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ORIGINAL ARTICLE

Whole blood versus red cells and plasma for exchange transfusion in ABO haemolytic disease

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SUMMARY. Records of 381 neonates who underwent exchange transfusion (ET) due to ABO haemolytic disease at the Division of Neonatology of Hacettepe University, Ankara, Turkey, between January 1977 and December 2003 were reviewed. Records were kept for the type of blood used in ET, the number of ETs for each infant, adverse event attributable to ET and bilirubin levels before, and 4 and 8 h after each ET. Of 381 infants, 300 were transfused with whole blood, whereas 81 infants were transfused with O red cells suspended in A or B plasma. The re-exchange rate was higher in the whole blood group, compared with the erythrocyte and plasma group. Use of erythrocyte and plasma provided 30% reduction in the number of ETs per patient. Eight hours after the first ET, mean bilirubin levels were 84% of the pre-exchange values in the whole blood group and 73% of the pre-exchange values in the erythrocyte and plasma group ($P = 0{\cdot}001$). As the use of O group red cells re-suspended in AB plasma decreased the re-exchange risk compared with O group whole blood, we suggest the use of O red cells re-suspended in AB plasma for the ET in cases of ABO haemolytic disease.

Key words: ABO incompatibility, erythrocyte and plasma, exchange transfusion, haemolytic disease of the newborn, newborn, whole blood.

ABO incompatibility and alloimmunization can occur when the mother has O blood type and the foetal red cells contain the A or B antigen. ABO incompatibility is the most common maternofoetal blood group incompatibility, which is usually a problem of the neonate rather than the foetus. Severe erythroblastosis fetalis or hydrops fetalis is extremely rare with isolated ABO alloimmunization, presumably because of the paucity or weaker expression of A or B antigen sites on foetal red blood cells (RBCs) at term and early in gestation, leading to a later activation of the maternal IgG immune system and later subsequent haemolysis of foetal RBCs (Miller & Petrie, 1963; Romano *et al*., 1978; Romano *et al*., 1983; McDonnell *et al*., 1998). ABO incompatibility occurs in 15–20% of all pregnancies and produces a spectrum of haemolytic disease, the extreme end of which is recognized as ABO haemolytic disease (Zipursky & Bowman, 1993; Herschel *et al*., 2002). Severe anaemia is uncommon; therefore, the therapy of severe haemolytic disease is directed towards the control of hyperbilirubinaemia. Phototherapy is widely used for this purpose (Peevy & Wiseman, 1978). However, there are some studies, indicating that phototherapy is not effective in reducing the number of exchange transfusions (ETs) in ABO alloimmune haemolytic disease (Keenan *et al*., 1985; Maurer *et al*., 1985). ET is a mode of therapy for the cases who do not respond to intensive phototherapy for the treatment of hyperbilirubinaemia. An increase in serum bilirubin levels despite the use of intensive phototherapy or bilirubin levels higher than 20 mg dL^{-1} are common indications for ET in ABO haemolytic disease. The classical approach regarding ET in ABO haemolytic disease was to administer group O whole blood of the infant's Rh type with low titre plasma anti-A and anti-B (Foerster, 1993). As whole blood contains natural anti-A or anti-B antibodies, the use of type O Rh-specific blood cells, which are reconstituted with type AB plasma, is recommended in cases of ABO

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incompatibility (Alverson & Izquierdo, 1996; British Committee for Standards in Haematology, 2004). However, to our knowledge, no study has evaluated the difference between the administration of whole blood and red cells + plasma during ET in ABO haemolytic disease. In this retrospective study, we investigated the effect of two different modes of ET on subsequent serum bilirubin levels and re-exchange rate in infants with ABO haemolytic disease.

