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REVIEW ARTICLE

Treatment and Prevention of Rh Isoimmunization

Krishna Agarwal · Anubhuti Rana · Anoosha K. Ravi

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Abstract Rhesus (Rh) isoimmunization is an important clinical entity in India and other developing countries, which is responsible for fetal anemia and hydrops fetalis, and if not treated, it can result in intrauterine fetal demise. Rh isoimmunization is responsible for severe jaundice in neonates, which can be severe enough to cause kernicterus with debilitating consequences, if not treated adequately. It can be prevented with simple measures and treated if recognized in time.

Keywords Rhesus D antigen · Isoimmunization · Fetomaternal hemorrhage · Prevention of Rh isoimmunization · Middle cerebral artery peak systolic velocity

Introduction

Isoimmunization is defined as the development of antibodies against the antigens of another individual of the same species. The antigens which are present on the human red blood cells (RBCs) are mainly ABO antigens (A, B, AB), rhesus D antigen (Rh-D) and infrequently other atypical rhesus (Rh) antigens like Cc, Ee, Kell (K), Duffy (Fy^a), Kidd (Jk^a, JK^b), M and S [1]. The presence of particular antigens on RBCs confers an individual a specific blood group status. For example, if the RBCs of a person carry A, B and Rh-D antigens, the person's blood group would be AB and Rh positive (expressed as AB positive) blood group. If the RBCs lack any antigen, the blood group status of that person would be O Rh negative (expressed as O negative).

Rh isoimmunization is the development of antibodies against the Rh antigens present on the surface of RBCs [1]. The important Rh antigen responsible for majority of cases of severe Rh isoimmunization is Rhesus D antigen. The other atypical Rh antigens with a potential to cause severe isoimmunization are c, E and Kell antigens. Rest of the Rh antigens (Duffy, Kidd, M and S) rarely cause significant problems.

Data on frequency of Rh typing from India are limited. A study from North India on 1,000 healthy blood donors (53 were women) showed that 93 % were D positive and 7 % were D negative. Of the D negative donors, 98 % had c antigen and 100 % of D positive had e antigen [2]. C antigen was present in 90 % D positives. The frequency of Kell antigen was found to be 2.8 %, Duffy (Fya) 7.3 %, Duffy (Fyb) 58.3 %, M 88 %, N 57.5 %, S 57.8 %, and s 87.5 %. Kidd antigen was not tested due to financial constraints. Another large study from Pune, India on 10,133 healthy voluntary donors revealed frequency of D—92 %, C—88 %, E—27 %, c—51 % and e—98 % [3].

The ABO blood group antigens may also sometimes cause isoimmunization in a mother having O positive blood group and carrying an A, B or AB positive fetus. But for practical purposes these antigens do not cause significant fetal hemolysis and fetal anemia.

Pathophysiology

An individual lacks the antibodies against the antigens, which are present on his own RBCs. However, if RBCs coated with different antigens (from another individual) gain entry into the circulation, the reticuloendothelial

K. Agarwal (✉) · A. Rana · A. K. Ravi
Department of Obstetrics and Gynecology, Maulana Azad Medical College, New Delhi 110002, India
e-mail: drkrishna.agarwal@gmail.com

system (RES) of the recipient identifies these antigens on RBCs as foreign and mounts immune response to eliminate these cells. A similar phenomenon occurs during Rh isoimmunization. The Rh positive RBCs of the fetus gain entry into Rh negative maternal circulation via fetomaternal hemorrhage (FMH) resulting into formation of anti-D antibodies, which in turn pass to the fetus through placental circulation and destroy fetal RBCs to produce fetal anemia. FMH occurs throughout pregnancy and the amount of this hemorrhage increases with increasing gestation. Kleiheur–Betke test can detect fetal blood cells into maternal circulation by acid elution test. It has been found that the amount of FMH is very minute (around 0.03 mL) during first and second trimester however it may be as high as 25 mL during third trimester [4, 5].

