The Journal of Obstetrics and Gynecology of India (September–October 2023) 73(5):381–390
https://doi.org/10.1007/s13224-023-01746-y

ORIGINAL ARTICLE

Double/Triple Intrauterine Blood Transfusion in Rh-isoimmunized Anemic Fetuses in Multiple Pregnancies with Favorable Outcome

Vandana Bansal[1] · **Meera Jayaprakash**[1] · **Akshay Gangurde**[1]

Received: 7 August 2022 / Accepted: 26 February 2023 / Published online: 25 March 2023

Abstract

Background Multiple pregnancies have increased with the use of assisted reproduction, and we expect more women reporting with Rh isoimmunization among multiple gestation in near future. Intrauterine transfusion in singleton itself is technically difficult and requires a lot of skill and precision. Performing double/triple transfusion in twins/triplets is expected to be more demanding.

Aim To create awareness on the technical difficulties encountered in intrauterine transfusion in twins and triplets.

Methodology We report a case series of four Rh-isoimmunized twins/triplets in 5 years who presented with severe anemia requiring intrauterine transfusion.

Results Each of the four sets of cases had their own intricacies that needed to be pondered before tackling them as not much was available in the literature. In Case 1, the first twin intrauterine transfusion in our 20-year-long experience, the difficulty in the approach to the first twin due to a posteriorly placed placenta has been highlighted. Case 2 was rare due to the concomitant presence of atypical antibodies in the mother in addition to Rh-D isoimmunization that made it difficult to cross match any donor blood for intrauterine transfusion. The third case was exclusive due to its monochorionic–diamniotic nature of the twins where the impact of inter-twin anastomosis on the transfusion was to be taken into consideration. Fourth case was a triplet gestation where the difficulty of which cord to be assigned to which fetus, the crowded space for intervention, as well as the risk of prolonged operative time and associated risk of preterm/premature rupture of membranes were our concern.

Conclusion Intrauterine transfusion (IUT) in twins/triplets is challenging. Difficulties encountered during IUT in multifetal gestation are due to different or uncertain chorionicity, intraplacental anastomosis between vessels, different degree of anemia in twins, difficult to ascertain cord–fetus relationship and difficulty to reach placental insertion site due to crowding by multiple fetal parts.

Keywords Fetal anemia · Intrauterine transfusion · Rh isoimmunization · Twin transfusion · Twin intrauterine transfusion

Introduction

Rhesus isoimmunization causes significant perinatal morbidity and mortality in the form of in utero anemia, hydrops, fetal demise and postnatal hyperbilirubinemia and cardiac failure. Intrauterine blood transfusion is the only proven lifesaving therapy for Rh-isoimmunized pregnancy with fetal anemia. Isoimmunization of sufficient severity requiring intrauterine transfusions in multifetal gestation is unusual and is expected to be a challenging task. There are very few cases in the literature describing intrauterine fetal blood transfusion in twins and triplets.

Dr Vandana Bansal (MD, DGO, DNB, MNAMS, FRCOG (UK), FICOG, FNB (High Risk Pregnancy and Perinatology)) Addl. Professor, Department of Obstetrics and Gynaecology and Fetal Medicine; Dr Meera Jayaprakash (MS (Obstetrics and Gynecology), FCPS) Fellow Fetal Medicine; Dr Akshay Gangurde, (MS (Obstetrics and Gynecology)), Senior Resident, Nowrosjee Wadia Maternity Hospital, Seth G. S. Medical College, Parel, Mumbai, Maharashtra, India.

✉ Vandana Bansal
drvandana_bansal@yahoo.co.in

Meera Jayaprakash
meera.jps@gmail.com

Akshay Gangurde
akshaygangurde42@gmail.com

[1] Department of Obstetrics and Gynecology and Fetal Medicine, Nowrosjee Wadia Maternity Hospital and Seth G. S. Medical College, Parel, Mumbai, Maharashtra, India

Methods

It is a retrospective analysis of four cases, in past 5 years of Rh-isoimmunized multifetal pregnancy (3 twin pair and one set of triplets) with fetal anemia resulting in successful outcomes after intrauterine blood transfusion. All these women were referred to our institute, following the identification of maternal anti-erythrocytic alloantibodies between 2016 and 2021. Each case was unique and had its own complexities to manage.

Results

Case 1

A 27-year-old patient (G3P1LIA1) was referred to our tertiary care hospital at 31 + 6 weeks of gestation, with an Rh-isoimmunized dichorionic–diamniotic (DCDA) twin pregnancy along with indirect Coombs test positive (ICT) and an Rh titer of 1:128. Her first pregnancy was an emergency cesarean section at term for cord around neck. Anti-D was given both at 28 weeks and postpartum. Her second pregnancy was a spontaneous abortion at 3 months for which a check curettage was done and anti-D was administered.

The present pregnancy was spontaneously conceived following ovulation induction. Her blood group was O negative, and her husband's blood group was A positive. Repeat Rh titer within a week of previous report had increased to 1:1024. Ultrasound revealed a DCDA twin pregnancy with normal growth, liquor and no evidence of hydrops in both twins. Middle cerebral artery peak systolic velocity was 70 cm/s (> 1.5MOM) in twin 1 and 55 cm/s (> 1.29MOM) in twin 2. At 32 + 3 weeks of gestation, in view of severe anemia of first twin and moderate anemia of twin 2, a decision was made to perform an intrauterine transfusion of both twins, in the same sitting. This was done in order to minimize number of procedures, reduce risk and utilize the same blood (divided into two) for both babies.