MATERIALS AND METHODS

This study was performed at the Division of Neonatology of Hacettepe University Faculty of Medicine, Ankara, Turkey. All records of the patients who underwent ET between January 1977 and December 2003 were reviewed. After eliminating the records of patients who underwent ET for reasons other than ABO haemolytic disease or who has glucose-6-phosphate dehydrogenase deficiency (G6PD), the medical records of the 383 remaining patients were reviewed in detail. Haematocrit and reticulocyte counts, blood group tests – including Rhesus, a direct antiglobulin test (Coombs' test) – and serum total and direct bilirubin levels were performed routinely in all cases. Double-volume ET was performed in cases where bilirubin levels exceed 20 mg dL^{-1}. ABO haemolytic disease was diagnosed in type A or B infants born to type O mothers, in the presence of clinical jaundice within the first 12–24 h, reticulocytosis and microspherocytosis on the peripheral blood smear. In our hospital from 1977 to 1992, all ETs were performed by administration of Rh (cCDeE) compatible O group whole blood, which underwent no testing for anti-A or -B titre. After a pilot study (Aydin *et al.*, 1992), since 1992, we have used Rh compatible O group red cells suspended in A or B plasma.

All blood used for ET was obtained from the Hacettepe Blood Center. The whole blood or red cells were anti-coagulated with citrate phosphate dextrose adenosine-1 and were <5 days old. Whole blood is obtained from donors whose haematocrit levels are over 40% due to the policy of the Blood Center for blood donation. The red cells with haematocrit levels of 70–75% are diluted with plasma until the haematocrit level of the mixture is reduced to 50–60%. ETs were performed by the paediatric residents under the supervision of the fellow or attending neonatologist. The double-volume ETs were generally completed within 1–1·5 h. All infants received phototherapy on admission and phototherapy treatment was continued, except ET periods, until bilirubin was reduced to the desired level. Records were kept for the number of ETs for each infant, adverse event attributable to ET and bilirubin levels before and, 4 and 8 h after each ET.

Data were expressed as the mean ± SD and evaluated for significance with Mann–Whitney *U*-test, χ^2-test and regression analysis.

RESULTS

A total of 383 newborns who underwent ET due to ABO haemolytic disease were enrolled in this study. Two infants who were transfused with whole blood died during ET and they were excluded from the study. They were both term infants 1 and 4 days old, respectively. They developed cardiac arrest in the course of the procedure and did not respond to resuscitation. Of 381 infants, 300 were transfused with whole blood, whereas 81 infants were transfused with O red cells re-suspended in A or B plasma. Table 1 shows the demographic characteristics and bilirubin levels of the two groups. The mean gestational age and birth weight were higher in the whole blood group. Higher bilirubin levels were found before the first and second ET in the whole blood group. Vaginal delivery was more common in the whole blood group. There was no difference between the groups regarding the presence of A antigen, a sibling who underwent ET and postnatal age at the time of the first ET.

Complications probably due to ET have been showed in Table 2. Fourteen infants (3·6%) had only asymptomatic complications, such as hypoglycaemia, hypocalcaemia and anaemia, whereas 15 infants experienced serious transient complications probably attributable to ET. There was no statistical difference between groups regarding the complications of ET.

The re-exchange rate was higher in the whole blood group, compared with the erythrocyte + plasma group. The mean ET number per patient was found as 1·8 in the whole blood group, whereas the mean ET number was 1·2 per patient in the red cells + plasma group. Use of erythrocyte + plasma provided 30% reduction in the number of ETs per patient. The number of ETs per patient has been showed in Table 3.

In order to identify the risk factors affecting re-exchange risk, a multiple logistic regression analysis was performed. Various risk factors, such as weight, sex and gestational age of the baby, mode of delivery (caesarean/vaginal), presence of a sibling who had undergone ET and a direct antiglobulin test positivity, did not significantly affect the re-exchange risk, whereas bilirubin level before the first exchange, the age of the baby, presence of the A blood group antigen and the blood type used in ET affected the risk of

Table 1. Characteristics and bilirubin levels of infants who were administered whole blood or erythrocyte + plasma