Kleihauer–Betke Test

It is the standard method to quantitate FMH [6]. The fetal blood has differential resistance to acid as compared to adult hemoglobin. A blood smear is prepared from the maternal blood and treated with the acid. This removes adult hemoglobin whereas fetal hemoglobin persists in the red cells. On subsequent staining, fetal cells appear rose-pink in color whereas adult RBCs appear as ghost cells. Two thousand cells are counted in the microscope and percentage of fetal to maternal cells is calculated.

The Rh-D antigen on fetal cells, which is circulating in maternal blood, is recognized by maternal RES as foreign antigen. The RES responds with the formation of IgM antibodies against Rh-D antigen in small amount (initial response). These antibodies can be detected in the blood of this individual within few weeks' time. When there is second time exposure to this foreign antigen (during second pregnancy with an Rh-D positive pregnancy), the previously primed memory B cells respond more strongly with the formation of IgG antibodies (booster response). Sometimes sensitization of an Rh-D negative mother may be because of the mismatched blood transfusion (Rh-D negative mother transfused with Rh-D positive blood).

The initial response could be prevented if the fetal RBCs entering the maternal circulation are removed before they are detected by the maternal RES. This can be achieved by giving anti-D immunoglobulins to the mother so as to counteract appropriately for the FMH. This prophylaxis has drastically brought down the cases of isoimmunization throughout the globe.

The IgM antibodies formed due to initial response cannot cross the placenta and gain entry into the fetal circulation and therefore do not affect the first sensitizing pregnancy. During the next pregnancy with Rh-D positive fetus, the IgG antibodies cross the placenta by binding on the Fcy receptors present on the syncytiotrophoblasts. Once in the fetal circulation, these antibodies bind to the Rh-D antigen on the fetal RBCs. These antibody-coated RBCs are trapped in the fetal spleen and are destroyed. This results in fetal anemia, the severity of which depends upon the amount of FMH and amount of antibody formation.

The Rh gene locus is located on chromosome 1p34-p36. This gene locus consists of two closely- linked genes that are 96 % homologous, each having 10 exons. One is labeled as RHCE (codes for Cc and Ee Rh antigens) and another is labeled as RHD (codes for Rh-D antigen). There are two isoforms for two antigens (Cc and Ee) but there is no isoform for D antigen and either absence or partial deletion of RHD gene is denoted as d in the genotype. One group of antigens would be inherited by the paternal chromosome and another group by the maternal chromosome which will result in one of the various possible combinations of genotypes. The genotype may be DCE/DCE (homologous D positive) or dCE/DCE (heterozygous D positive) or dCE/dCE (Rh-D negative) and other combinations of Dd, Cc and Ee [1, 7].

Epidemiology

Since the introduction of prophylactic anti-D immunoglobulin given to all unsensitized Rh-D negative women after termination of pregnancy or delivery with an Rh-D positive fetus, there has been a dramatic decrease in the rate of Rh isoimmunization. In UK, prophylactic anti-D immunoglobulin has been in use since 1971. The stillbirths or neonatal death rate due to Rh-D isoimmunization reduced from 120 per 100,000 births in 1971 to 1.3 per 100,000 in 1992. Still there are around 600–700 new cases of Rh-D isoimmunization happening every year in UK. The reason suggested is either due to insufficient dose or failure to cover a potentially sensitizing event. Incidence of hemolytic disease due to antibodies against the other Rh antigens like Kell, Duffy and kidd is around six cases per year in the UK [8]. In North America, there is policy of prophylactic immunization to all Rh-D negative women and there has been much more decline in the number of cases of Rh-D isoimmunization [9]. In India, out of 26,000,000 births which occur every year, 13,00,000 (5 %) births occur in women with Rh negative blood group. Prevalence of Rh negative blood group in population from different parts of India from various reports is shown in Table 1.

