Study of Exchange Transfusion by Reconstituted Blood in Hemolytic Disease of Fetus & New Born

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ABSTRACT

Introduction: In newborns with hemolytic disease of fetus and newborn, exchange transfusion is one of the treatments. The main objective of this study was to review and establish the practice of exchange transfusion with reconstituted blood in neonates and to observe fall of bilirubin and also rise in hemoglobin and its comparison with related studies.

Material and methods: Total 31 neonates with hemolytic disease of fetus and newborn were included in this study and exchange transfusion was carried out to treat hyperbilirubinemia. Exchange transfusion with O Rh negative cells suspended in AB plasma were done for neonates having Rh hemolytic disease of fetus and newborn and O Rh positive cells suspended in AB plasma were used for exchange transfusion to ABO hemolytic disease of fetus and newborn. The pre and post exchange transfusion blood samples were tested for serum bilirubin and hemoglobin.

Result: Out of the 31 cases, 20 were of Rhesus (Rh) hemolytic disease of fetus and newborn, while ABO and other blood groups constituted 08 and 03 hemolytic disease of fetus and newborn cases respectively. The average post-exchange fall in serum indirect bilirubin was (53.47%) and average rise in hemoglobin level was 3.06 gm/dl in all 31 cases.

Conclusion: The reconstituted blood is 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: Exchange Transfusion, Reconstituted Blood, Hemolytic Disease of Fetus ans New Born (HDFN).

INTRODUCTION

Hemolytic disease of the fetus and Newborn (HDFN) is characterized by 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.[1] Early detection and treatment of neonatal hyperbilirubinemia is important in prevention of bilirubin-induced encephalopathy.[2] It is classified as RhD HDFN, ABO HDFN and HDFN due to other blood group antibodies (non-ABO, non-RhD) according to the specificity of causative IgG antibodies. RhD incompatibility is still the most common cause of HDFN, although other RBC incompatibilities are increasing in incidence.[3]

Exchange transfusion (ET) with or without phototherapy is one of the choice for treating the newborn with ongoing hemolysis. ET removes indirect serum bilirubin, circulating mother's antibodies and antibody coated neonate's red blood cells (RBCs) from the circulation and provides RBCs compatible with neonate's serum and albumin with new bilirubin binding sites.[4] For ET in HDFN, whole blood either compatible with neonates' serum or mother's serum is commonly used.[5]

ET can be performed using many different combinations of blood components, including fresh whole blood and packed RBCs reconstituted with fresh frozen plasma (FFP). Fresh whole blood of the appropriate blood group is not always readily available, reconstituted blood is an alternative blood component for exchange transfusion. In the present study, reconstituted blood was used for ET. The objective of this study was to establish the role of reconstituted blood for ET in HDFN to decrease indirect bilirubin level and to increase hemoglobin level.

MATERIAL AND METHODS

This study was carried out in 31 cases suffering from HDFN in the Department of Immunohematology and blood transfusion of the medical college for the period of twenty months after getting approval from the ethical committee of the institute and consent of the patient's guardian for the same. The blood bank of the department was having all necessary equipment like refrigerated blood bag centrifuge, deep fridge of -40˚c and -70˚c, water bath, sterile tube connecting

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device, plasma extractor, dielectric tube sealer, laminar air-flow for preparing reconstituted blood. Reconstituted blood was supplied for ET to neonates with HDFN hospitalized in the new born care unit attached with the institute following AIIMS protocol for pre-term neonates and following Bhutani's protocol for term neonates for bilirubin exchange range. Neonate with high bilirubin level but not fit for exchange transfusion as per above mentioned protocol and neonate with physiological jaundice were excluded from the study. All the cases of HDFN were diagnosed by testing cord blood samples for ABO group, Rh type, Direct Antiglobulin Test (DAT), total and serum bilirubin. Mother's samples were tested for ABO group, Rh type and Antibody screening and identification whenever screening was positive with the help of commercial cell panels (Tulip Diagnostics Pvt. Ltd., India). Based on these tests cases were divided into the three groups:

1. ABO HDFN group - when mother is O and neonate is A or B group
2. RhD HDFN group - when mother is Rh negative and neonate is Rh positive
3. Other blood group HDFN (except ABO and RhD system) - when irregular antibodies are present in mother's serum and corresponding antigen present on neonate's RBCs.