	Whole blood ($n = 300$)	Erythrocyte + plasma ($n = 81$)	*P*-value
Gestational age (weeks)	39·3 ± 1·9 (30–42)	38·1 ± 2·8 (30–41)	<0·001
Weight (grams)	3015 ± 552 (1300–4700)	2802 ± 596 (1400–3800)	0·01
Sex (male/female)	182/118	37/44	0·01
Coombs positive cases (%)	19 (6·3)	8 (9·8)	0·27
Re-exchange rate (%)	172 (57·3)	14 (17·3)	<0·001
Bilirubin before first ET (mg dL^{-1})	23·3 ± 4·3 (13·4–44·7)	21·5 ± 4·5 (10·6–37·8)	<0·001
Bilirubin before second ET (mg dL^{-1})	22·4 ± 2·8 (16·1–38·8) *n*: 172	21·3 ± 4·9 (14·9–33·7) *n*: 14	0·09
Delivery mode (vaginal/caesarean)	271/29	61/20	0·001
Blood group of baby (A/B)	235/65	58/23	0·2
Mean age for first ET (day)	4·1 ± 1·8 (1–10)	3·8 ± 1·9 (1–9)	0·1
Any sibling who underwent ET present/absent)	64/236	14/67	0·5

re-exchange. A 1-mg increase in bilirubin levels on admission, the presence of the A antigen and administration of whole blood increased the re-exchange risk 1·2, 1·8 and 6·7 times, respectively. On the other hand, a 1-day increase in the age of the baby decreased the re-exchange risk 0·7 times (Table 4).

The reduction rate in bilirubin levels after the first ET was similar in both the whole blood and erythrocyte + plasma groups (54 versus 55%). However, the increase rate in bilirubin levels 4 and 8 h after the first ET was significantly different between the groups. Four hours after the first ET, mean bilirubin levels reached 82% of the pre-exchange value in the whole blood group, and 72% of the pre-exchange value in the erythrocyte + plasma group ($P = 0·001$). Eight hours after the first ET, mean bilirubin levels were 84% of the pre-exchange values in the whole blood group and 73% of the pre-exchange values in the erythrocyte + plasma group ($P = 0·001$, Fig. 1). Similarly, the bilirubin levels after 4 and 8 h following the second ET were 79 and 80% of the pre-exchange values, respectively, in the whole blood group and 69 and 69% of the pre-exchange values, respectively, in the erythrocyte + plasma groups ($P = 0·001$, $P = 0·006$, Fig. 2).

DISCUSSION

Hyperbilirubinaemia caused by haemolytic disease of the newborn is the most common indication of ET in newborn patients. It has been reported that although the incidence of blood group O mothers delivering babies of blood group A or B is approximately 15%, ABO haemolytic disease is estimated to occur in only 3% of pregnancies and requires treatment with ET in <0·1% of pregnancies (Zipursky & Bowman, 1993). However, the incidence of ABO incompatibility, significant hyperbilirubinaemia in ABO incompatibility and severe haemolytic disease of the newborn as a result of ABO incompatibility were found to be 14·8, 21·3 and 4·4%, respectively, in a previous study performed in Hacettepe Hospital (Sarici *et al.*, 2002). The incidence of a significant hyperbilirubinaemia in this study (21·3%) is much higher than that reported in the general population (Bhutani *et al.*, 1999; Seidman *et al.*, 1999), probably due to the ethnic and geographical characteristics of the population in Turkey. Because some of the infants were born elsewhere and admitted to the hospital with a significant hyperbilirubinaemia due to ABO incompatibility, we could not give data concerning the incidence of a significant hyperbilirubinaemia in the present study.

The diagnosis of ABO haemolytic disease of newborn is complicated. Mothers with a high titre of IgG anti-A or anti-B are more likely to have affected babies, but there is no direct relationship with the antibody titre of the mother and the incidence of having affected babies. A high titre IgG anti-A or anti-B in the mother is supportive evidence, but a low

Table 2. Complications probably due to exchange transfusion (ET)

Exchange complications	Whole blood ($n = 300$)	Erythrocyte + plasma ($n = 81$)
Asymptomatic complications treated (e.g. anaemia)	12 (4·0%)	2 (2·4%)
Treated serious complications (e.g. sepsis, bradycardia, apnea)	13 (3·3%)	2 (2·4%)