Unfortunately there are no reports regarding the real magnitude of Rh isoimmunization from India. In 1980, Bhatnagar et al. from Patiala, Punjab in northern India, reported the incidence of Rh-D negative cases to be 5.60 % among 1,500 pregnant women with the incidence of isoimmunization to be 1.33 % in the total sample and 23.80 % in the Rh-D negative women [5]. Gupta et al. from

Table 1 Prevalence of Rh negative blood group in population from different parts of India

Author	Total no. of people tested	Rh negative males	Rh negative females
Chandra and Gupta, Lucknow, 2012 [10]	140,320	5,980 (4.28 %)	1,093 (10.93 %)
Giri, Maharashtra, 2011 [11]	11,554	504 (4.58 %)	32 (6.51 %)
Koram, Hyderabad, 2014 [12]	15,140	711 (4.63 %)	17 (4.63 %)
Patel, Ahmedabad, 2012 [13]	5,316	246 (4.85 %)	17 (7.08 %)
Latoo, Kashmir, 2006 [14]	100,980	Rh negative subjects 4,136 (4.09 %)	

Maharashtra also reported the incidence of Rh-D negativity to be 5 % among the antenatal population, however, they reported incidence of isoimmunization among Rh-D negative women 3.1 % during 1981–1983 which declined to 1.7 % during 1990–1992 [15]. A recent hospital-based study from New Delhi reported overall incidence of isoimmunization to be 1.25 % [16]. The isoimmunization rate was 10.7 % among Rh-D negative mothers and 0.12 % among Rh-D positive mothers. The non Rh-D antibodies responsible for isoimmunization were anti-C, anti-M, anti-S and anti-c. Thakral et al. have reported two cases of hemolytic disease of newborn by maternal alloimmunization to anti-c and anti-E [17]. The newborn babies had features of hemolytic disease of newborn with Rh positive status of mother. A positive direct Coombs test led to suspicion which was confirmed by definitive testing for atypical antigens.

Manifestations

Rh isoimmunization of a pregnant mother may be responsible for varying severity of anemia in the fetus and newborn. Usually it is in the second or subsequent pregnancies that the fetus is affected. Such a fetus will initially have fetal anemia, this may manifest clinically as decreased fetal movements. If the condition persists and becomes more serious there would be extramedullary erythropoiesis in the liver and spleen. This can be seen on ultrasound as hepatosplenomegaly. In a profoundly anemic fetus initially there is increased cardiac output but the hypoxic heart can no longer sustain and finally culminates in heart failure. This is manifested sonographically as hydropic changes like pleural effusion, pericardial effusion, ascites, subcutaneous edema and scalp edema. To compensate for the reduced oxygen supply, the placenta also enlarges which could be seen as placentomegaly on ultrasonography [1, 7]. Fetal anemia is reflected on USG as increased middle cerebral artery (MCA) peak systolic velocity. By the time these hydropic changes are evident on ultrasound, it is quite late and fetus is very sick with a high fetal mortality. Therefore our aim remains to identify fetal anemia much before this terminal stage [1, 7].

Management

An algorithm for management for Rh isoimmunization is depicted in Fig. 1.

Screening for Rh Isoimmunization

Blood grouping and crossmatching is performed in all pregnant women at the first visit. If the woman is Rh-D positive no further testing for blood groups is required. In western countries testing for antibodies against all the Rh red cell antigens (D, Ee, Cc, Kell, Duffy, Kidd, Jka, Jkb and M) is also performed irrespective of the blood group and if a woman is positive for these antibodies she is managed as a case of isoimmunized pregnancy. However because of the high cost, this test is not routinely performed in India. It is reserved for pregnant women, who are Rh positive or Rh-D negative with negative indirect Coombs test (ICT) for Rh-D antibodies, with a past obstetric history suggestive of isoimmunization (birth of a baby with features of hydrops, neonatal jaundice or history of postnatal exchange transfusion).