Other laboratory investigations carried out were hemoglobin estimation, hematocrit and ABO and Rh status of father, if not done during pregnancy. All blood products are acquired from voluntary donors and underwent routine screening for infectious agent as per guidelines of food and drug administration government of India.

The reconstituted blood was prepared in the blood bank by standard method of preparation of component, i.e., centrifugation and separation method followed by mixing fresh frozen plasma (FFP) and Red cell concentrates / saline-washed RBCs as below mentioned:

1. Take fresh O negative red cells which are preferably less than 5 days old.
2. Add 0.9% normal saline equal to the amount of red cell with the help of sterile connective device and laminar air flow under aseptic condition.
3. Centrifuge at 3500 rpm for 10 minutes in refrigerated blood bag centrifuge at 4˚ c.
4. Once the spin is over, put the bag in plasma expresser. Break the seal and express out the entire plasma and saline under laminar air flow. Seal the segment with the tube sealer.
5. Repeat the whole procedure three times.
6. Thaw AB positive/negative FFP at 37^0 C in a plasma bath and add FFP in red blood cell bag with the help of sterile tube connecting device.
7. Transfer the volume of FFP which is equivalent to $1/3^{rd}$ volume of red cell. Mix well and issue as early as possible.
8. It has to be used within 24 hours.

Hematocrit was adjusted to 45% to 60% depending upon the desired end result. Higher range was used for correction of severe anemia.

In Rh HDN, O RhD negative cells were suspended in AB plasma. In ABO HDN; O RhD positive packed RBCs were suspended in AB plasma; while in other (non-ABO and non-RhD) group HDFN; Indirect Antiglobulin Test (IAT), cross-matched O cell compatible with neonates' serum suspended in AB plasma is given.

Volume of RBCs and FFP to be ordered

The volume required is dependent on the reason for exchange and is determined by the formula below.

(1) Single volume exchange (anaemia with normovolaemia)
Estimated blood volume depends on gestational age and timing of cord clamping ranging from 53 - 105 ml/kg/min. Mean blood volume was 70 ml/kg (early cord clamping) versus 90ml/kg (delayed cord clamping) for infants weighing 480-2060 gram.
Estimated single blood volume = 85ml x weight (kg)

(2) Double volume exchange (for established hyperbilirubinaemia or to prevent hyperbilirubinaemia)
Estimated double volume to be exchanged (ml) = 85ml x {2 x weight (kg)}
= 170 ml x weight (kg)

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

Under all aseptic precautions, exchange transfusions were performed under antibiotic coverage in the pediatric neonatal intensive care unit by CONTINUOUS technique **w**here 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. In the present study, the volume of blood used for exchange transfusion was calculated as 160 ml/kg in term neonates and 180 ml/kg in preterm neonates.

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 HDFN.

STATISTICAL METHODOLOGY

All values were expressed as percentage. *P* value less than 0.05 were used as cutoff to reject null hypothesis and all calculations were done using Microsoft Excel 2010 software.

RESULT

ET was done in total 31 cases by reconstituted blood. The total numbers of male cases were 21 (68%) whereas total numbers of female cases were 10 (32%) as shown in figure 1. In this study average age of newborn was 3 days (range 0 - 9 days), average weight of newborn was 2.40 kg (range 1.1 – 3.2 kg), and average volume of reconstituted blood used was 384 ml (range 176 - 512 ml). (Table 1)

	Age in days	Weight in kg	ET volume in ml
Range	0-9	1.1-3.2	176-512
Average	3	2.40	384

