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EXCHANGE TRANSFUION IN HEMOLYTIC DISEASE OF NEWBORN:EFFICACY OF WHOLE BLOOD RECONSTITUTED (WBR) OVER WHOLE BLOOD

Pathology

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ABSTRACT

INTRODUCTION: hemolytic disease of newborn; also known as erythroblastosis fetalis, is an preventable blood group disorder that occurs when the blood types of mother and fetus are incompatible. **MATERIAL AND METHOD:** A prospective study was carried out on the cases suffering with HDN in the year 2018-2020 in the Blood Bank of the Department of Pathology, G.R. Medical College, Gwalior. The affected cases were given double volume exchange transfusion by reconstituted blood or whole blood and the outcome was studied. **RESULT:** 150 cases of HDN were studied out of which RhD HDN was found to be the most common cause seen in 61.3% and exchange transfusion by reconstituted blood was found to be an effective therapy. **CONCLUSION:** The reconstituted blood is found to be immunologically much safer and better than whole blood for purpose of exchange transfusion in hemolytic disease of fetus and newborn because of its superiority in minimizing transfusion reactions and in achieving all the therapeutic effects of exchange transfusion in better way.

KEYWORDS

INTRODUCTION: Hemolytic disease of New Born (HDN) is featured by detection of IgG antibody in maternal circulation which causes hemolysis in fetus by crossing the placenta and sensitizing red blood cells for destruction by macrophages in fetus spleen resulting in hyperbilirubinemia[1]. The spectrum of adverse effects of hemolysis occurring in fetus ranges from minimal hyperbilirubinemia to severe anemia with hydrops fetalis and/or kernicterus. Bilirubin induced encephalopathy which is one of the adverse effects of hemolysis can only be prevented by early detection and treatment of neonatal hyperbilirubinemia[2]. According to the specificity of causative Immunoglobulin G (IgG) antibodies HDN is classified as

1. **Rhesus D HDN (RhD HDN),**
2. **ABO group HDN and**
3. **HDN due to other blood group antibodies (non-ABO, non-RHD).**

Several fetal Antigen may cause alloimmunisation like ABO, Rh (in this D, d, C, c, E and e antigen) Kell, Duffy and other blood group systems. A large number of cases of hemolytic disease used to be caused by rhesus D antigen but its incidence has significantly reduced with the administration of Rh immununoglobulin to Rh negative women during pregnancy and shortly after the birth of rhesus positive baby[3]. Consequently, ABO incompatibility now comprises the single largest cause of HDN in western world[4].The illustrious rhesus alloimmunization begins with red cells from rhesus -positive fetus crossing the placental barrier during pregnancy and delivery and entering the maternal blood circulation. The Kleihauer -Betke acid election technique determines the proportion of fetal RBC's in maternal circulation which shows that incidence and degree of feto-maternal hemorrhage increases with gestation being 7%, 16% and 29% in first, second and third trimester respectively. Procedures such as aminocentesis, chorionic villus sampling and cordocentesis also increase the risk of alloimmunization. An exchange transfusion is destined for sequential removal of aliquots of patient blood and simultaneously replacing it with donor blood in order to get rid of the abnormal blood components and circulating toxins while maintaining adequate circulating blood volume. Present comparative study was undertaken to see superiority of whole blood reconstituted over whole blood in exchange transfusion.