When the expectant mother is Rh negative, the husband's blood is tested for ABO grouping and Rh typing. If the husband is Rh positive, virtually all guidelines [18–21] recommend performing genotype of the father for Rh-D coding gene. A homozygous father will inherit Rh-D gene to all his offspring and all the pregnancies will have potential for sensitization. If the father is heterozygous, there is 50 % chance of fetus being Rh-D positive. Most of the western guidelines recommend finding the fetal blood group from circulating cell free fetal DNA in maternal blood. When fetus is Rh-D negative no further testing required. If fetus is Rh-D positive, further follow- up is done. However in India, the facilities for testing of zygosity for Rh-D gene and fetal blood group from circulating cell-free fetal DNA in maternal circulation are available only in few centers. Therefore a pregnancy, when mother is Rh-D negative and father is Rh-D positive, is considered potentially at risk of immunization.

Small amount of FMH (total of less than 15 mL) is inevitable during the course of pregnancy. To detect sensitization of mother, presence of anti-D antibodies in

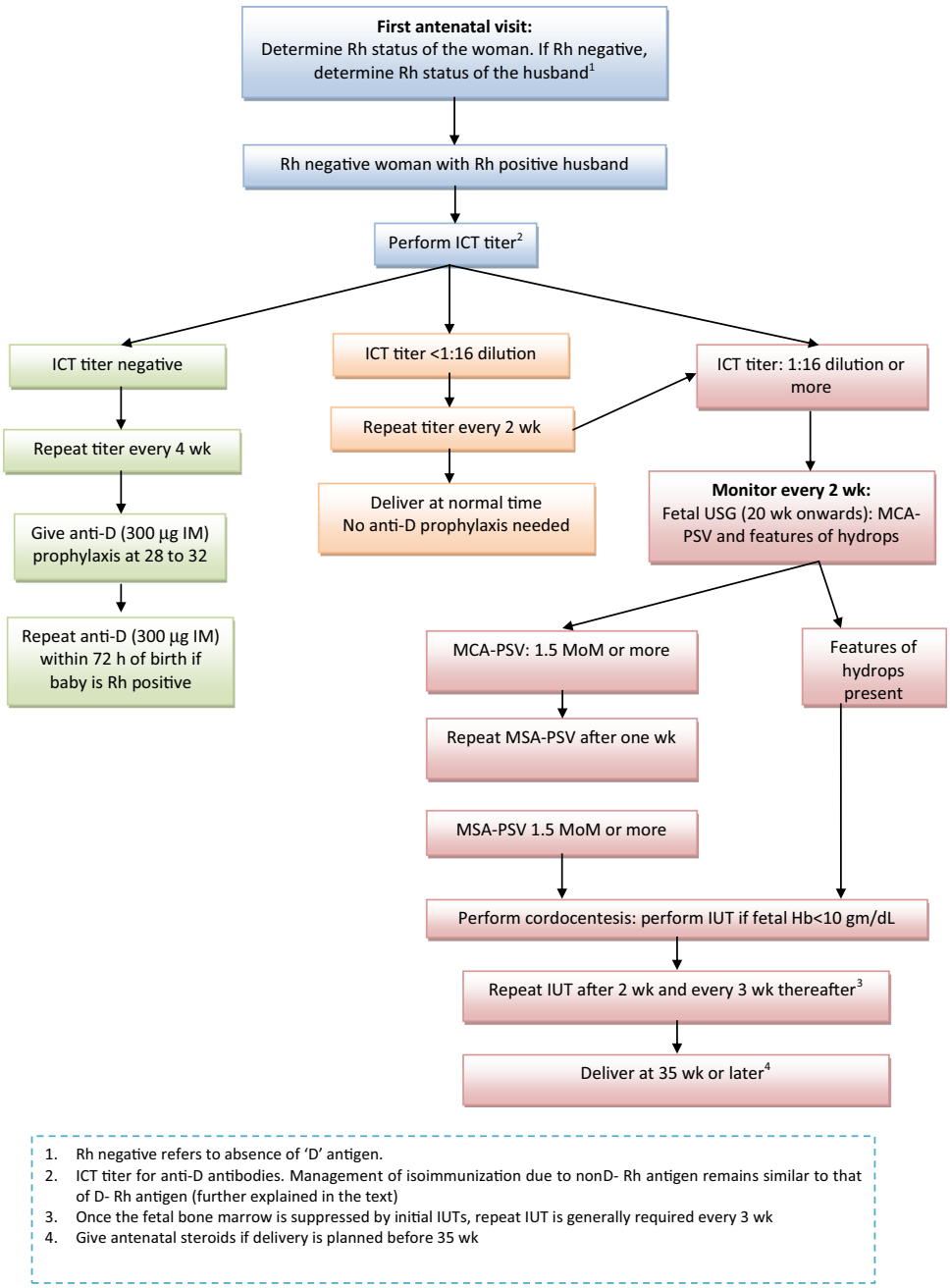

Fig. 1 Algorithm for management for Rh isoimmunization

maternal circulation is usually detected by ICT. It involves incubation of maternal serum with the RBCs carrying the particular Rh antigen against which the antibodies are being tested. Anti-human immunoglobulin is then added. It will cause agglutination of RBCs if they have adsorbed the antibodies. The serial dilutions of maternal serum are mixed with the RBCs carrying D antigen and the reverse of maximum dilution which causes clumping of RBCs is denoted as the titer. Mostly 1:16 or 1:32 is considered as the critical titer (potential to cause significant fetal anemia) which may vary with the laboratory. In UK, many labs use automated analyzers which give quantitative values in international units and invasive testing is indicated at levels above 10 IU/L. When ICT is negative the test is repeated every 4 week and if ICT is positive, mother is managed as an isoimmunized pregnancy. It is to be remembered that ICT as commonly performed, tests only for Rh-D antibody and not the minor Rh alleles. Separate tests using specific Rh allele are required for detecting antibody to minor alleles. In some women, the test for Rh-D typing may be weak Rh-D positive. These women are genetically Rh-D positive and are at low risk of producing anti-D antibodies.