Table 3. The number of exchange transfusions (ETs)

	Whole blood ($n = 300$)		Erythrocyte + plasma ($n = 81$)	
Number of ETs per patient	Patients (%)	Transfusions	Patients (%)	Transfusions
1	128 (42·6)	128	67 (82·7)	67
2	111 (37)	222	12 (14·8)	24
3	54 (18)	162	2 (2·5)	6
4	6 (2)	24	–	–
5	1 (0·4)	5	–	–
Total	300 (100)	541	81 (100)	97

titre does not exclude diagnosis. The direct antiglobulin test should be at least weakly positive for anti-A or anti-B; however, because of the sparse distribution of antigenic sites on a newborn's RBCs, ABO haemolytic disease may be present even without a positive result on the direct antiglobulin test (Luchman-Jones *et al*., 2002).

Group A or B babies who have maternal anti-A or anti-B in their plasma may convert to DAT positivity and develop haemolysis when they are transfused with blood of their own group. This is due to the increased expression of A and B antigens on adult cells of those groups. Group O blood, compatible with the maternal plasma, should be used for transfusion in both groups (British Committee for Standards in Haematology, 2004). The selection of blood type is important in the case where ET is required in ABO haemolytic disease. The use of group O red cells with low titre plasma anti-A and anti-B or group O red cells suspended in AB plasma is recommended (Alverson & Izquierdo, 1996; British Committee for Standards in Haematology, 2004). However, the tests used for the determination of low titre plasma anti-A and anti-B in O type whole blood are expensive and time consuming. In addition, the definition of low titre is not well defined internationally and the tests used in order to measure it are not easily reproducible. For this reason O type whole blood without testing for anti-A and anti-B is used for ET in ABO haemolytic disease in some centres. Although the use of type O Rh-specific red cells suspended in AB plasma is recommended, a more rational approach is the use of type O Rh-specific red cells suspended in the selected plasma according to the babies' ABO blood group types.

This study shows the results of ET when these two types of blood (O type whole blood versus type O Rh-specific red cells suspended in the selected plasma according to babies' ABO blood group types) were used for ET in ABO haemolytic disease. The reduction rate of bilirubin was similar at the completion of the ET. However, the increase in bilirubin levels 4 and 8 h after ET was significantly higher in the whole blood group. This result can be explained with the presence of anti-A and anti-B in O type whole blood. Probably, these antibodies in addition to the maternal antibodies, which cannot be totally cleared with ET, increased ongoing haemolysis. As a result of this increase, the recurrent ET rate was high in the whole blood group. When the selected fresh plasma according to the babies' ABO blood group types was used, additional anti-A or anti-B antibodies, targeting babies' red cells, have not been introduced. Although colloid or crystalloid replacement rather than plasma may seem to be advantageous in terms of infectious agent transmission and immunological reactions that may be caused by the infusion of donor-derived antibodies, A or B blood group

Table 4. Factors affecting re-exchange risk

	Univariate			Multivariate		
	Risk ratio	CI (95%)	*P*-value	Risk ratio	CI (95%)	*P*-value
Age of baby (day)	0·912	0·8–1·0	0·09	0·698	0·6–0·8	<0·001
Weight of baby (kg)	1·11	0·8–1·6	0·5	0·726	0·5–1·1	0·14
Bilirubin level before first ET (mg dL^{-1})	1·154	1·1–1·2	<0·001	1·245	1·2–1·3	<0·001
Blood group of baby (group A)	1·712	1·1–2·8	0·03	1·814	1·0–3·2	0·04
Blood type used in ET (whole blood)	6·431	3·5–12	<0·001	6·72	3·4–13·2	<0·001