MATERIAL AND METHOD : This study was carried out on the cases suffering with HDN in the year 2018-2020 in the Blood Bank of the Department of Pathology, G.R. Medical College, Gwalior. Reconstituted blood and whole blood was given to the babies suffering from HDN and requiring double volume exchange transfusion for their illness, admitted in the Department of Pediatrics of G.R. Medical College and associated J.A. group of Hospitals at Gwalior. All the cases of HDN were diagnosed by considering cord blood/neonate blood for ABO grouping and Rh typing, Direct Coomb's test (DCT), Indirect Coomb's test (ICT), total and indirect serum bilirubin along with mother's sample for ABO grouping, RhD typing and ICT. Based on these above tests cases were divided into following three groups: 1) RhD HDN group—Where mother was RhD negative and neonate was RhD positive and ICT & DCT in neonates and ICT in mother were positive. 2) ABO HDN group—Where mother was of O group and neonate was A/B/AB group and IgG antibodies of ABO group were detected in neonates. 3) Other Blood group HDN (Non-ABO, Non-RhD)— where DCT and ICT in neonates and ICT in mother were positive by using polyspecific coomb's serum. Other laboratory investigations carried out are hemoglobin (Hb), hematocrit, peripheral smear, reticulocyte count in neonates and ABO and RhD status of father if not done during pregnancy. All blood products were obtained from relative/voluntary donors and then rendered for routine screening for infectious agents as per guidelines of Food & Drug Administration Government of India. The whole blood reconstituted was prepared in Blood Bank by standard method of preparation of component i.e. centrifugation and separation method by separating and mixing of Fresh Plasma (FP)/Fresh Frozen Plasma (FFP) and Packed RBC (PC)/ Leuco-reduced RBCs (LC)/Saline Wash RBC (SC)[5]. The volume required for exchange is given by formula

Estimated double volume to be exchanged (ml) = 85ml x {2 x weight (kg)} = 170 ml x weight (kg)

Double volume exchange removes approximately about 85% of the infant's red blood cells. After the exchange blood transfusion is over the bilirubin should be about 50% of pre exchange level. It is expected to rebound at about 4 hours to 2/3rds the pre-exchange level.

The hematocrit of the WBR is adjusted to the range of 45% to 60% depending on the desired end result. Higher range is being used for correction of severe anemia[6]. WBR was prepared by suspending O RhD positive/ negative cells (PC/LC/SC) in AB plasma (FP/FFP) and was supplied for ET. In RhD HDN; O RhD negative cells[7], in ABO HDN; O RhD positive/negative cells (depending on the RhD status of neonate), while in other group HDN (non-ABO and non-RhD); ICT cross matched O positive/negative cells (depending on the RhD status of neonates) were used. After keeping all the aseptic precautions in mind, exchange transfusions were performed under antibiotic coverage in the pediatric neonatal intensive care unit by CONTINUOUS

PUSH AND PULL technique using a TRI-WAY CANULA where access was via an umbilical venous catheter (blood in) and an umbilical arterial catheter (blood out). Blood withdrawal at each cycle is about 10ml/kg. Blood exchange at each cycle varied with the weight, maturity and general condition of the newborn. Post-exchange blood was collected for estimation of hemoglobin, hematocrit, indirect serum bilirubin and direct antiglobulin test. This was an observational study in which the fall of serum indirect bilirubin level and rise of hemoglobin level after using reconstituted blood for ET was observed in neonate suffering from HDN.

Inclusion criteria: Diagnosed cases of HDN where double volume exchange transfusion is required will be included in the study.

Exclusion criteria: Cases associated with other complications will be excluded.

RESULTS: Present study was conducted on 150 cases in which 167 exchange transfusions were performed. This study was mainly conducted to evaluate the usefulness of exchange transfusion in cases of HDN. In 132 cases(88%) exchange transfusion was done by reconstituted blood whereas 18 cases (12%) were given whole blood. in which 135 cases (90%) ET has been performed once while 13 cases (8.7%) required ET twice and only 2 cases (1.7%) were given ET three times. The average age of neonate was 5 days and average weight was 2340 gm. Average volume of blood transfused was 398ml. We found male child to be more commonly affected by HDN in 96 cases (64%) as compared to female child who were affected in 54 cases (36%). The average pre-ET bilirubin was 29.1mg/dl (range 18.1-43.6 mg/dl) and the average pre-ET hemoglobin was 12.13gm/dl (range 6-15 gm/dl). The average post-ET bilirubin was 14.9 mg/dl (range 9.1-23.8mg/dl) and the average post-ET hemoglobin was 16.2gm/dl (range 10-20 gm/dl). The overall post-ET average fall in indirect bilirubin was 52.1% whereas rise in hemoglobin was 4.12. In this study RhD HDN was found to be the most common cause seen in 92 cases (61.3%) followed by ABO HDN and other group HDN seen in 49 cases (32.7%) and 09 cases (6.0%) respectively.