Table-1: Details of cases of HDFN (hemolytic disease of fetus and new born

Type of HDFN	Pre exchange bilirubin range (mean) (mg/dl)	Post exchange bilirubin range (mean) (mg/dl)	Pre exchange hemoglobin range (mean) (gm/dl)	Post exchange hemoglobin range (mean) (gm/dl)
ABO	22-39 (29.21)	10.3-15 (12.53)	6-15 (12.1)	10-20 (14.5)
RhD	18-44.2 (29.06)	9.2-24 (14.11)	7-16 (11.6)	11-18 (14.72)
Other	26-32 (29.24)	10.3-16 (14.06)	12-15 (13.33)	17-20 (18)

Table-2: Range of bilirubin and hemoglobin in various type of HDFN (hemolytic disease of fetus and new born)

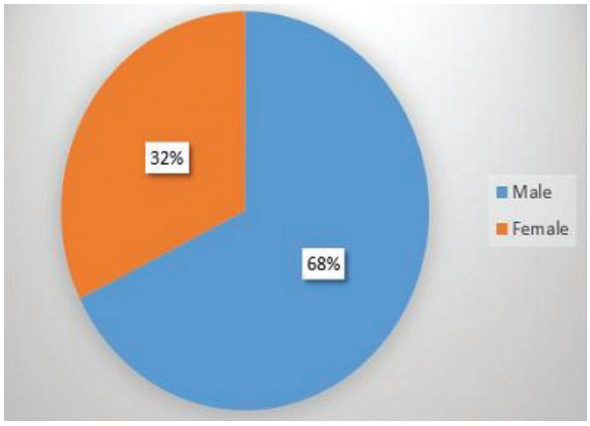

Figure-1: Gender distribution of newborns with HDFN (hemolytic disease of fetus and new born)

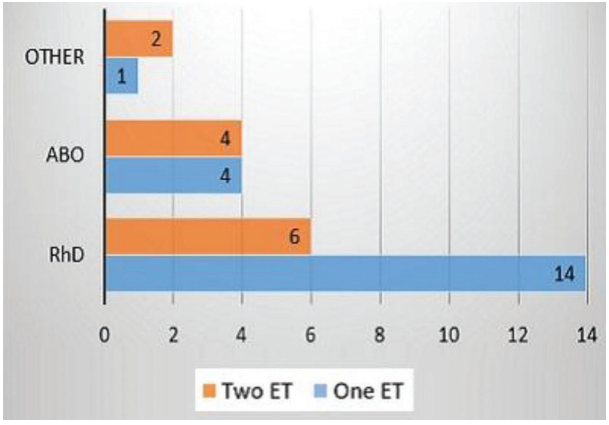

Figure-3: Numbers of ET (exchange transfusion) done

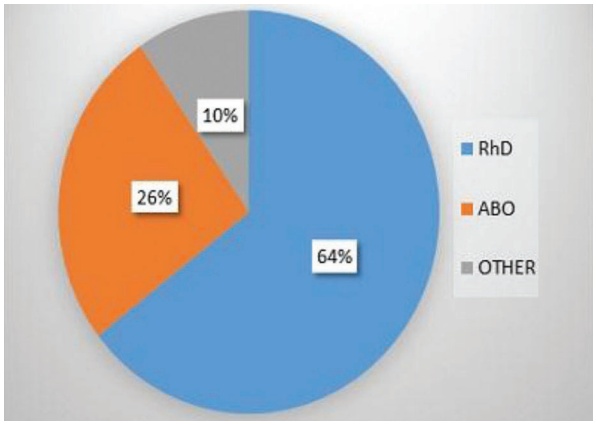

Figure-2: Types of HDFN (hemolytic disease of fetus and new born)

