Antenatal Maternal Serum IAT Titer and Fetal Outcome in Rh Isoimmunized Pregnancies

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Abstract The incidence of Rh negativity in India is about 1–5 % and the rate of Rh sensitization to be approximately 0.79 % of live births. This study evaluated the role of antenatal maternal serum Indirect Antiglobulin Test (IAT) titre in predicting the feto-neonatal outcome. The study was conducted from Jan 2007 to Dec 2012 at our centre in Pune, Maharashtra, India. This study reports our experience with 75 IUTs carried out for 42 cases of severe Rh isoimmunization. IAT was performed by ID gel cards and test tube method was utilized for titration. Results were analysed by odds ratio (OR) with 95 % Confidence Interval. IAT titre was found to have a direct correlation with the maternal parity, requirement of number of IUT's and adverse fetal outcome. Of the 42 cases of severe Rh isoimmunization who underwent IUT, 11 (26.2 %) had hydropic fetus resulting in 08 (73 %) live babies, two intrauterine and one neonatal death. The remaining 31 (73.8 %) non-hydropic fetuses who received IUT, one intrauterine and one neonatal death were observed. In the 11 hydropic cases, who received IUTs, two intrauterine and one neonatal death were observed in which the IAT titre was ≥ 512 , which was found statistically significant with OR of 9.77 & *P* value of 0.05. The overall survival rate was 37/42 (88.1 %). Severity and fetal outcome in Rh isoimmunized pregnancies, showed a significant association with antenatal maternal serum IAT titre. More the antibody titre

more would be the fetal and/or neonatal severity with respect to immune hemolytic anemia. Requirement of multiple IUTs are also associated with high antenatal serum IAT titre.

Keywords Rh isoimmunization · Intrauterine blood transfusion · Indirect Antiglobulin Test

Introduction

Intrauterine Transfusion (IUT) is a procedure in which blood is given to the fetus to provide a better chance of survival from life threatening conditions associated with severe fetal anemia. It is an accepted method for the management of pregnancies, complicated with fetal haemolytic disease due to maternal red cell isoimmunization. The majority of such cases are associated with Rhesus haemolytic disease [1–3]. The incidence of Rh (D) negativity in India is about 5 % [4, 5]. Maternal isoimmunization occurs in approximately 1–2 % of Rh (D) negative women [6, 7]. Approximately 10 % of isoimmunized women have a fetus affected by severe haemolytic anemia [8]. Antenatal serological tests including ABO/Rh (D) typing and IAT plays an important role to identify Rh-negative women and to detect maternal isoimmunization. In order to assess the strength of the antibody and its likelihood of causing significant hemolysis, antibody titration is usually performed [9]. Estimations of maternal antibody titre are useful in two ways: a low value indicates that the infant will be mildly affected, or unaffected and an increase in titre values is a warning that the severity of the haemolytic process may be increasing [10].

This study was conducted to assess the outcome of Rh iso-immunized pregnancies with severe fetal anemia,

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which underwent IUTs based on standard criteria (Spectrophotometric assessment and Doppler study) and its relationship with a rising maternal serum anti-D titre levels.

Materials and Methods

The study was conducted from Jan 2007 to Dec 2012 in the Department of Transfusion Medicine, in association with the Obstetrics and Gynaecology department in the tertiary care hospital of western India. A written consent was taken from the patient and institutional ethical committee has granted the Permission to conduct the study.

75 IUTs were carried out in 42 cases of severe Rh isoimmunization in which a single IUT was done for 20 cases, twice for 14 cases, thrice for 5 cases and 4 times for 3 cases. Indication to perform IUT, up till 2008 was based on, amniocentesis [amniotic fluid optical density (AOD) at 450 nm in Lileys upper zone II or Zone III, was considered indicative of severe fetal hemolysis]. From 2008 onwards a more sensitive, non-invasive method, Color Doppler USG system, by which fetal blood flow velocity [Middle Cerebral Artery-Peak Systolic Velocity (MCA-PSV)] can be calculated, is available. MCA-PSV could predict severity of anemia better.

At first antenatal visit, evaluation of all isoimmunized pregnant women was done, which involved a detailed obstetric history, ABO/Rh blood grouping, IAT with titration and confirmation of anti-D antibody by using 11 cell identification panel from Biorad GmbH, Switzerland. An ultrasound examination was done to detect the presence of hydrops.