Post-ET percentage fall of indirect serum bilirubin among different groups was 50.9% in RhD HDN, 56.2% in ABO HDN and 49.7% in other group HDN, while average increase in Hb was 3.87 gm/dl in RhD HDN, 4.57 gm/dl in ABO HDN and 4.67 gm/dl in other group HDN

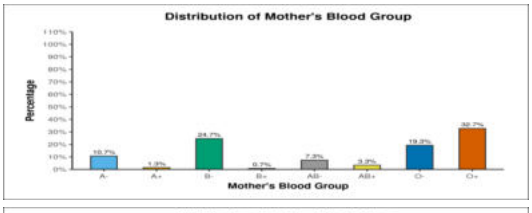
Three deaths were reported in which two deaths were of RhD HDN and one of ABO HDN leading to a case fatality rate of 2.1% and 2.0% respectively, with an overall death rate of 2% in the entire study. Deaths occurred due to inborn complications and severe neonatal sepsis. Septicemia following the course of exchange transfusion was not observed.

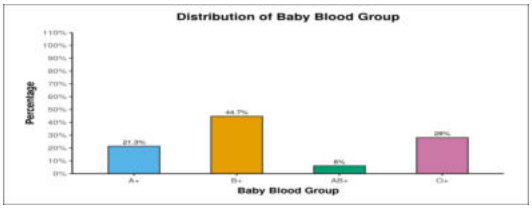
Table 1: Distribution of the Cases in Terms of Age (n = 150)

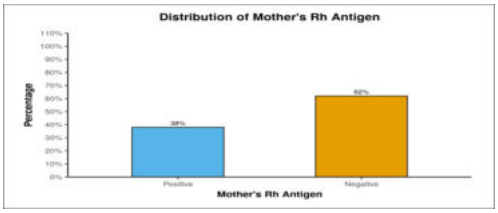
Age	Frequency	Percentage
0-10 Days	130	86.7%
11-20 Days	17	11.3%
21-30 Days	3	2.0%
Total	150	100.0%

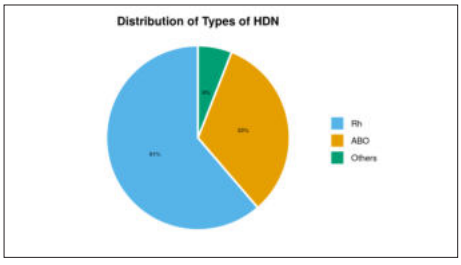
Table 2 : Distribution of the Cases in Terms of Gender (n = 150)

Gender	Frequency	Percentage
Male	96	64.0%
Female	54	36.0%
Total	150	100.0%









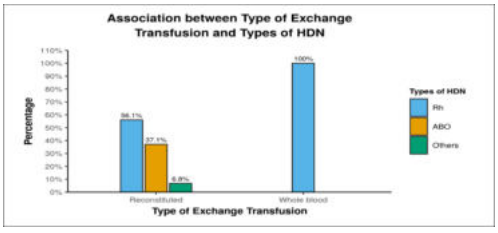
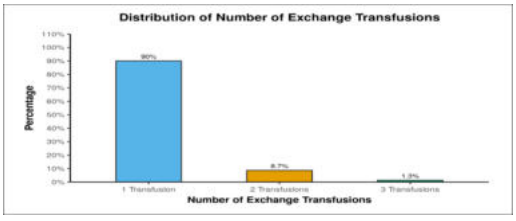
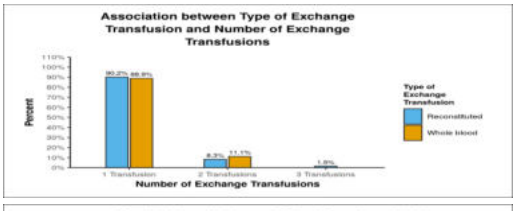
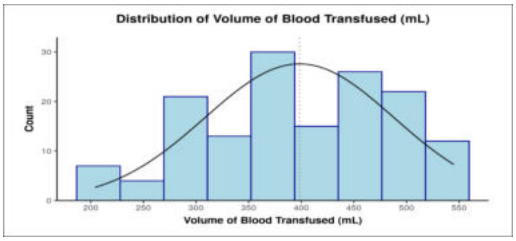