The placenta for twin 1 was posterior with a marginal cord insertion, and approachability was an issue; hence, we decided to perform an intra hepatic portal vein transfusion. 2nd twin was anterior and toward the right wall. In order to reach an appropriate angle for the placental cord insertion of twin 2, the position of the patient was changed to right lateral tilt at the time of twin 2 transfusion (Fig. 1).

A paralyzing agent (pancuronium) was given intramuscularly to gluteal muscles of both fetuses, to minimize fetal movements during transfusion. Under ultrasound guidance intrauterine transfusion was done with freshly collected (within 72 h), double packed (Hematocrit of 75%), O negative, leuco-depleted, CMV negative, gamma irradiated, cross-matched blood with mothers blood, into the hepatic portal vein of twin 1 and intravascular placental cord insertion site of twin 2.

Twin 1, who was expected to be more anemic, was transfused first as its procedure would be technically more difficult. Although we expected the 2nd twin to have better hemoglobin as the MCA PSV was < 1.5 MOM, to our surprise it was also severely anemic. We could not take post-transfusion hemoglobin of twin 2 as the fetus moved and displaced the needle from its site, probably because the anesthetic effect had washed off due to double time taken for two transfusion. Pre- and post-transfusion hematocrit, MCA PSV, and the amount transfused are mentioned in Table 1.

MCA PSV dropped significantly post-transfusion in both babies. Both twins were monitored with weekly MCA PSV (Table 2). There was a steady rise in MOM values with twin 2 always showing a better MCA PSV, and at

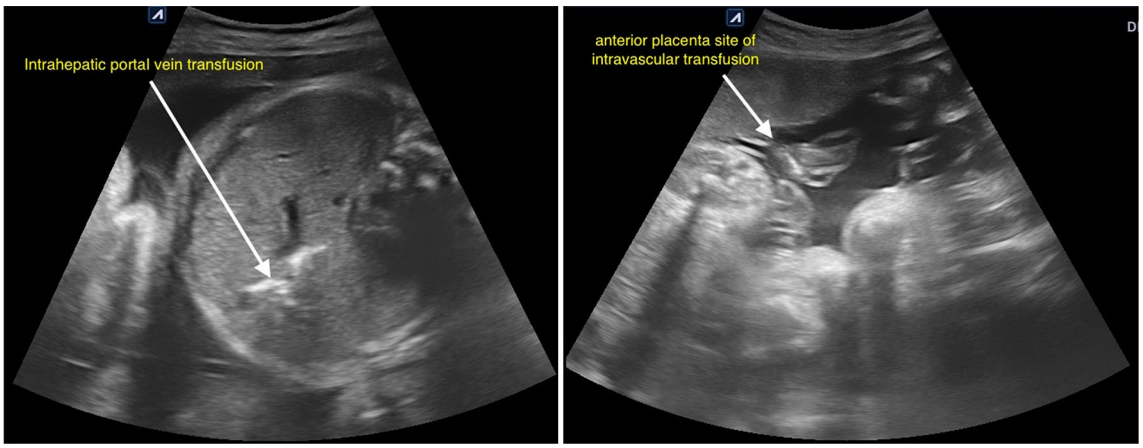

Fig. 1 Case 1 ultrasound image of twin 1undergoing intrahepatic portal vein transfusion and twin 2 with anterior placenta having placental cord insertion transfusion

Table 1 Correlation of fetal hematocrit of pre- and post-intrauterine transfusion and MCA PSV and effect of volume transfused

Case Time of transfusion	Twin		Hemoglobin (g%)	Hematocrit (%)	MCA PSV	Blood volume transfused
Case 1 32 weeks	Twin 1	Pre-IUT	8.1	27.3	70 cm/s	80 ml Donor HCT 75%
		Post-IUT	12.9	41.7	56 cm/s	–
	Twin 2	Pre-IUT	7.9	26.1	55 cm/s	100 ml
		Post-IUT	NA	NA	43.7 cm/s	–
Case 2 33 weeks	Twin 1	Pre-IUT	9.1	31.1	58 cm/s	75 ml Donor HCT 85%
		Post-IUT	12.1	38	48.3 cm/s	–
	Twin 2	Pre-IUT	11.3	34.1	44 cm/s	50 ml
		Post-IUT	13.5	41.5	39.1 cm/s	–
Case 3 32 weeks	Twin 1	Pre-IUT	6.4	22	55	85 ml Donor HCT 75%
		Post-IUT	18.3	41.7	24	–
	Twin 2	Pre-IUT	5.8	19.2	98	70 ml
		Post-IUT	11.6	30.6	65	–
Case 4 28 weeks	Triplet 1	Pre-IUT	12.6	38	50	70 ml
		Post-IUT	21.6	65.4	38	
	Triplet 2	Pre-IUT	12.9	38.9	45	60 ml
		Post-IUT	–	–	32	
	Triplet 3	Pre-IUT	13	40	48	50 ml
		Post-IUT	20.7	62.5	29	

Table 2 MCA PSV monitoring in Case 1 pre- and post-transfusion till delivery

TWIN 1	32 weeks Pre-transfusion	33 weeks Pre-transfusion	34 weeks Post-transfusion	34 + 3 weeks Post-transfusion
Twin 1				
MCA PSV	56	70	64	75
MOM	1.3	1.5	1.5	> 1.5
Twin 2				
MCA PSV	42.2	55	48	50
MOM	< 1	1.29	< 1	1–1.29

34 + 3 weeks of gestation, fetus 1 became severely anemic needing either another transfusion or delivery. A decision for Emergency LSCS after steroid cover was taken, and two boy babies were delivered at 35 weeks with the NICU team ready to manage.