These women are given status of weak D positive and Rh typing is represented by Rh-D^{u}. There are no clear recommendations for management of these women and at present these women are not given any anti-D prophylaxis.

Primary Prevention of Rh Isoimmunization

For women who are not yet isoimmunized the aim is to prevent sensitization. It can be achieved by giving prophylactic dose of anti-D immunoglobulins to cover for the spontaneous fetomaternal hemorrhages and also any antepartum event which has potential to cause additional FMH. If no prophylaxis is given, it is estimated that 1 % of Rh-D negative women would develop antibodies by the end of first Rh-D positive pregnancy. Around 7–9 % of additional women would be sensitized at the time of delivery. Another 7–9 % would develop antibodies during 6 months following delivery. Therefore around 17 % women would become sensitized by the second pregnancy [1, 7].

The most effective strategy to reduce the incidence of Rh isoimmunization has been the introduction of antenatal and at birth anti-D prophylaxis. The occurrence of Rh-D sensitization in last few week of an uncomplicated pregnancy has been stated to be the single most reason for remaining cases of isoimmunization. It may be due to either the inability to cover the potential events causing FMH or inadequate dose of anti-D. Therefore, clear instructions regarding the event-specific doses and timing could almost eliminate this condition. Also transfusion of Rh-D positive blood to Rh-D negative woman should be avoided and blood should be properly crossmatched before transfusion so as to avoid possibility of isoimmunization against other minor red cell antigens.

Prophylaxis

Antenatal Prophylaxis

If ICT is negative at the first visit, it is repeated at four weekly interval and if it remains negative on subsequent testing, prophylactic dose of anti-D immunoglobulin is given (300 μg deep intramuscularly) at 28–32 week of pregnancy. This will take care of the small amount of FMH and prevent isoimmunization. Since anti-D injection is a human blood product obtained from plasma, a written informed consent should be taken before its administration.

1. One dose versus two doses

There are various thoughts regarding the one dose of 300 μg at 28 week versus two doses of 100–120 μg each at 28 and 34 week. But most of the guidelines have preferred single dose and have mentioned that two dose schedules could be used as an alternative regime [18, 22].