Table 3: Distribution of the Cases in Terms of Type of Exchange Transfusion (n = 150)

Type of Exchange Transfusion	Frequency	Percentage
Reconstituted	132	88.0%
Whole blood	18	12.0%
Total	150	100.0%

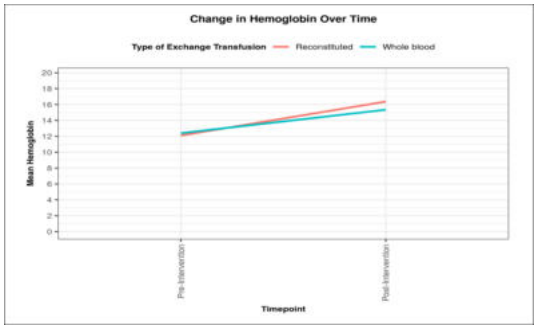






The following is a line diagram depicting the change in T. Bilirubin over time in both the groups.

The following is a line diagram depicting the change in Hemoglobin over time in both the groups.



DISCUSSION :

In this study, Rh HDN was the main cause of HDN with 92 cases (61.3%) while incidence of ABO- HDN and other group HDN (nonABO, non Rh) were 49 cases (32.7%) and 09 cases (6.0%) respectively. Similar findings were seen in other three comparative studies done by Sharma DC et al published in the year 2007 and 2013[8,9]. Study which constituted 110 cases, had 61 (55.5%) cases of RhD HDN whereas 30 (27.3%) and 19 (17.3%) cases of ABO HDN and other group HDN respectively. Study which was having 25 cases of HDN, 15 (60%) cases were of Rh HDN and 06 (24%) cases were of ABO HDN, whereas 04 (16%) cases were of other group HDN. Another similar study done by Kamal A. Patel and Kruti A. Raja et al which constituted of 31 cases, in which the most common cause of HDN was RhD HDN, which constituted 20 cases (64.51%) while ABO HDN and other group HDN (non-ABO, non-RhD) were 08 (25.80%) and 03 (9.6%) respectively[10]. But another study done by Badiee Z and Gharehbaghi M M et al had ABO incompatibility as most common cause of HDN[11,12].

In our study the average age of neonate was 5 days and average weight was 2340 gm. This was comparable by the study done by Sharma DC et al published in the year 2013 which constituted 110 cases, where the average age of neonate was 4 days and average weight was 2160 gm. In previous other studies average age and weight were not described. In our study for age we further divided the cases in groups where the maximum cases 129 (86%) were seen in the age group of 0-10 days, 18 (12%) cases in 11-20 days age group and only 3 (2%) cases were observed in newborn of 21-30 days old. We also found the male child to be more commonly affected by HDN 96 cases (64%) as compared to female child who were affected in 54 cases (36%).

In the present study, in all cases mean fall in post ET indirect serum bilirubin was 52.1% which was comparable with other studies who showed a fall of 53.47%, , 52.01%, and 52% respectively[9,13,14] while it was comparable to mean fall in post ET indirect serum bilirubin level (54.6%) of study done by Sharma DC et al. (2013)[8] In 92 cases of RhD HDN the average fall in the total serum bilirubin percentage was 50.9% which was comparable with 52.1% of the study done by Kamal A. Patel and Kruti A. Raja et al and a fall of 51.7%[10] and 55.66% in study done by Sharma DC et al (2007) and Sharma DC et al (2013) respectively[8,9].

In 49 cases of ABO-HDN where average post-ET indirect serum bilirubin fall was 56.2% which was totally comparable with the study done by Kamal A. Patel and Kruti A. Raja et al where the fall was of 56.59%[10]. This fall was much more than the previous studies done by Sharma DC et al (2007) in which it was 54.3% and Sharma DC et al (2013) where it was 54.12%[8,9].