- (a) Indirect antiglobulin test (IAT) and antibody titration:
IAT was done by low ionic strength solution (LISS) Coomb's gel card from Biorad GmbH, Switzerland with "O" Rh (D) positive pooled cells. The titration was performed by test tube method through Anti human globulin (AHG) phase with 60-min incubation at 37 °C. In all IAT positive cases, antibody titre was repeated every 4–6 weeks till delivery. IAT titre of >16 was considered critical, which was used as a preliminary marker to undergo fetal monitoring procedure (Cordocentesis or MCA-PSV) [11].
- (b) Fetal monitoring and decision making for intra uterine transfusion:
Fetal monitoring for early diagnosis of fetal anemia was done at regular intervals from 14–16 weeks onwards by serial ultrasound and MCA-PSV using Color Doppler. Cordocentesis was performed when >1.5 multiples of the median (MOM) of the Doppler peak velocities of the MCA was found. Blood was collected for evaluating hemoglobin, hematocrit,

blood group (ABO & Rh) & DAT status. IUT was carried out at the same sitting when fetal hemoglobin was found less than 10 gm/dL or hematocrit was less than 30 %.

Flow chart for the evaluation & management of Rh isoimmunization is shown in Fig. 1.

- (c) Calculation of blood volume to be transfused:
The total amount of red cells required to be transfused depends on the initial fetal haematocrit, desired final haematocrit, hematocrit of the donor unit and feto-placental blood volume (an estimated volume is about 100 mL/kg $\pm$ 14 mL). The fetal weight (in grams) was estimated using ultrasonography [12]. The formula used to calculate the volume of red cells to be transfused is as follows [13].

$$\frac{\text{Desired HCT} - \text{fetal HCT}}{\text{Donor HCT}} \times (\text{Feto} - \text{placental blood vol})$$

The final target haematocrit in non-hydropic foetuses was 40–50 %, whereas in the group of hydropic foetuses it was about 25 %, to avoid post-transfusion cardiac overload. The mean gestational age at first IUT was 29 ± 2 weeks. The amount of blood transfused varied from 40 to 110 mL (Table 1).

- (d) Preparation of a blood unit:
In all cases "O" Rh (D) negative, leucodepleted, irradiated and fresh packed red blood cells (collected within 72 h) with Haematocrit of 75–80 % was transfused. Haemoconcentration of packed RBC unit (to achieve Hct 75–80 %) was done by centrifugation at 2,000 rpm for 9 min and removal of supernatant. Hematocrit was measured by hematology analyzer (Sysmex KX-21). Transfer bag connection and separation was done by sterile connecting device (SCD) and sterile tube sealer respectively. Compatibility tests with fresh maternal serum were performed before each transfusion.
- (e) Intrauterine transfusion procedure:
Intra-Vascular Transfusion (IVT) mode of IUT was performed invariably in all cases. The blood was transfused slowly into the fetal circulation through umbilical vein under ultrasound guidance. 22-gauge spinal needle was utilized for injecting blood. The fetus was monitored carefully throughout the procedure.

Data analysis

Data was analyzed statistically by using Odds Ratio (OR) with Confidence Interval (CI) of 95 %. Data entry software Epi-Info (Version-7) was used to determine the association

Fig. 1 Evaluation & management of Rh isoimmunization

of high IAT titres with the fetoneonatal morbidity & mortality. *P* value of <0.05 was considered statistically significant.

Results

The IAT titre range varied according to the degree of fetal anemia, requirement of number of IUTs as shown in Table 1 and the presence of hydrops. IAT titre was found to have a direct correlation with the maternal parity. More the parity, higher would be the IAT titre (Table 1). In all cases, initial IAT titres were low at 16–20 weeks of gestational age, but subsequently the titre was increased at around 28–30 weeks of gestational age i.e. at the time of first IUT. Out of 42 cases, 11 (26.2 %) had hydropic fetus with IAT titre ranging from 256 to 1,024. Three adverse