2. Doses for different types of procedures

In addition to routine prophylaxis, different situations which are considered to increase risk of FMH should be covered by anti-D prophylaxis. After checking for maternal blood type and antibody screening, 120–150 μg of intramuscular anti-D injection is given for the following obstetric conditions within 12 week of pregnancy:

Threatened abortion; miscarriage; induced abortion; ectopic pregnancy; molar pregnancy; and chorionic villus sampling.

After 12 week of pregnancy, 300 μg of intramuscular anti-D for following conditions:

All above conditions after 12 week; amniocentesis; external cephalic version; antepartum hemorrhage; retained placenta; and blunt trauma over the abdomen.

Usually these doses prevent development of isoimmunization and take care of up to 15 mL of fetal RBCs. Where severe FMH is suspected, Kleihauer–Betke test could be done to measure the amount of FMH and accordingly dose can be adjusted. Additional 10 μg of anti-D should be given for every additional 0.5 mL of fetal RBCs in maternal circulation.

A few investigators have observed that if Rh negative mother does not deliver by 40 week and she has received prophylaxis at 28 week (12 week ago), the circulating anti-D is not enough to take care of FMH occurring at this time and advise a second dose of anti-D at 40 week. However, there is not enough evidence and it is not a routine practice.

Postpartum Prophylaxis

The FMH which occurs at the time of delivery is covered by prophylactic anti-D within 72 h of birth. A dose of 300 μg of anti-D is given when the baby's blood group is Rh-D positive. If anti-D dose is missed within 72 h, it can be given up to 28 days of delivery with some benefit [18, 23].

Secondary Prevention of Rh Isoimmunization

Early Diagnosis

Once ICT for Rh-D antibodies becomes positive in critical titer, pregnancy is managed as isoimmunized pregnancy. The aim is to detect fetal anemia at the earliest and also to avoid events likely to increase the FMH (like external cephalic version, external trauma over the abdomen or invasive testing). Weekly monitoring is performed with the antibody titer and ultrasonography. Ultrasound is performed for MCA peak systolic velocity (MCA-PSV) and for any evidence of fetal hydrops (scalp edema, pleural effusion, pericardial effusion, ascites, and skin edema).

Antibody titers of 1:128 are considered to cause significant fetal anemia and once these titers are reached, the pregnancy is monitored closely by MCA-PSV [24, 25].

Before 1995, isoimmunized pregnancies were monitored by serial amniocentesis to detect bilirubin levels in amniotic fluid with the help of spectrophotometry. The Liley's and Freda's graphs are available to manage these pregnancies on the basis of amniotic fluid optical density at 450 mμ. The value of OD450 indicates degree of hemolysis and the fetal outcome [26]. Management of isoimmunized pregnancies with serial amniocentesis has been now given up in favor of measurement of MCA-PSV because it requires invasive testing with a potential to cause FMH and increase in antibody titers.

MCA-PSV normograms are available and values are grouped according to multiples of median (MOM). Median values for MCA-PSV is for healthy nonimmunized pregnancies at different gestations are recorded and 1.5 MOM values are calculated. MCA-PSV above 1.5 MOM is considered to be associated with significant fetal anemia. In a case where MCA-PSV has reached 1.5 MOM level, MCA-PSV is repeated after 1 week and if it remains at this level or rises, it is an indication for cordocentesis and intrauterine transfusion (IUT). If titer has increased, but MCA-PSV is less than 1.5 MOM, patient is still managed with weekly MCA-PSV measurement. Using cut-off of 1.5 MOM, fetuses with moderate to severe anemia are identified with a positive predictive value of 65 %, negative predictive value of 100 % and false positive rate of 12 % [27].

Intrauterine Transfusion (IUT)

IUT is considered to be most effective in management of isoimmunized pregnancy where fetus is anemic and not mature enough to be delivered. If IUT is not done, fetus is at the risk of developing hydrops and dying in utero.

Procedure

This is usually performed as an outdoor procedure.

Preparation Adult blood of O-Rh negative group from which white cell component has been removed is packed to hematocrit of 80–85 %. Preferably, blood should be freshly collected within 24 h and sterilized by irradiation and tested for hepatitis B and C, HIV, cytomegalovirus.