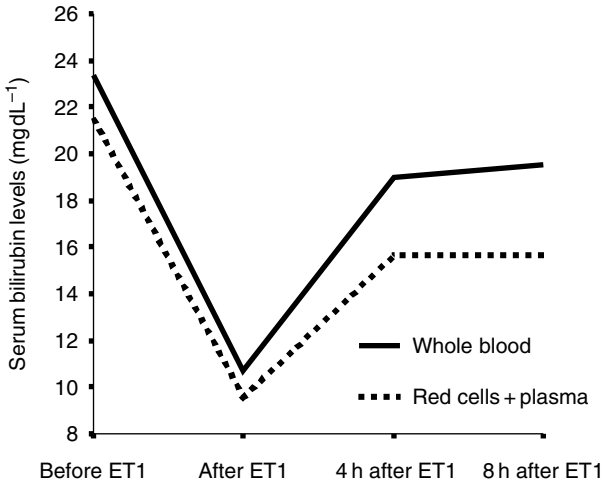

Fig. 1. Serum bilirubin levels before and after the first exchange transfusion (ET1).

substances, existing in the selected plasma, may play an additional neutralization role on the remaining maternal antibodies in the babies' circulation (Hostrup, 1963; Denborough *et al*., 1969). When we had investigated the factors affecting re-exchange risk, the most important factor was found to be the blood type. The administration of O type whole blood increased re-exchange risk 6·7 times, compared with O red cells + A or B plasma.

Reduction in the number of ETs per patient by 30% (the mean ET number per patient was found as 1·8 in the whole blood group, whereas it was 1·2 per patient in the red cells + plasma group) means a reduction in the rate of complications related to ET. On the other hand, the use of component therapy provides some advantages, compared to whole blood. Red cells, plasma and platelets can be administered to three different patients and, components' shelf life is longer. The main disadvantage of red cells + plasma, compared to whole blood, is two-donor exposure of the patient. However, if this means that the risk of requiring re-exchange is decreased, this may be offset.

The other factors affecting re-exchange risk were the age of the baby, the presence of A antigen and bilirubin levels before ET. There was no difference between the groups regarding the presence of A antigen and the age of the baby at the time of ET. The only difference between the groups was found in the bilirubin levels before the first ET. Bilirubin levels were higher in the whole blood group, and this may be explained by the fact that an increased family awareness of the risks of hyperbilirubinaemia resulted in an early admission of patients in recent years. In addition, there was no statistical difference in bilirubin levels before the second ET between the two groups.

ET is a high-risk procedure. Jackson (1997) reported 1·2% serious complication rate attributable to ET in healthy infants. The 4·2% serious clinical problems associated with ET reported by Keenan *et al*. (1985) are similar to the serious complication rate (3·9%) found in the present study. Two infants died during the ET procedure and were excluded from our study. Jackson (1997) reported 2% mortality rate (two in 106). The mortality rate in the present study, 2 in 383 patients (0·52%) or two in 638 procedures (0·31%), is similar to that (0·53%) reported by Keenan *et al*. (1985).

In conclusion, this study shows the importance of blood group selection for ET in ABO haemolytic disease. As the use of O group red cells suspended in A or B plasma decreased the re-exchange risk significantly, compared with O group whole blood, we suggest the use of O red cells suspended in A or B plasma for the ET in cases of ABO haemolytic disease.

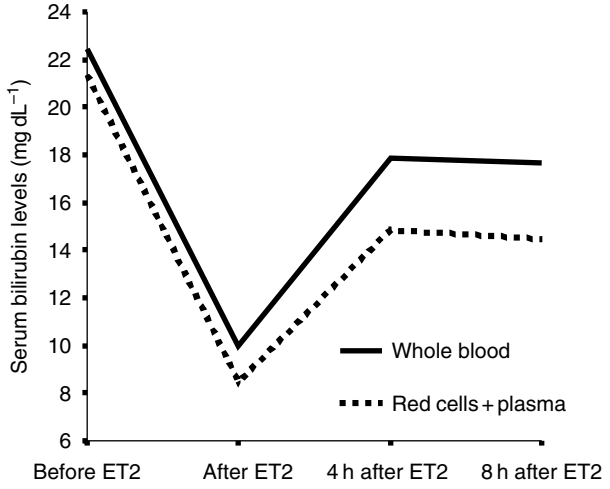

Fig. 2. Serum bilirubin levels before and after the second exchange transfusion (ET2).

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