Twin 1 was 1.8 kg with cord hemoglobin of 6.4 g%, cord bilirubin 12.6 g%, direct Coombs test (DCT) positive, hepatomegaly with signs of heart failure. Neonate was shifted to NICU, intubated, received 2 intravenous immunoglobulin (IVIG) and 2 packed cell blood on day 1 and 2 of life and exchange transfusion on day 3 along with double-surface phototherapy. Post-exchange transfusion, hemoglobin was 12.4 g% and bilirubin 3.4 g% on day 4. Regression of liver size was noticed. Neonate was kept in NICU under observation and discharged on day 15 in stable condition.

Twin 2 was 1.4 kg, growth restricted with cord hemoglobin of 10.3 g%, DCT positive, cord bilirubin of 4.3 g% who received single dose of IVIG. On day 3, hemoglobin was 11.4 g% but bilirubin increased to 11 g%, for which phototherapy was given for 6 days and the neonate was discharged on day 13 from the NICU. At one year follow-up, both babies were healthy.

Case 2

A 27 years old, G4P3L3, referred for clinical management at 26 weeks, with Rh-isoimmunized DCDA twin pregnancy, with indirect Coombs test positive and a titer of 1:32. Anti-D was given after all her vaginal deliveries with healthy children and no history of blood transfusion. Ultrasound at our fetal medicine center showed MCA PSV in Zone C (33 cm/s and 28 cm/s—1.1 MOM) in both the twins which was suggestive of mild anemia and Doppler monitoring was done with MCA PSV every 2 weeks. At 31 weeks, MCA PSV showed fetal anemia significant enough to require transfusion in both twins (58 cm/s—1.5 MOM and 44 cm/s, 1.3 MOM). However, no O negative blood from our blood bank or from across the city would cross match with the maternal blood for fetal intrauterine transfusion. On further evaluation of the maternal sample, multiple atypical alloantibodies

to minor blood groups were detected. Case was discussed with various hematology experts, but no clear-cut guidelines were documented in the literature. The option of utilizing mother's own blood (auto-transfusion to the fetus) was considered, but we were worried of the effect of maternal blood with multiple antibodies on the already compromised fetuses. A decision was made to cross match Rh-negative donor blood with cord blood drawn from both fetuses. However, this would entail an extra cordocentesis of both in utero fetuses. Hemoglobin of twin 1 was 9.1 g% (hematocrit 31.1) and twin 2 was 11.3 g% (hematocrit 34.2) and both fetuses were A positive with direct Coombs test positive. At 33 weeks, intrauterine transfusion (75 ml to twin 1 and 50 ml to twin 2 as per calculated amount) was performed in both the twins with A negative donor blood (hematocrit 85%) cross-matched with cord blood collected previously from both twins.

Post-IUT, monitoring was done with MCA PSV weekly, and at 35 + 4 weeks, she went into labor with severe anemia noted in twin 1 (MCA PSV twin 1—61 cm/s, Twin 2—43 cm/s). In the presence of multiple atypical antibodies in the maternal serum, compatible blood was again unavailable in all blood banks across the city that may be required for both the mother and the neonate. With multidisciplinary team of obstetricians, neonatologist and transfusion medicine specialist, decision to keep least compatible blood in lifesaving situation was made. The risks and consequences were explained to the patient and her husband. Fortunately, she delivered vaginally and had no postpartum hemorrhage.

Twin 1 was a 2.2 kg female child with a cord bilirubin of 7.19 g% and hemoglobin of 9.3 g% with DCT positive. Twin 2 was a 2.1 kg male child, cord bilirubin was 4.5 g%, and hemoglobin was 13.8 g% and DCT positive. Both babies were initially managed with intravenous immunoglobulin, methyl prednisolone and double-surface phototherapy. However due to continued postnatal hemolysis, hemoglobin dropped to 4 gm % in twin 1 and 9 gm % in Twin 2. Both neonates required 2 units of packed cell transfusion each, with least compatible blood, after informed consent during their stay in NICU for 25 days.

Case 3

A 26-year-old patient, G3 P1L1, Rh-isoimmunized monochorionic–diamniotic (MCDA) twins, was referred at 31 + 6 weeks with Rh titers of 1: 128 and severe fetal anemia (MCA PSV A—55 cm/s, 1.5 MOM and B—98 cm/s, 1.8 MOM) in both the twins. She had received anti-D prophylaxis in her previous pregnancy, post-LSCS. Both fetuses required transfusion, but the issue in this case that needed to be resolved was whether we transfuse one or both fetuses as there could likely be intraplacental anastomosis in our monochorionic twins. We were unsure whether a larger dose to one twin might take care of both fetuses. On the other hand, we were worried that this top up transfusion of both fetuses simultaneously, with the required individual dosage, could lead to high output cardiac failure of one or both twins due to additional auto-transfusion across inter-twin anastomosis. Additionally, the 2nd twin was already compromised with severe anemia with signs of hydrops in the form of minimal ascites, with mild pericardial effusion but with no cardiac dysfunction.

Since we found no answers in the literature on dosage in MCDA twin gestation, we decided to transfuse both fetuses at the same sitting but keep hematocrit under-corrected to be safe. As a routine procedure prior to transfusion fetal blood sample was obtained to measure fetal hemoglobin, hematocrit, blood group and Rh factor. The value of hematocrit was ascertained immediately to calculate amount of blood to be transfused, and while awaiting the report, transfusion was started to twin 1.