Volume of blood to be transfused The volume of blood to be transfused will depend on the fetal gestation, hematocrit of the donor blood and hematocrit of fetal blood. The normograms are available to calculate the volume of blood needed to increase the fetal hematocrit to 40 % [28].

It may also be calculated by using the formula:

$$\text{Volume of blood to be transfused} = \frac{\text{Desired hematocrit} - \text{Fetal hematocrit}}{\text{Donor hematocrit} - \text{Desired hematocrit}} \times \text{Fetoplacental blood volume}$$

The aim is to increase hematocrit to 35–40 % in early mid-trimester and after that to 45–55 %.

Preliminary calculation for amount of blood transfusion is kept ready on a paper and as soon as fetal hematocrit value is available, third person can quickly do the final calculation.

Maternal sedation If mother is apprehensive, she can be given sedative like tab lorazepam, 2 mg 1 h before the procedure. Under continuous ultrasound guidance, procedure is performed using free hand technique. Three trained persons are required—one to perform the procedure, second person for withdrawing and pushing the blood and third person getting the fetal hematocrit and for the estimation of blood to be transfused.

Site of puncture It is much safer to go in the umbilical vein. When the placenta is anterior, 22G spinal needle is traversed through placenta and needle is guided in the umbilical vein near the cord insertion. Once the needle tip is seen in the umbilical vein, 1 mL of blood is withdrawn. Blood is immediately checked for the hematocrit. If automatic analyzer is available, it is preferred. The needle is attached to three-way connector which, in turn, is connected to the donor blood bag. Blood is slowly pushed at a rate of 10 mL per min. Once the total desired amount is transfused, 1 mL blood is collected for checking post-transfusion hematocrit [29, 30]. If the placenta is posterior, approach to placenta may be difficult and in these cases, there is an option of using a free loop of umbilical cord, hepatic vein or to perform intraperitoneal transfusion. After the procedure, CTG tracings are taken and monitored for at least half an hour for any fetal heart decelerations.

Subsequent transfusions Usually, the second transfusion is performed within 10–14 days. The subsequent transfusions usually are required at 3 week interval.

Intraperitoneal transfusion This is performed when transfusion is required in early second trimester or approach to the umbilical vein is difficult. The donor blood is infused into the peritoneal cavity of fetus from where it gets absorbed by subdiaphragmatic lymphatics and thoracic duct into the fetal circulation.

Intravenous immunoglobulins (IVIg) High doses of IVIg to mother have been tried in the management. However, the results are not promising and the fetus still requires transfusion. Deka et al. studied the effect of high dose of IVIg in iso-immunized mother as primary therapy in six patients and found it to be beneficial [31, 32]. Deka et al. had also studied the effect of direct fetal intravenous transfusion of immunoglobulins and reported that these

fetuses required less number of transfusions and the rate of fall in fetal hemoglobin was slower in the intervention group [31, 32].

Tertiary Prevention of Rh Isoimmunization

Once the baby has delivered, baby is carefully monitored for severe anemia and jaundice, and if required, postnatal exchange transfusion would save the baby.

Prognosis

Rh isoimmunization is one condition which had got a very good prognosis if managed properly and in expert hands.

Management of Isoimmunization Due to Other Rh Antigens

This is suspected when there is past history of hydrops, neonatal jaundice and exchange transfusion. On Rh typing these mothers will be either Rh-D positive or Rh D negative with negative ICT for Rh-D antibodies. When suspected, ICT for Rh c, E, Kell and Duffy antigens is performed and a positive ICT will denote isoimmunization due to a specific antibody. The management is essentially same as with Rh-D isoimmunization (monitoring with antibody titer and MCA-PSV). The only difference is that antibody titer is not very informative and management depends on the MCA-PSV. In Kell isoimmunization the fetal anemia is due to erythroid cell depression and not because of the hemolysis. However the management remains same as for Rh-D isoimmunization.

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Conflict of Interest None.

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