

Enhancement of Antibody Titre and Development of Additional Red Cell Alloantibodies Following Intrauterine Transfusion

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Received: 21 August 2013 / Accepted: 28 October 2013 / Published online: 6 December 2013
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Abstract Intrauterine blood transfusion is the mainstay of managing foetuses with severe anemia. It may however result in fetomaternal hemorrhage, which in cases of Rh isoimmunisation may increase the severity of the disease by enhancing the maternal immunological response to fetal antigens. This study was conducted to determine the frequency, specificity and origin of additional red cell antibodies which developed after IUT. The change in the titre of allo anti-D following IUT was also determined. Antibody detection and titration was done on the blood samples of all the patients before and after intrauterine blood transfusion to check for the development of additional antibody and change in the titre of existing anti-D. Severe anemia was found in 17 (58.6 %) fetuses who received a total of 42 ultrasound-guided IUTs. Development of antibodies additional to anti-D in maternal serum was seen in 5 (29.4 %) cases. The specificity of additional alloantibodies was anti-C in four cases whereas it was anti-E in one case. Four fold or greater increase in existing allo-anti D titre was seen in 6 (35.3 %) cases after IUT. Enhancement of maternal sensitisation leading to an increase in maternal antibody titre is particularly seen after the first IUT. Matching of the donor RBCs particularly for Rh antigens

might prevent the induction of additional alloantibodies against these antigens. IUT as a treatment modality should be given judiciously and only when the need is inevitable.

Keywords Intra uterine transfusion · Fetomaternal hemorrhage · Anti-D · Antibody titre

Introduction

Administration of intrauterine transfusion (IUT) is the mainstay of treating fetal anemia in pregnancies which are complicated by alloimmunization to red cell antigens. Transplacental ultrasound guided IUT may result in small amount of fetomaternal hemorrhage (FMH) and transfused donor red cells may also enter the maternal circulation. Women whose fetus has undergone IUT treatment are high alloresponders to RBC antigens [1] Thus, the maternal immunological response to fetal red cell antigens leads to increase in the existing antibody titre. Exposure to donor antigens also causes induction of additional allo-antibodies against red cell antigens. The development of these antibodies causes problems in obtaining compatible packed red blood cells (PRBC) for further fetal transfusions and has a potential of causing delayed hemolytic transfusion reactions. In addition, increased antibody titre may worsen the disease in future pregnancies and accelerate the destruction of fetal RBCs in present pregnancy. Approximately 90 % of all severe hemolytic disease of fetus and new born (HDFN) in need of IUT treatment is caused by anti-D [2]. This study was designed to determine the frequency, specificity and origin of additional red cell antibodies which developed after IUT in patients alloimmunized by anti-D. The change in the titre of allo anti-D following IUT was also determined.

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Table 1 List of patients who developed additional antibody after IUT

Case	Gravida	History of blood transfusion	Total no. of IUT given	No. of IUT after which additional antibody developed	Specificity of additional antibody	Probable source of antigen
1 ^a	2	No	3	1	C	Fetus
2	3	Yes	2	2	E	Donor
3	2	No	4	3	C	Fetus
4	4	No	2	1	C	Fetus/donor
5	1	No	4	1	C	Donor

^a Same in Table 2, case 1

Materials and Methods

Blood samples of all antenatal women referred to the department of Maternal and reproductive health at our institute were sent to the department of Transfusion Medicine for antibody screening, as a part of routine antenatal follow up protocol. If a sample was positive on antibody screening using three cell panel (ID-DiaCell Bio-Rad, DiaMed GmbH, Switzerland), antibody identification was performed using 11 cell panel (ID-DiaPanel Bio-Rad, DiaMed GmbH, Switzerland). Further antibody titration was done using R₁R₁ cells by conventional test tube technique. Foetuses were monitored by ultrasonography and middle cerebral artery-peak systolic velocity (MCA-PSV). Those with features of severe HDFN received ultrasound-guided IUTs of PRBC from unrelated donors after crossmatching. The specificity and titre of red cell allo-antibodies in maternal serum was determined after each IUT on a biweekly basis. If additional allo-antibodies, not existing earlier in maternal serum, were detected, their origin was determined by phenotyping the fetal, donor, and paternal RBCs for minor clinically significant antigens (Rh, Kell, Kidd, Duffy, Lewis & MNSs).