events were reported after IUT procedure, in which mother developed a low grade fever which subsided within 12–24 h. Intrauterine transfusion enabled pregnancies to be carried on successfully to 32–36 weeks in 92.86 % of the cases. Mode of delivery was elective Caesarean Section (CS) in 10 (23.8 %) cases, emergency CS in 11 (26.2 %) cases and vaginal delivery with intra-partum electronic monitoring was carried out in 21 (50 %) cases. Overall fetal and neonatal outcome in relation to their corresponding IAT titre are shown in Table 2. In the 11 hydropic cases, who received IUTs, two intrauterine and one neonatal death were observed (IAT titre ≥ 512), which was found to be statistically significant with OR of 9.77 & *P* value of 0.05. Eight live babies were discharged from hydropic category. In 31 non-hydropic fetuses who received IUT, there were one intrauterine and one neonatal death, with a survival rate of 93 %. Neonatal death was

Table 1 Number of IUTs required, mean volume of blood transfused in each IUT and maternal parity in relation to IAT titer

Indirect antiglobulin test (IAT) titer range	No. of IUTs per case	No. of cases (42)	Mean volume of blood transfused in each IUT (range)	Maternal parity (no. of cases)
>1:16 to 1:64	1	20	57 mL (50–100)	G2P1A0 (14) G3P1A1 (5) G3P0A2 (1)
1:128 to 1:256	2	14	68 mL (40–110)	G2P1A0 (5) G3P1A1 (3) G3P0A2 (2) G4P2A1 (4)
1:512 to 1:1024	3	05	72 mL (65–110)	G3P2A0 (3) G3P1A1 (1) G4P1A2 (1)
≥1:1024	4	03	85 mL (70–95)	G5P1A3 (1) G4P1A2 (2)

G gravida, P para and
A abortion

Table 2 Foetal & neonatal outcome in relation to Indirect Antiglobulin Test (IAT) titre

Fetal & neonatal outcome	IAT titer	Odds ratio (OR)	P value
Hydropic fetuses (11)			
Fetal death (2)	≥1:1024	9.77	0.050
Neonatal death (1)	1:512		
Non-hydropic fetuses (31)			
Fetal death (1)	1:512	5.70	0.161
Neonatal death (1)	1:256		
Newborn required blood transfusion (8)	1:128 to 1:512	10.54	0.004
Healthy live babies at discharge (29)	1:16 to 1:256		

observed 48 h after delivery. There were no procedure related death observed. The overall survival rate was 88 %. Thirteen newborns required blood transfusion 1–3 weeks after delivery.

Discussion

Our study showed the correlation of IAT titre in maternal serum and feto-neonatal outcome. Of the five deaths (three intrauterine & two neonatal) and thirteen newborns requiring post natal transfusions, all showed very high levels of maternal anti-D titres (Table 2). Besides, risk factors like multiparity, advanced age of the mother and hydrops fetalis which contributed to fetal anemia, a high value of antenatal maternal serum IAT titre was found to be the most important risk factor with respect to fetal and neonatal outcome. This study has shown the direct association of antenatal IAT titre with the severity of fetal and neonatal anemia. There have been no other similar studies conducted to emphasize the importance of maternal IAT titre to predict the fetal outcome.

Maternal isoimmunization promotes the passage of IgG type antibodies across the placenta. These antibodies

reach the fetal circulation and cause progressive hemolysis and severe anemia in the fetus leading to heart failure and effusion of fluid into body cavities (fetal hydrops). If no measures are taken to correct the anemia, progression to death is inevitable [14, 15]. Management of fetal anemia is not possible without ultrasound monitoring and ultrasound guided intrauterine fetal blood transfusion. It is however a procedure requiring a lot of skill and precision, with a considerable risk of procedure related complications and volume overload. Hence it is important to determine the volume of blood to be transfused very carefully. Perinatal survival after intrauterine transfusion varies by center and the experience of the operator. In one review series, overall survival was noted to be 84 %. Survival of nonhydropic fetuses (92 %) was markedly improved over those with hydrops (70 %). Kamp et al. reported that when intrauterine reversal of hydrops occurs as a result of the treatment, 98 % of fetuses survived [16].

In conclusion, severity and fetal outcome in Rh isoimmunized pregnancies, has got a significant association with antenatal maternal serum IAT titre. More the antibody titre more would be the fetal and/or neonatal severity with respect to immune hemolytic anemia. Requirement of

multiple IUTs are also associated with high antenatal serum IAT titre.

Conflict of interest None.

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