Volume of blood to be transfused to each twin was calculated depending on the initial fetal hematocrit, donor hematocrit and estimated fetal weight and fetoplacental blood volume using Moise et al. calculation [1]. A target hematocrit to be achieved at the end of procedure was 45–50%. With a cord blood hemoglobin of 6.4 g% (hematocrit 22%) in twin 1, with estimated fetal weight of 2 kg, the requirement of transfusion was 90 ml with a donor hematocrit of 75%. Similarly calculating the transfusion requirement of twin 2 (hemoglobin 5.8 g%, hematocrit 19.2%, fetal weight 1.9 kg) was 100 ml. There was a single posterior placenta with one central and the other marginal cord insertion. Under ultrasound guidance and under fetal immobilization with pancuronium, 85 ml of fresh O negative doubly packed blood was transfused through vascular access of umbilical vein at placental cord insertion site very slowly at 3–5 ml/min, using 20 gauge spinal needle to twin 1. There was technical difficulty in transfusion of twin 2, which was hydropic, and only 70 ml was transfused, although the required amount was 100 ml (under-correction).

Immediately following both fetal transfusion, MCA PSV values dropped from 55 to 24 cm/s in twin 1, and less so in twin 2, from 98 to 65 cm/s. Initially the hydrops subsided in twin 2 but within 15 days of transfusion at 34 weeks, twin 2 worsened with MCA PSV reaching 100 cm/s (1.8 MOM), while twin 1 was stable at MCA PSV of 54 cm/s (1.4 MOM).

Decision to deliver after administration of steroid for lung maturity and magnesium sulfate for neuroprotection was taken. Emergency lower segment cesarean section was done at 34 weeks in view of previous LSCS, both breech with severe fetal anemia and hydrops of twin 2.

Twin 1, a male child weighing 2.3 kg, cried immediately, had cord hemoglobin of 14.4 g% and bilirubin 4.7 g% was managed in the NICU with only phototherapy and discharged on day 7 of life. However, the 2nd twin, male,

weighing 2 kg required intubation with 5 min APGAR < 5, succumbed within 6 h. His cord hemoglobin was 10 g%, and bilirubin was 5.1 g% with features of hydrops (Table 3).

Case 4

A 32-year-old, G3P2L1NND1, trichorionic–triamniotic (TCTA) triplets, Rh-isoimmunized pregnancy was being followed up elsewhere with MCA PSV and referred at 28 + 4 weeks with Rh titers of 1:64 and ultrasonography suggestive of all three fetuses weighing around one kg with MCA PSV between 1.4 and 1.5 MOM for intrauterine transfusion. Obstetric history revealed previous 2 full-term deliveries with 1st living female child with no significant antenatal or postnatal complications. She was not given postnatal anti-D prophylaxis. In her 2nd pregnancy, patient delivered at term with neonate having significant jaundice on day 3 of life and was admitted to NICU but eventually expired on day 4 of life.

All three fetuses were transfused intravascularly at the placental cord insertion site of 70, 60 and 50 ml of O negative blood at one sitting. Due to overcrowding in triplets, there was technical difficulty in assigning cord to fetus during the transfusion and each cord which was transfused was traced to the fetus. Intrauterine transfusion helped in prolonging the pregnancy by 5 weeks while being monitored noninvasively by MCA PSV (Table 4, Fig. 2). Timely correction of fetal anemia prevented hydrops, prolonged pregnancy to 34 weeks, and no postnatal exchange transfusion was needed.

She underwent LSCS at 34 weeks in view of triplets with severe preeclampsia after steroid and magnesium cover, with triplet 3 having MCA PSV of 1.5 MOM. 1.5 kg, 1.4 kg and 1.6 kg male babies were born. Postnatally in the NICU all three triplets were given triple-surface intensive phototherapy and IVIG at 1 gm/kg after ensuring serum bilirubin in phototherapy range but not in exchange range. Phototherapy surfaces tapered and phototherapy discontinued by 72 h and all three babies were discharged from the NICU at 96 h of life.

Discussion

Each of the four cases we managed had their own intricacies that needed to be pondered before tackling them as not much was available in the literature due to the rarity of the disorder in multiple pregnancies. Case 1 was the first case of twins that we ever encountered in our experience of 20 years in managing intrauterine transfusion in our tertiary referral institute. Here, the difficulty in the approach to the first twin has been highlighted. Case 2 was rare due to the concomitant presence of atypical antibodies in the mother, in addition to

Table 3 Neonatal outcome in twins and triplet in relation to the timing of delivery, time since transfusion, post-transfusion hematocrit and MCA PSV just prior to delivery

Case	Gestational age at delivery	Post-IUT hemoglobin	Time since last transfusion days	MCA PSV at delivery	Weight	Apgar	Hemoglobin	Bilirubin	Phototherapy	IVIG	Exchange transfusion	Top-up transfusion	NICU stay
1/1	35 + 4	12.9	17	75	1.8	8	6.4	12.6	+	+	+	+	15
1/2	35 + 4	–	17	50	1.4	7	10.3	4.3	+	+	–	–	13
2/1	35	12.1	18	61	2.2	8	9.3	7.1	+	+	–	+	25
2/2	35	13.5	18	43	2.1	8	13.8	4.5	+	+	–	+	25
3/1	34 + 2	18.3	15	54	2.3	8	14.4	4.7	+	–	–	–	7
3/2	34 + 2	11.6	15	100	2	4	10	5.1	–	–	–	–	NND Day 1
4/1	33 + 4		26	48	1.5	8	17.8	6.3	+	+	–	–	4
4/2	33 + 4		26	52	1.4	8	18.1	5.2	+	+	–	–	4
4/3	33 + 4		26	55	1.6	8	16.9	4.4	+	+	–	–	4

Table 4 Triplet noninvasive monitoring by MCA PSV for fetal anemia throughout pregnancy