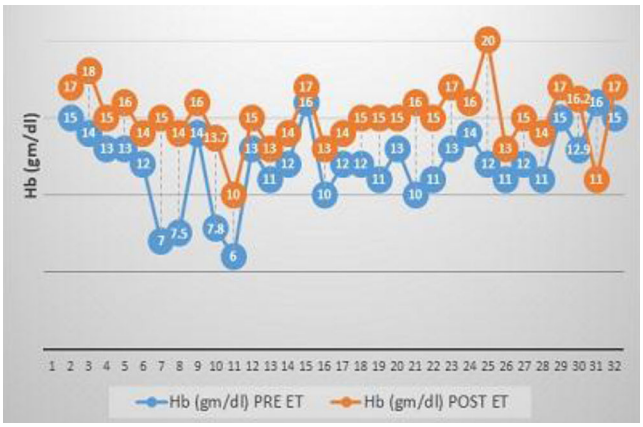

Figure-4: Fall in billirubin after ET (exchange transfusion)

Most common cause of HDFN in this study was RhD HDFN, which constituted 20 cases (64.51%) while ABO HDFN and other group HDFN (non-ABO, non-RhD) were 08 (25.80%) and 03 (9.6%) respectively. (Figure 2)

The range of pretransfusion indirect bilirubin in all the cases was 18.0 - 44.2.0 mg/dl. Out of 31 cases, in 19 cases (61%) ET has been performed once while 12 cases (39%) required ET twice. Among 20 (64.51%) cases of Rh HDN, 12 cases (60.00%) in which indirect bilirubin was < 30 mg%, required exchange transfusion only once; and for 08 (40.00%) cases, in which pretransfusion indirect bilirubin was ≥30 mg%, required two ET. (Figure 3)

Pre and Post Exchange transfusion; indirect bilirubin and hemoglobin values are shown in table 2. Post-ET mean of fall of indirect serum bilirubin among different groups was 52.12% in RhD HDFN, 56.59% in ABO HDFN and 54.12% in other group HDFN while average increase in Hemoglobin was 3.06 gm/dl in RhD HDFN, 2.4 gm/dl in ABO HDFN and 4.67 gm/dl in other group HDFN. (Figure 4 and 5)

Among three cases of other-group HDFN; in two cases, antibody identified was anti C and in one case antibody was

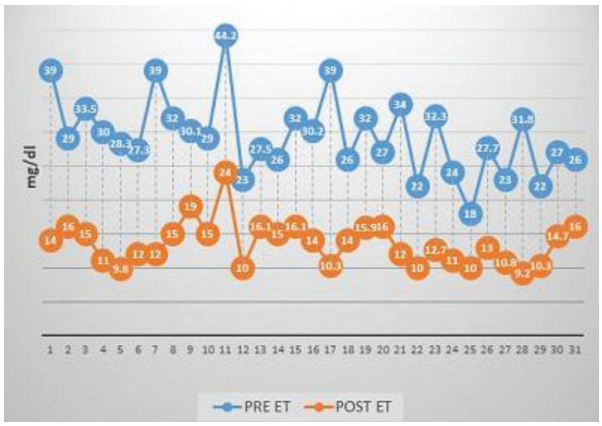

Figure-5: Rise in Hb (hemoglobin) after ET (exchange transfusion)

anti c.

DISCUSSION

Present study was conducted to evaluate the usefulness of reconstituted blood in ET in HDFN. By using reconstituted blood in the study, the author further strengthened the concept and practice of using reconstituted blood in neonates for ET. Other authors also reported usefulness of reconstituted blood over whole blood and component therapy for pump priming in heart surgery in infants.[6,7]

In ABO-HDFN, red cells for ET must be of O group and re-suspended in AB plasma or O group whole blood with low Anti A, Anti B titer.[8,9] Since these studies showed several severe to fatal hemolytic reactions in ABO HDFN cases during ET by O group whole blood, reconstituted blood is a better and safe option.