Third group was of other blood group HDN categorized as non-RhD and non ABO HDN. Total numbers of such cases were nine in which pre-ET indirect bilirubin was 25.8 - 36 mg/dl. The post-ET average fall in indirect bilirubin was by 49.7%, which was comparable with the previous studies in which it was 50% and 51.83% respectively[8,9] a study done by Kamal A. Patel and Kruti A. Raja et al showed a much higher fall of 54.12%[10].In the present study the mean total serum bilirubin level before ET was 29.19 ± 5.86. mg/dl and immediately after ET was 14.94 ± 3.45mg/dl. P value was < 0.05, so the difference was statistically significant so the ET was effective treatment.

In the present study out of 150 cases,167 transfusions were given in which 135 cases (90%) ET has been performed once while 13 cases (8.7%) required ET twice and only 2 cases (1.7%) were given ET three times. The range of pre transfusion total serum bilirubin was 18.1-43.6 mg/dl. These results were found similar to a study done by Sharma DC et al (2013) in which out of 110 cases, in 101 cases (91.81%) ET has been performed once, while 09 (8.18%) cases required ET twice. The range of total serum bilirubin in all cases was 16.2 - 45 mg/dl. In other study of Sharma DC et al (2007), out of 25, in 20 cases (80%) ET has been performed once while 05 cases (20%) required ET twice. The range of total serum bilirubin in all the cases was 22.0 - 45.0 mg/dl. In study conducted by Kamal A. Patel and Kruti A. Raja et al, were only 31 cases were taken into account, out of which 19 cases (61%) ET has been performed once while 12 cases (39%) required ET twice and the range of total serum bilirubin was 18.0 - 44.2 mg/dl and 30 - 39 mg/dl respectively[10]. Badiee Z had found that out of 68 cases, in 60 cases (88%) ET has been performed once while 08 cases (12%) required ET twice[8,9,11].

In the present study, the mean total serum bilirubin levels in the patients who required more than one ET was higher than in patients with single ET and the difference was statistically significant (35.8±4.51 vs. 24.32±4.88, P = 0.0003). similar results were seen study of Kamal A. Patel and Kruti A. Raja et al where the mean total serum bilirubin levels in patients who required more than one ET was higher than in patients with single ET, which was also statistically significant (33.7±3.36 vs. 26.68±5.21, P = 0.0003) (140), whereas opposite results were seen in study done in northwest Iran by Hosseinpour SS et al (35.66±12.21 vs. 29.12±6.30, p=0.09) in which the difference was not statistically significant[15]. The study conducted in Iran concluded that ET with whole blood reconstituted (WBR) or fresh whole blood was safe and efficient method for reducing hyperbilirubinemia. The concept of WBR is immunologically valid and can be transfused irrespective of the ABO group of neonate and mother. This concept is easy to understand, simple to follow and desired results are better than whole blood. The immunological complications and risks are very low and hematocrit, volume and leuco-reduction of WBR can be adjusted as per the requirement of the new born[12].

In the present study, pre transfusion mean hemoglobin (Hb) is 12.13 gm/dl and post transfusion mean Hb is 16.25 gm/ dl, concluded an average increase of post transfusion Hb by 4.12 gm/dl, which was comparable with the study done by Sharma DC et al (2013) in which pre transfusion mean Hb was 12.46 gm/dl and post transfusion mean Hb was 16.17 gm/dl, so average increase in post transfusion Hb was 3.71 gm/dl[8]. Study done by Kamal A. Patel and Kruti A. Raja et al showed pre transfusion mean hemoglobin (Hb) is 12.00 gm/dl and post transfusion mean Hb is 15.06 gm/ dl, concluded an average increase of post transfusion Hb by 3.06 gm/dl[10].

In the present study an average increase of Hb in RhD HDN by 3.87 gm/dl, in ABO HDN by 4.57 gm/dl and in other group HDN by 4.67 gm/dl. In other comparable study by Sharma DC et al (2013), in RhD HDN it was 3.70 gm/dl, in ABO 3.83 gm/dl and in other blood group it was 3.60 gm/dl[8]. in study done by Kamal A. Patel and Kruti A. Raja et al an average increase of Hb in RhD HDN by 3.12 gm/dl, in ABO HDN by 2.4 gm/dl and in other group HDN by 4.67 gm/dl[10].