	23 + 6 Weeks	24 + 4 Weeks	25 + 4 Weeks	26 + 3 Weeks	27 + 3 Weeks	28 + 3 Weeks	28 + 6 Weeks	30 + 5 Weeks	31 + 6 Weeks	32 + 6 Weeks	33 + 2 Weeks
TRIPLET 1											
MCA PSV	37	37	38	42	44	56	50	38	43	48	50
MOM	1.2	1.1	1.1	1.2	1.2	1.5	1.3	< 1	< 1	1.0	1.1
TRIPLET 2											
MCA PSV	37	37	37	41	46	56	45	32	45	52	54
MOM	1.2	1.1	1.1	1.1	1.2	1.5	1.1	< 1	1.0	1.1	1.2
TRIPLET 3											
MCA PSV	29	37	32	40	38	53	48	29	54	55	56
MOM	< 1	1.1	< 1	1.1	1	1.4	1.2	< 1	1.2	1.1	1.5

Rh-D isoimmunization that made it difficult to cross match any donor blood for intrauterine transfusion. The third case was exclusive due to its monochorionic–diamniotic nature of twins, where the impact of inter-twin anastomosis on the transfusion was to be taken into consideration. Fourth case was a triplet gestation where the difficulty of which cord to be assigned to which fetus, the crowded space for intervention, as well as the risk of prolonged operative time and associated risk of preterm/premature rupture of membranes was our concern.

Twin births have increased with the use of assisted reproduction methods, and we expect more women with twin Rh isoimmunization in the near future, hence the need for initial experiences to be reported.

The incidence of Rh isoimmunization has reduced significantly following the universal implementation of pre- and postnatal anti-D immunoglobulin prophylaxis in Rh-negative pregnant women. Despite standardized use of anti-D prophylaxis, alloimmunization may occur due to unrecognized fetomaternal hemorrhagic events, inadequate dosing or missed prophylaxis for antenatal sensitizing events, poor patient compliance and absence of prophylaxis for other atypical RBC antigens. Three of our patients were sensitized in spite of receiving anti-D prophylaxis in all their prior pregnancies. We do not have information on the type of anti-D used. Benefits of monoclonal vs polyclonal, dosages used, timing and need for additional Kleihauer–Betke test to assess large fetomaternal bleed are beyond the scope of our case series, and they have not been addressed.

Impact of Advances in Technology on Treatment Outcomes

Although the technique of intrauterine transfusion has not significantly changed, the management of these pregnancies has evolved significantly in the last 30 years. There are very few cases documented in the literature describing intrauterine fetal blood transfusion in twin pregnancy. In a meta-analysis of all case reports till 1985, Manning et al. analyzed 15 sets of twins complicated by Rh alloimmunization. At the time, the only twin pair to survive had received 15 amniocentesis and 8 intrauterine transfusions [2]. Most of these cases were intraperitoneal transfusions aided with X-rays or low-resolution ultrasound imaging. Outcomes, especially of hydropic and very young anemic fetuses remained poor. Better and higher resolution in ultrasound technology to guide invasive procedures and refining the technique by vast clinical experience has improved success rates. In our case series, 8 of our 9 twin/triplet sets (88%) who received intravascular intrauterine transfusion survived with only one or two procedure per patient.

Intraperitoneal transfusion relies on injecting red cells into the peritoneal cavity that are transported through the

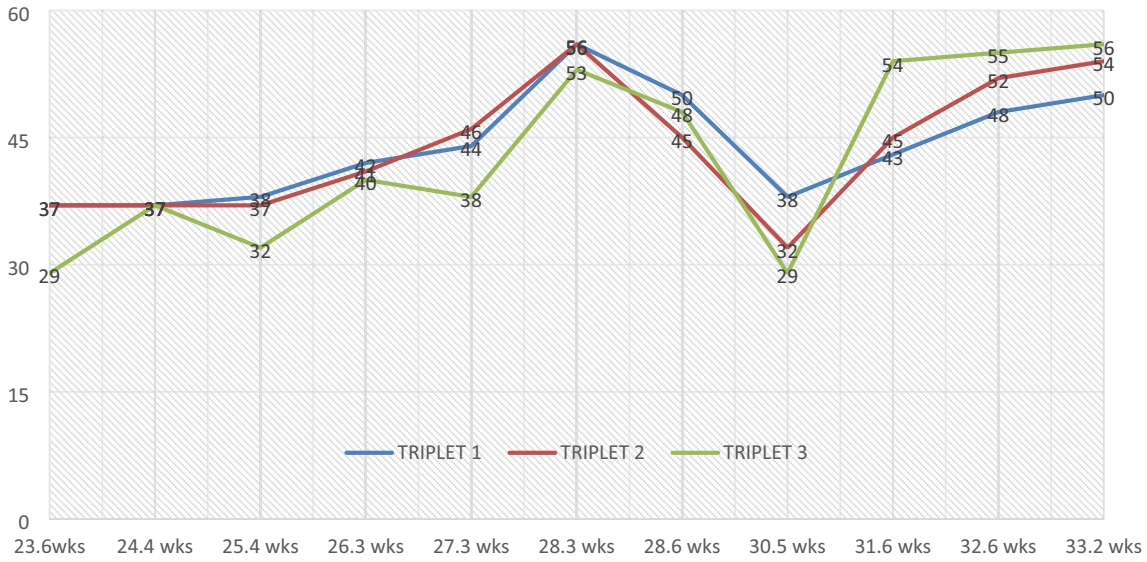

Fig. 2 Trends in MCA PSV in all three triplets pre- and post-transfusion

lymphatic system to the fetal circulation. Because of the erratic absorption, especially in hydropic fetuses, direct intravascular fetal transfusion has largely replaced the intraperitoneal technique. All our reported twin/triplet cases underwent intravascular transfusions.

