have evidence of jaundice. Infants in these three groups were compared for their sex, birth weight, gravidity and blood group of mother and baby. Also recorded was etiology of hemolysis in these patients. Bilirubin was measured by Van den bergh reaction[6]. Direct and Indirect antiglobulin tests were performed using the standard operating procedure of the blood bank using gel card method on three times cell washed sample for Direct Antiglobulin Test[7] and pooled O cell prepared fresh. The gel system is based on the principle that the Sephadex gel matrix which serves as a filter thorugh which large erythrocyte agglutinates get entrapped in the gel. When a clear pellet of cells settle at the bottom of the microtube, it indicates a negative reaction. The reactions are graded according to the allocation of erythrocytes at the bottom of the gel. This technique is simpler to carry out and hence overcomes the practical problems of performing DCT by tube method. Other laboratory investigations carried out were hemoglobin (Hb), hematocrit, peripheral smear, reticulocyte count in neonates and ABO and RhD status of father if not done during pregnancy.

Result:

Out of 50 patients studied 23 belonged to group A and remaining 27 to group B. group C (control group) comprised of 50 healthy infants. On evaluating the cause of hemolysis, we found that Alloimmune HDN due to ABO incompatibility was the leading cause of hemolysis (48%). Alloimmune HDN due to Rh incompatibility accounted for hemolysis in 22% patients. Of the remaining cases septicemia was responsible for hemolysis in 26% and G6PD deficiency in 4%.

There were 65 % males in group A, 66 % in group B and 64% in group C. However, we found that the $\chi 2$ value of this table to be 0.055 with $P > 0.1$ which was highly insignificant which indicated that gender of the baby is insignificant as an etiological factor for HDN. Similarly we also found that the gravidity of the mother is also is insignificant as an etiological factor for HDN.

In patients with ABO incompatibility, 37.5% of patients were primigravida, 42 % were second gravid and rests were multigravida. However, in patients with Rh incompatibility, the maximum patients were multigravida (54%), followed by second gravid (36%) and only 9% patients were primigravida.

In the case of relation of HDN to birth weight & blood group of the patient, we found that their etiological implications were insignificant. The maximum number of patients in the test groups, 70% in group A and 63% in group B, had a positive Direct Antiglobulin Test. Whereas only 1% of patients in the control group had a positive Direct Antiglobulin Test. Blood cultures in case of septicemic patients revealed Klebsiella, Staphylococcus aureus, Streptococcus pneumonia, and Clostridium perfringens.

Table 1: Summary of HDN due to ABO incompatibility

	ABO HDN present	ABO HDN Absent
Mother of blood group O	22	24
Mother of blood group other than O	2	10

Table 2: Causes of HDN other than ABO incompatibility

Due to Rh anti D	11
Rh antigen other than anti D	Nil
Other than ABO or Rh antigen	Nil
Non immune etiology of hemolysis	15

Table 3: Result of Direct antiglobulin test on various groups

Group	Positive Direct Antiglobulin Test	Negative Direct Antiglobulin Test
A	16	7
B	17	10
C	2	48

Discussion:

In this study ABO incompatibility was the commonest cause of HDN in contrast to the study conducted by Dharmesh Chandra Sharma et al [8] in whose study Rh incompatibility was the commonest causeof HDN. It is more common in "O" blood group mothers because "O" blood group mothers have been shown to have high titers of IgG than "A" or "B" group mothers. In type A and B individuals, naturally occurring anti-B and anti-A isoantibodies which are largely IgM molecules; that do not cross placenta. In comparison, the alloantibodies present in type O patients are mainly of IgG antibodies. For this reason, ABO incompatibility is largely limited to type O mothers having fetal blood group A or B. The occurrence of IgG anti-A or anti-B antibodies in type O mothers also explains why hemolysis caused by ABO incompatibility frequently occurs during the first pregnancy without prior "sensitization". The pathophysiology of alloimmune hemolysis resulting from Rh incompatibility includes an Rh-negative mother, an Rh-positive fetus, leakage of fetal RBCs into the maternal circulation, and maternal sensitization to D antigen on fetal RBCs. The D antigen is the most immunogenic of the Rh antigens and there are no naturally occurring antibodies to Rh antigens. Immunization occurs almost exclusively during pregnancy. Small volumes of fetal RBCs enter the maternal circulation throughout the pregnancy. However, the main fetomaternal transfusion responsible for sensitization occurs during delivery. Rh hemolytic disease rarely ever occurs during the first pregnancy. However, once sensitization occurs, re exposure to Rh (D) RBCs in subsequent pregnancies leads to an anamnestic response and there is a rise in the maternal anti-D titer and an increased incidence of affected infants. We also deduced that for HDN of the newborn due to ABO incompatibility, gravidity does not appear to be a major criterion. Primigravida are affected as seriously as multigravida as sensitization does not occur in ABO HDN. However in Rh HDN out of 11 suspects 1 was born to primigravida and 10 were born to multigravida since Rh antibodies are uncommon in first pregnancy and tend to occur in later pregnancy after fetomaternal bleed. On further evaluating one primigravida it was revealed that she had previous history of accident for which she had blood transfusion in a local hospital. Antibodies are seen in 0.3% after first pregnancy and 6.6% after two. Sex of the patient also did not seem to influence outcome of the patients in our study. However both patients of G6PD deficiency were males as this disorder is X-linked. Birth weight and Blood group of the baby also did not affect the outcome of disease in our series. Antiglobulin test performed on the infants' sample correlates well with the occurrence of disease. We got P value for the Table – 9 is $P < 0.001$ which shows high predictive value of Antiglobulin test for occurrence of disease but for patients affected by disease, P value > 0.1 which is highly insignificant. Direct Antiglobulin test is thus highly nonspecific in predicting severity of disease.

Conclusion:

All immune hemolytic anemia due to ABO incompatibility is the most common cause of hemolytic disease of newborn. Gender of the baby does not have significant effect on the outcome of disease saving G6PD deficiency which is more common in males as disease is X-linked. Gravidity of mother does not affect the outcome of disease process. However HDN due to Rh antibodies is uncommon in primigravida. Blood group of patient does not affect the disease outcome. Birth weight of the patient also does not have any effect on the outcome of disease. Direct Antiglobulin test of baby has a strong predictive value determining the outcome of alloimmune hemolytic disease of newborn but it does not predict the severity of disease.

Acknowledgments:

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References:

1. Rowley SD. Hematopoietic stem cell transplantation between red cell incompatible donor–recipient pairs. Bone Marrow Transplant 2001;28(3):15–21.

2. A. G. Hadley. Laboratory Assays for Predicting the Severity of Hemolytic Disease of the Fetus and Newborn. Transplant Immunology, 2002;10:191-198.

3. A. Petrova, R. Mehta, G. Birchwood, B. Ostfeld and T. Hegyi. Management of Neonatal Hyperbilirubinemia: Pediatricians Practices and Educational Needs. BioMed Central Pediatrics, 2006;6:6-10.

4. J. M. Bowman, M. S. Kennedy, S. Wilson and J. G. Kelton, Eds. Historical Overview: Hemolytic Disease of Fetus and Newborn. Perinatal Transfusion Medicine, American Association of Blood Banks, Arlington, 1990;1:10-20

5. Levine PK, Katzin EM. Isoimmunization in pregnancy: its possible bearing on the etiology of erythroblastosis fetalis. JAMA 1941; 116: 8-25

6. Griffiths William James, Kaye Geoffrey. A study of the bile pigments in relation to the van den bergh reaction. Biochem J. 1930;24(5):1400–1407.

7. Lapierre Y, Rigal D, Adam J, Joseph D, Meyer F, Gerber S, Drot C. The gel test: a new way to detect red cell antigen–antibody reactions. Transfusion, 1990; 30:109–113.

8. Dharmesh Chandra Sharma, Sunita Rai, Sudha Iyengar, Bharat Jain, Satya Sao, Ajay Gaur, Rahul Sapra. Efficacy of Whole Blood Reconstituted in Exchange Transfusion in Hemolytic Disease of New Born-A Study of 110 Cases, Open Journal of Blood Diseases, 2013;3:15-20

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