In RhD HDFN and other group HDFN; O cells are preferred over neonates ABO group cells because of the fact that ABO blood group antigens are not fully developed in newborns and sub-groups of A and B antigens are frequently encountered. In the present study, AB plasma was used for reconstituted whole blood to prevent the transfusion of ABO antibodies, which was not yet developed in neonates.[10]

In this study, Rh HDFN was the main cause of HDFN with 20 cases (64.51%) while incidence of ABO- HDFN and other group HDFN (non ABO non Rh) were 08 cases (25.80%) and 03 cases (9.6%) respectively. Similar findings are seen in other two comparative studies by Sharma DC et al published in the year 2007 and 2013.[11,12] Study which constituted 110 cases, had 61 (55.5%) cases of RhD HDFN whereas 30 (27.3%) and 19 (17.3%) cases of ABO HDFN and other group HDFN respectively. Study which was having 25 cases of HDFN, 15 (60%) cases were of Rh HDFN and 06 (24%) cases were of ABO HDFN, whereas 04 (16%) cases were of other group HDFN. But another studies done by Badiee Z and Gharehbaghi M M et al had ABO incompatibility as most common cause of HDFN.[13,14]

In all cases mean fall in post ET indirect serum bilirubin was 53.47%, which was better than the other studies in which fall in post ET indirect serum bilirubin was, 52.01%, 51.9% and 52% respectively[12,15,16] 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)[11] In 20 cases of RhD HDFN where average post-ET indirect serum bilirubin fall was 52.12%, which was comparable with 51.7% and 55.66% in study done by Sharma DC et al (2007) and Sharma DC et al (2013) respectively.[11,12] In 08 cases of ABO-HDFN where average post-ET indirect serum bilirubin fall was 56.59%, which is more than the previous studies done by Sharma DC et al (2007) in which it was 54.3% and Sharma DC et al (2013) 54.12%.[11,12] Third group was of other blood group HDFN categorized as non-RhD and non ABO HDFN. Total numbers of such cases were three in which pre-ET indirect bilirubin was 26 - 39 mg/dl. The post-ET average fall in indirect bilirubin was by 54.12%, which was better than previous studies in which it was 50% and 51.83% respectively.[11,12]

In the present study the mean total serum bilirubin level before ET was 29.59±6.88 mg/dl and immediately after ET was 11.51±3.72 mg/dl. *P* value was < 0.05, so the difference was statistically significant so the ET was effective treatment. In present study, out of these 31 cases, in 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. In 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. Badiee Z h ad found that out of 68 cases, in 60 cases (88%) ET has been performed once while 08 cases (12%) required ET twice.[11,12,13]

In the present study, the mean total serum bilirubin levels in patients who required more than one ET was higher than in patients with single ET and the difference was statistically significant (33.7±3.36 vs. 26.68±5.21, *P* = 0.0003), 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.[17]

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 Hct, volume and leuco-reduction of WBR can be adjusted as per the requirement of the newborn.[14]

In the present study, 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, 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.[11] Study done by Sharma DC et al (2007) showed

average 20% fall in post ET Hb level in comparison to pre ET Hb level in some cases.[12] In the present study an average increase of Hb in RhD HDFN by 3.12 gm/dl, in ABO HDFN by 2.4 gm/dl and in other group HDFN by 4.67 gm/dl. In other comparable study by Sharma DC et al (2013), in RhD HDFN it was 3.70 gm/dl, in ABO 3.83 gm/dl and in other blood group it was 3.60 gm/dl.[11]

In the present study, the third group was of other blood group HDFN categorized as non-RhD and non ABO HDFN, in which irregular antibodies were anti C in two cases and in one case it was anti c. Irregular antibodies in the mother's serum were detected by Indirect Antihumanglobulin Test (IAT) and corresponding antigen present on neonate RBCs were detected by DAT using poly and mono specific Coomb's sera.

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[18], 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.[19] 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 the present study, an average fall in indirect serum bilirubin was 53.47%, and an average increase of Hb was 3.06 gm/dl which was resulted from removal of sensitized RBCs and circulating mother's antibodies. Fresh whole blood of the appropriate blood group is not always readily available, reconstituted blood is an alternative blood component for exchange transfusion. Hence it can be concluded that exchange transfusion in HDFN should be carried out by reconstituted blood, provided that reconstitution of the blood components done as per standard guidelines.

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