With the availability of noninvasive technique for assessing fetal anemia by Doppler evaluation of middle cerebral artery peak systolic velocity (MCA PSV), the number of invasive procedures per patient has reduced. The landmark study by Mari et al. in 1995 showed that increased velocity in the middle cerebral artery above 1.5 MOM is an extremely accurate method for diagnosing fetal anemia noninvasively with a sensitivity of almost 100% and a false-positive rate of 12% [3]. Oepkes et al. [4] thereafter confirmed the superiority of MCA PSV over amniotic-fluid OD450, making serial amniocenteses for fetal anemia screening essentially obsolete. The correlation between MCA peak systolic velocity and fetal hemoglobin concentration when the fetus is not anemic or mildly anemic is not strong [5]. All our cases were monitored noninvasively by middle cerebral artery peak systolic velocity (MCA PSV) making all other ultrasound predictors of anemia including amniocentesis for bilirubin OD 450, redundant. However, it may be difficult in multifetal gestation to obtain the angle of insonation of MCA close to 0 degrees for accurate velocity assessment due to little space for manipulation. In our twin cases also, although the MCA velocities were apparently below 1.5 MOM in one each of the twin pairs, the fetuses were severely anemic (Table 1) suggesting a poor MCA correlation due to technical difficulties in getting the right angle in twins/triplets as compared to singleton.

Hydrops presents once the cardiac dysfunction starts, as end-stage sign of fetal anemia, and is observed once the hemoglobin deficit is 7 g/dl below the mean for the gestational age or absolute hemoglobin value below 5 g% when treatment results are poor [7]. Our only fetus with hydrops showed mild ascites with minimal pericardial effusion and had a hemoglobin of 5.4 g%.

It is believed that in the first immunized pregnancy, Rh antibody titers correlate well with the severity of disease. Only if the titers cross the critical values (most lab use 1:16) at any time of gestation or if there is a significant rise in the titers between 2 consecutive samples (> two tube dilutions), further assessment of fetal anemia is done noninvasively using middle cerebral artery peak systolic velocity [6]. Rh antibody levels as seen in all four of our cases were varying although all of them were above critical levels and required transfusion. Three of our four cases did not have a previously affected pregnancy, but values of the Rh titers did not correlate with the fetal hematocrit. Rh titers above critical level may not help in defining severity of fetal affection and deciding the time of transfusion. It may be safer to monitor middle cerebral artery peak systolic values from 18 weeks in all Rh sensitized multifetal pregnancy, even if it is the first affected pregnancy, once Rh titers are positive.

The antibody titers although are not predictive of the severity of disease, they help in timely referral to fetal therapy center for institutional management. In all our cases, critical Rh titers, and not the ultrasound, warned the clinician for need for fetomaternal specialized opinion and further management.

Although the Rh antibodies crossing the placenta in each pregnant woman carrying twins/triplets are expected to be

same, there was varying impact on co-twin/triplet sets with different values of hemoglobin and cardiac dysfunction specially in Cases 2 and 3. These differences in the severity of fetal anemia, amount of hemolysis and the varying fetal response clearly show that each fetus in multiple pregnancy has to be managed individually.

Noninvasive fetal genotyping from maternal blood using cell-free DNA testing for Rh (D) is clinically available [8]. However, it will be futile to assess it in Rh-isoimmunized DCDA/TCTA pregnancy as in our cases.

Atypical Antibodies and Their Effect on Management

The introduction of Rh (D) immunoglobulin in 1968 has greatly decreased the incidence of fetal anemia caused by Rh (D) alloimmunization. As a result, alloantibodies to other antigens of the Rh blood group system (c, C, e, E) and other atypical antibodies such as Duffy, Kidd, Kell, MNS and Lewis have increased in relative importance. These atypical IgG antibodies can cross the placenta and may cause hemolytic disease of the fetus and newborn even in Rh-positive mothers. Routine screening for atypical antibodies is not done in India as they are rare. The disease can be severe enough to warrant antenatal intervention, as was seen in our second case that had multiple atypical antibodies in addition to Rh-D isoimmunization. The presence of multiple antibodies in the mother may make blood type cross-matching difficult, and this can be problematic if she requires intrauterine fetal transfusion or transfusion at delivery or later.

Intrauterine therapy itself is associated with a risk of immunization to additional antigens, as has been noted in a large cohort where 25% of women formed additional antibodies after IUT, and more than 70% had multiple red blood cell antibodies postpartum [9]. The risk is highest when the IUT requires transplacental passage of the needle. The prevalence of additional maternal red cell antibodies may complicate present and subsequent pregnancies and future transfusions and is also capable of inducing delayed hemolytic transfusion reactions. Multiple atypical antibodies in our case were prior to and not secondary to any intrauterine transfusion. The presence of additional antibodies caused problems in obtaining compatible red blood cells for fetal and maternal transfusions. We utilized donor blood cross-matched with the fetal blood by cordocentesis. Though it required additional invasive procedure, it was the safest decision.

Technique of Fetal Therapy for Rh Isoimmunization

Intrauterine transfusion (IUT) is the treatment of choice for fetal anemia. It is indicated if the MCA PSV reaches above 1.5 MOM suggesting severe fetal anemia or development of immune hydrops or a fetal hematocrit of < 30% and the fetus is not mature enough to be delivered (< 35 weeks). Two sites commonly used for direct vascular access are intrahepatic portion of umbilical vein or the umbilical vein at the cord insertion site into the placenta. Hepatic portal vein approach is safer as it avoids arterial puncture and cord tamponade due to the absence of umbilical artery in near vicinity. But it is technically difficult in late gestation as manipulation of the needle within the liver is not possible and special long needles are needed to access the vein. Ultimately, the choice depends on operator preference and experience. Abassi et al. [10] in his experience found intrahepatic transfusion particularly useful in multiple pregnancy or in advanced gestation when the placenta is posterior. In our first case with low unapproachable cord insertion in a posteriorly placed placenta of twin 1, access to the portal vein was good and successful transfusion was possible at late gestation using a 5-inch needle. Entry to the abdominal wall should be accurately angled and fast before the baby moves under the pressure of a hesitant needle or incomplete anesthesia.