Results

During a period of 6 months, antibody screening was performed on samples of 127 women out of which 29 (22.8 %) were found to have anti-D alloimmunization. Severe HDFN was found in 17 (58.6 %) fetuses who received a total of 42 ultrasound-guided IUTs (Mean 2.4 IUTs per patient). Development of antibodies additional to anti-D in maternal serum was seen in 5 (29.4 %) cases (Table 1). Mean number of IUTs received by each patient were three. The source of the immunizing antigen was donor in two cases and fetus in other two cases. The fetus and the donor shared the immunizing antigen in one case. The specificity of additional alloantibodies was anti-C in four cases whereas it was anti-E in one case. Out of all 17 cases, four fold or greater increase in existing allo-anti D titre was seen in 6 (35.3 %) after IUT (Table 2). Mean

Table 2 List of patients whose anti-D titre increased after IUT

Case	Gravida	History of blood transfusion	Total no. of IUT given	No. of IUT after which antibody titre increased	Initial titre	Final titre
1 ^a	2	No	3	1	64	256
2	1	No	2	1	128	512
3	2	No	3	2	64	512
4	3	No	2	1	256	1,024
5	1	No	4	3	128	1,024
6	2	No	3	1	64	256

^a Same in Table 1, case 1

number of IUTs received by each patient were 2.8. In one case (Case no.1 of Tables 1 and 2), there was development of anti-C antibody as well as fourfold increase in antibody titre. Overall, 10 (58.8 %) cases were found to have adverse serological outcomes after IUT.

Discussion

Invasive obstetric procedures may result in fetomaternal haemorrhage, which in cases of Rh isoimmunisation may increase the severity of the disease by enhancing the maternal immunological response to fetal red cell antigens. This study was conducted to determine the frequency and origin of additional alloantibodies directed against red cells and change in titre of existing anti-D after IUT.

In the present study, 5 (29.4 %) out of 17 women developed additional alloantibodies after IUT. In a study done by Watson et al. [3], 23 % patients with anti-D developed additional antibodies after IUT. A study from Germany [4] has reported the incidence of secondarily induced antibodies detected after the onset of IUT to be 33 %. This group had a significantly higher rate of transplacental punctures than the control group which was considered to be causative for the secondary sensitization. In another study from Germany [5], 19 % incidence of additional antibodies has been reported. The authors

concluded that many of these patients were ‘high responders’. Therefore, fetomaternal transplacental hemorrhage induced by invasive intrauterine examination methods and transfusions was considered as the main cause.

In 2/5 (40 %) cases, the additional antibody was directed against fetal antigens. Both of these women had prior pregnancies and none had a history of blood transfusion. In addition, their partners were positive for the corresponding antigen as well. Allo-immunization could therefore have occurred during previous pregnancies in these cases. Boosting of the pre-existing, originally undetectable alloantibodies would have been resulted from FMH after IUT. In two cases, additional alloantibodies were directed against donor RBCs. However, in only one case we could definitely attribute the production of new alloantibody as a primary response to antigens of the donor, since this patient had no history of blood transfusion and her husband was negative for the corresponding antigen. The other patient had history of blood transfusion, a few months back, which could not be ruled out as a sensitizing event. In one case, the corresponding antigen was shared by both fetus and donor, so the root cause of alloimmunization could not be deciphered.

O Rh(D) negative PRBC units issued for IUT were subjected to random cross matching with maternal serum sample and no matching for other (C,c, E,e) Rh antigens, was done with maternal red cells which may have contributed to the development of these antibodies against donor red cells. However, a study done by Schonewille et al. [6] to determine the antibody formation after introduction of preventive Rh and K matching of IUT donors showed no decline in the incidence. Twenty-five percent of the women formed new antibodies after Rh and K matched IUT and out of total, additional antibodies in 48 % cases were directed against Rhesus and K antigens induced by the fetus or as natural antibodies. The authors concluded that more extensive IUT donor red cell matching should be done to reduce the formation of new red cell antibodies.

Enhancement of maternal sensitisation results in an accelerated fall in fetal packed cell volume by an increase in maternal antibody titre, which is particularly seen after the first IUT when fetal erythropoiesis is generally not yet suppressed [7]. In the present study also, the increase in antibody titre occurred after first IUT in 4/6 (66.6 %) cases and additional antibodies developed after first IUT in 3/5 (60 %) cases. This is because, first and to a lesser extend a second IUT will lead to increased haemorrhage of fetal RBC, whereas in later IUTs the child’s erythropoiesis is completely suppressed and replaced by donor RBCs. A FMH of incompatible RBCs as is the case in the first IUT, to which the mother has strong antibodies, likely induces cytokine release that may enhance other immunizations [8,

9]. A previous study has also shown that women who already had allo anti-D and who subsequently produced an additional alloantibody after an IUT showed a significantly higher anti-D titre than those who had not produced an additional alloantibody [10]. In our study however, there was only one case (Case 1 in both Tables 1 and 2) in which there was development of anti-C antibody along with four-fold increase in anti-D titre. Subsequently, patient required two more episodes of IUT before her full term delivery.

In summary, IUT in alloimmunized women is associated with high incidence of adverse serological reactions in the form of increase in titre of existing antibody and additional antibody development. The determination of these additional alloantibodies is imperative for providing antigen negative blood in further intrauterine transfusions. Matching of the donor RBCs for Rh antigens, might prevent the induction of additional alloantibodies against these antigens. IUT as a treatment modality should be given judiciously and at latest possible gestational age before fetal anemia becomes severe.

Conflict of interest None.

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