Third group was of other blood group HDN categorized as non-RhD and non ABO HDN, where other blood group irregular antibodies in the mother's serum were detected by indirect coomb's test (ICT) and corresponding antigen present on neonate RBCs were detected by DCT & ICT using polyspecific Coomb's serum. Other than RhD and ABO antibodies, the most common and significant antibodies causing HDN are RhC, Rhc, RhE, Kell I (K) Kell II (k), Jsb, Fya M, S & V[16,17]. Antibodies usually associated with mild to moderate HDN are Kpa, Kpb, LW, JKa, JKb, JK3, Jsa, Ula, (Kel 10) Fy3, etc.[16,17]. Vengelem Tyler V[18] studied and discussed 64 different RBC antibodies specificities reported to cause HDN. Antibodies, which are not associated with HDN, are N, s, PI, Leb, Lea, Lua and Lub. As our center has no facilities to specify these antibodies, these cases were grouped under non-ABO and non-RhD cases. On the contrary, a study done by Kamal A. Patel and Kruti A. Raja et al, the third group of HDN categorized as non-RhD and non-ABO HDN, in which irregular antibodies were anti C in two cases and in one case it was anti c[10].

ET was carried out on neonates, who were full term healthy/pre-term/ill neonates and few had associated complications. Three deaths were reported in which two deaths were of RhD HDN and one of ABO HDN leading to a case fatality rate of 2.1% and 2.0% respectively, with

an overall death rate of 2% in the entire study. These results were comparable to the study by Sharma DC et al (2013) in which two deaths of RhD HDN reported in the study comprised of 3.2% case fatality in this group and 1.8% death rate in the entire study, and an other study done by Jackson JC.[19] where they have two deaths out of 106 cases (1.88%). Author(s) used whole blood reconstituted (WBR) instead of whole blood and preferred O group cells to neonate's ABO group cells[20]. However, the study conducted in Iran[21] concluded that ET with whole blood reconstituted or fresh whole blood was safe and efficient method for reducing hyperbilirubinemia. The concept of WBR is immunologically valid and can be transfused irrespective of the ABO group of neonate and mother. This concept is easy to understand, simple to follow and desired results are better than whole blood. The immunological complications and risks are very low and HCT, volume and leuco-reduction of WBR can be adjusted as per the needs of neonate.

Prior to the introduction of ET, liveborn infants with severe rhesus hemolytic disease had a 35-40% mortality, with a 90% risk of severe neurological damage among survivors[22]. A reduction in mortality to 20% and reduction in adverse neurological outcome to 30% was observed following the introduction of double volume ET. The efficacy of ET with regards to long term neurological outcome is related to the etiology of hyperbilirubinemia and the population in which ET is undertaken. ABO incompatibility often results in a less severe hemolytic disease, needing fewer ET compared to rhesus incompatibility[23]. In developing countries, babies with severe jaundice may be referred late (with babies already showing signs of kernicterus), and therefore ET may not be useful in preventing neurological damage. Bilirubin encephalopathy occurs at lower serum bilirubin levels in preterm infants and the threshold for ET is usually lower.

CONCLUSION: In spite of wide use administration of anti-D in Rh negative mother, still in greater Gwalior region Rh HDN was found to be the most common cause seen in 61.3%. As Such cases in their subsequent pregnancies need a close monitoring and require expertise in various infrequently performed procedures, these should be coordinated with the Perinatal Centre.

In this study, reconstituted blood has shown superiority over the whole blood in exchange transfusion in HDN. The therapeutic indications fulfilled by reconstituted blood were far better than the whole blood in terms of: 1) Fall in bilirubin 2) Rise in hemoglobin 3) Removal of all the sensitized cells from neonate circulation 4) To ensure the better survival of transfused cells. Results of our study showed the average fall of bilirubin being 52.1%, average rise in hemoglobin was about 4.12, removal of sensitized cells from circulation was 90-95% (owing to double volume exchange transfusion), and a 100% survival of the transfused cells. As fresh matched whole blood is not always available for use reconstituted blood has now proved to be an good alternative as the components can be prepared easily. Hence, we conclude that exchange transfusion in cases of HDN should be taken over by reconstituted blood, provided all aseptic precautions are taken during component preparation.

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