The only single-center large study on Rh-isoimmunized twin pregnancy is from France, where 9 twin pregnancies were managed between 1987 and 1996. Eight pregnancies were dizygotic, and the fetal blood groups were different in 3 cases [11]. It may be prudent to transfuse O negative blood to both twin pair when the blood group is not known, before the transfusion, as was done in our first and third twin pair and fourth triplet sets. However, in our second twin we already knew the blood group as A positive for both and hence we transfused A negative blood to both babies.

Chorionicity is an important factor for antenatal management of Rh alloimmunization. Dizygotic/dichorionic twin fetuses have separate placentas, and each fetus must be managed as separate individual. The presence of transplacental vascular communications in monochorionic twin pregnancies might be beneficial to the second twin, who thus would share in the absorption of adult red cells. On the other hand, such sharing would dilute the general effect of these cells and diminish their effectiveness for the first twin. In the lone case of monochorionic placentation, Jacques and colleagues alternately transfused every 2 weekly due to the presence of shared circulation [11]. In our monochorionic pair, both the fetuses were severely anemic on MCA PSV with one fetus additionally had hydrops. Both fetuses at the time of transfusion showed severe anemia (pre-transfusion hemoglobin 6.4 g% and 5.6 g%). Transfusing both fetuses was a practical decision as it would be risky to only transfuse the severely anemic hydropic fetus and risk volume overload in an already compromised heart. Additionally, transfusing to supraphysiological hematocrit levels has been associated with a theoretical increased risk of complications. On the other hand, delaying its transfusion would worsen it. We transfused both fetuses with a blood volume slightly lower

than required, specially in the hydropic fetus, expecting a reduced cardiac reserve and higher susceptibility to volume overload.

Procedure-related fetal loss ranges from 0.9 to 4.9% per procedure and is more associated with fetal hydrops, early gestation at transfusion, severity of fetal anemia and experience of operator [12, 13]. The risk in twin pregnancies has not been calculated but should be considered more than double due to the need for double transfusions, technically more demanding and the inherent risk in twins/triplets of preterm labor and premature rupture of the membranes. We encountered no complications in all our cases during or within 2 weeks post-transfusion.

Several studies have demonstrated worse outcomes following IUT in hydropic (survival 78%) compared with non-hydropic fetuses possibly due to their reduced cardiac reserve and increased susceptibility to volume overload [14, 15]. Our single neonatal demise of the three pair of twins and one set of triplets (survival 88%) was hydropic to start with, improved initially with intravascular transfusion, but worsened fast and could not be salvaged postnatally. Prevention of fetal hydrops by timely detection and treatment may improve survival as well as long-term outcome.

Repeat transfusion is usually required in 1–2 weeks and is calculated by a 1% drop of hematocrit per day from the final post-transfusion hematocrit or when MCA PSV reaches 1.5 MOM again. Alternatively, the timing of the next transfusion can be calculated using the expected decline in fetal hemoglobin using 0.4 g/L/day, 0.3 g/L/day and 0.2 g/L/day hemoglobin decline for the first, second and third IUT intervals, respectively [16]. In all our twin/triplet cases, the drop in values of hemoglobin was different in each set of twin/triplet with one of the pair faring better than the counterpart (Tables 2, 4). It may be prudent in twin gestations to consider post-transfusion hematocrit along with the MCA PSV of the more severely anemic fetus to dictate the timing of next transfusion. In addition, it may be beneficial to perform the procedure at one sitting in order to reduce risk of multiple and frequent procedures as well as reduce the cost to the patient by utilizing the same cross-matched blood divided into two for both fetuses.

Delivery and Postnatal Outcome

Unfortunately, there are no high-quality data regarding the optimal timing of delivery in the fetus receiving in utero therapy for anemia. Most centers perform fetal transfusions up to 35 weeks of gestation, with delivery anticipated at 37–38 weeks in singleton pregnancy based on balancing the risk of stillbirth, the consequences of fetal anemia and the risks of another fetal intrauterine transfusion, against the risks of prematurity and the additional morbidity of anemia and hyperbilirubinemia prior to term delivery. The inherent additional procedure-related risk of twin/triplet transfusion as well as increased baseline tendency of preterm and premature rupture of membranes forced us to deliver all our four Rh-isoimmunized multiple pregnancies at 34–35 weeks, after steroid cover for lung maturity.

The obstetric philosophy need not be altered for the transfused fetus. Two of our twin pairs and the triplet had an LSCS for obstetric indication, and one pair delivered vaginally. Although neonatal services should be involved early in the care of these patients, it is particularly critical for them to be prepared for delivery so that blood for transfusion can be ready if needed and the proper personnel can be present at delivery to optimize neonatal management of alloimmune anemia.

In the intrauterine period, fetal hyperbilirubinemia is cleared by the maternal system. However, once delivered, the neonatal hepatic system is overwhelmed with the excessive bilirubin to be metabolized, which may lead to kernicterus. Phototherapy followed by double volume exchange transfusion forms the mainstay of treatment for hyperbilirubinemia. The greater the number of intrauterine transfusions performed, the less severe the hyperbilirubinemia postnatal and longer the suppression of erythropoiesis. All our surviving 8 neonates required double-surface phototherapy as prophylaxis, while only one needed exchange transfusion. Top-up transfusion was needed in three neonates postnatally. IV immunoglobulin is also preferred modality in our neonatal ICU to reduce the need for exchange transfusion.

Neonates with Rh-mediated hemolytic disease of the new born need to be managed promptly by intensive phototherapy and if needed exchange transfusion. Intravenous IVIG is an alternative to reduce the number of exchange transfusions and associated risks. A Cochrane Systemic Review included seven studies with 371 neonates and compared phototherapy alone and phototherapy with IVIG in these neonates. IVIG resulted in a reduction in need of exchange transfusion, lowered maximum bilirubin levels, shortened duration of phototherapy but had similar need for RBC transfusions during the first week and thereafter [17]. Seven of our nine neonates received IVIG. Only Case 3 MCDA twins did not receive IVIG as one of the twins had mild hyperbilirubinemia requiring only phototherapy and the other had a poor APGAR at birth and succumbed within 6 h.

Neonatal survival overall, in the era of IUT, is 84%: 70% in hydropic and 94% in non-hydropic fetuses [10]. Neonatal survival in our multifetal gestation with Rh alloimmunization who had anemia severe enough to require transfusion was 88% (8/9). We have not included twin cases where MCA PSV never peaked above 1.5 MOM and intrauterine transfusion was not required.

Conclusions

Intrauterine transfusion in twins/triplets is a very challenging task. It requires a skilled and experienced specialist. Difficulties encountered during IUT in multifetal gestation are due to different or uncertain chorionicity, intraplacental anastomosis between vessels, different degree of anemia, difficult to ascertain cord–fetus relationship and difficulty to reach placental insertion site due to crowding by multiple fetal parts.

The presence of hydrops due to severe anemia is the main prognostic factor affecting survival after IUT therapy. Values of Rh titers above critical level may not help in defining severity of fetal affection and deciding the time of transfusion but are extremely useful for timely referral.

It needs to be pondered why despite exposure to the same maternal Rh antibody in the same concentration, the fetal affection and treatment response are different in twin/triplet sets.

Since the number of patients with alloimmunization has declined, it is important to centralize these procedures in centers of excellence to maintain enough needling experience per operator. It has favorable outcome when managed by experienced hands at tertiary care center.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This work has not been published before and is not under consideration for publication anywhere else.

Human and Animal Rights Research involves human participants but since it is a retrospective study and clinical work has been analyzed, there has been no direct risk to participants. Study has been approved by the hospital ethics committee.

Informed Consent Informed consent has been taken from all participants at the time of writing the article.

References

1. Giannina G, Moise KJ Jr, Dorman K. A simple method to estimate volume for fetal intravascular transfusion. Fetal Diagn Ther. 1998;13:94–7.
2. Manning FA, Bowman JM, Lange IR, et al. Intrauterine transfusion in an Rh immunized twin pregnancy: a case report of successful outcome and review of literature. Obstet Gynecol. 1985;65:2S.
3. Mari G, Deter RL, Carpenter RL, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. N Engl J Med. 2000;342:9–14.
4. Oepkes D, Seaward PG, Vandenbussche FP, et al. Doppler ultrasonography versus amniocentesis to predict fetal anemia. N Engl J Med. 2006;355:156–64.
5. Mari G. Middle cerebral artery peak systolic velocity for the diagnosis of fetal anemia: the untold story (Level III). Ultrasound Obstet Gynecol. 2005;25:323–30.
6. Mari G, Norton ME, Stone J, et al. Society for Maternal-Fetal Medicine (SMFM); Clinical Guideline #8: the fetus at risk for anemia diagnosis and management. Am J Obst Gynecol. 2015;212:697–709.
7. Nicolaides KH, Thilaganathan B, Rodeck CH, et al. Erythroblastosis and reticulocytosis in anemic fetuses. Am J Obstet Gynecol. 1988;159:1063–5.
8. Chitty LS, Finning K, Wade A, et al. Diagnostic accuracy of routine antenatal determination of fetal RHD status across gestation: population based cohort study (Level II-2). BMJ. 2014;349:g5243.
9. Schonewille H, Klumper FJ, Watering LM, et al. High additional maternal red cell alloimmunization after Rhesus- and K-matched intrauterine intravascular transfusions for hemolytic disease of the fetus (Level II-2). Am J Obstet Gynecol. 2007;196(2):143.e1-6.
10. Abassi N, Johnson JA, Ryan G. Fetal anemia. Ultrasound Obstet Gynecol. 2017;50:145–53.
11. Jacques L, Poissonnier MH, Coutanceau MJ, et al. Management and outcome of Fetomaternal alloimmunization in twin pregnancies. Fetal Diagn Ther. 1999;14:26–30.
12. Lindenburg IT, Smits-Wintjens VE, Klink JM, et al. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am J Obstet Gynecol. 2012;206(141):e1-8.
13. Lindenburg IT, Van Kamp IL, Oepkes D. Intrauterine blood transfusion: current indications and associated risks. Fetal Diagn Ther. 2014;36:263–71.
14. Radunovic N, Lockwood CJ, Alvarez M, et al. The severely anemic and hydropic isoimmune fetus: changes in fetal hematocrit associated with intrauterine death. Obstet Gynecol. 1992;79:390–3.
15. Van Kamp IL, Klumper FJ, Bakkum RS, et al. The severity of immune fetal hydrops is predictive of fetal outcome after intrauterine treatment. Am J Obstet Gynecol. 2001;185:668–73.
16. Scheier M, Hernandez-Andrade E, Fonseca EB, et al. Prediction of severe fetal anemia in red blood cell alloimmunization after previous intrauterine transfusions. Am J Obstet Gynecol. 2006;195:1550–6.
17. Zwiers C, Scheffer-Rath EAM, Lopriore E, et al. Immunoglobulin for alloimmune hemolytic disease in neonates. Cochrane Database Syst Rev. 2018;3:1–64.