

Alloimmunization due to red cell antibodies in Rhesus positive Omani Pregnant Women: Maternal and Perinatal outcome

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Abstract:

Objective: This study is aimed to determine the prevalence of alloimmunization due to antibodies to red blood cell (RBC) antigens (other than rhesus [Rh] antigen) and report the maternal, perinatal, and neonatal outcomes. **Materials and Methods:** A retrospective review of medical records of all patients with minor RBCs antibodies alloimmunization who were followed and delivered at Sultan Qaboos University Hospital, Oman from June 2011 to June 2013. Maternal characteristics, antibody type, antibody titer in addition to perinatal and neonatal outcomes were reviewed. **Results:** There were 1160 patients with Rh positive status in the study. The most common ABO blood group was O, followed by A, B, and AB. We found 33 out of 1160 Rh positive women alloimmunized with minor RBCs antibodies that gave a prevalence of minor RBCs alloimmunization of 2.7%. The most frequent antibody was anti-E 38%, followed by anti-c 17% and anti-kell 17%. 6 of these 33 patients were identified to have significant antibody titer, and two cases showed evidence of fetal anemia. Only one case required an intrauterine blood transfusion. The most common neonatal complication was jaundice in 53%, followed by respiratory distress syndrome in 28%. Two cases complicated by neonatal anemia required a postnatal blood transfusion. **Conclusion:** Alloimmunization with anti-E, anti-c, and anti-kell were the most common antibodies among the study group. Minor RBCs alloimmunization was an important cause of neonatal morbidity.

Key words:

Alloimmunization, perinatal medicine, red cell antibodies, transfusion

Introduction

Hemolytic disease of the fetus and newborn (HDFN) due to alloimmunization is a result of the transfer of IgG1 or IgG3 through the placenta from the mother to the fetus.^[1,2] Mixing of blood between the mother and fetus can occur due to miscarriages, ectopic pregnancy, ante-partum bleeding. It can also occur due to procedures like amniocentesis/cordocentesis or due to external factors like, abdominal trauma. The mother may also get sensitized due to blood transfusion during pregnancy^[2] and it is the most common risk factor for alloimmunization.

Anti-D remains the strongest antibody that causes HDFN.^[1,3,4] However, the widespread use of rhesus (Rh)-D immunoglobulin - "rhogam" - as a protection against anti-D alloimmunization has led to an increasing prevalence of alloimmunization due to other red blood cell (RBCs) antibodies.^[5-8] These antibodies are classified into 29 systems like kell (K, k, Js^a, Js^b), Rh (C, c, Cw, E, e), Duffy (Fy^a, Fy^b), and Kidd (Jk^a, Jk^b)... etc., collectively known as minor RBCs antibodies.^[9] From these antibodies, anti-kell is known to be associated with severe anemia, hydrops fetalis, and fetal death.^[10] Moreover,

anti-kell antibody can cause severe anemia regardless of antibody titer because it has ability to cause bone marrow suppression along with RBC destruction.^[10] Following anti-kell, anti-c and to a lesser extent other Rh antibodies (C, Cw, E, e) causes severe HDFN in a vast majority of cases.^[4]

The management of minor RBCs alloimmunized pregnant women is similar to anti-D alloimmunization. Since anti-kell has the ability to cause bone marrow suppression in addition to RBC hemolysis at any titer serial middle cerebral artery peak systolic velocity of blood flow (MCA-PSV) Doppler should be performed regardless of antibody titer done.^[10]

Materials and Methods

This study was a retrospective review of electronic medical records of pregnant women who attended the obstetrics and gynecology department in Sultan Qaboos University Hospital (SQUH) between June 2011 and June 2013. They were tested for blood group, Rh factor and antibody screen in their first visit as part of their routine antenatal care. The study group included all women registered and delivered in the obstetrics and gynecology department in SQUH.

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Data were collected based on the blood group (ABO) and the Rh factor to determine the frequency of each blood group and estimate the prevalence of Rh-positive blood group among the study population. Patients who had positive Rh factor were further screened for the presence of minor RBC antibodies like: anti-K, anti-c, anti-C, anti-E and anti-Fy^a... etc. In women who were tested positive for these antibodies, antibody titer was performed (the antibody detection was performed by the BIORAD® ID-System Gel cards to evaluate the severity of their cases. The antibody titer of 1:16 or more was considered as critical titer except anti-kell.^[11]

Though 1 in 16 is usually used for anti-D antibodies, we used it for some of the other antibodies as well with close clinical monitoring of the fetus. High-risk patients who had critical antibody titer were followed up by serial MCA Doppler every 1-2 weeks. Patients who showed high MCA Doppler were treated either by fetal intrauterine blood transfusion or delivery depending on fetal status and gestational age. Data was collected regarding maternal age, gravidity, parity, previous miscarriage, previous blood transfusions, type of minor RBC antibodies and their titer, obstetric and medical history, gestational age at critical titer, gestational age at first MCA Doppler and gestational age at which MCA Doppler showed evidence of fetal anemia.

Information regarding including intrauterine blood transfusion, fetal complications, gestational age at delivery, and mode of delivery, birth weight, Apgar score, hemoglobin and hematocrit at birth, neonatal complications, postnatal blood transfusion and neonatal death was also gathered from the medical records. The study was approved by the Research and Ethics Committee of the College of Medicine and Health Sciences.

Results

A total of 1251 pregnant women were included in the study of a period between June 2011 and June 2013. 1160/1251 (92.7%) of these women were Rh positive while the remaining 91/1251 (7.2%) had Rh negative blood group. The distribution of ABO blood group among Rh positive pregnant women is as shown in Table 1. Of 1160 pregnant women, 33 were alloimmunized with minor RBCs antibodies with a prevalence of 2.8%.

Types and frequency of minor red blood cell alloimmunization

Minor RBC antibody types among this group of population was as follows: 38% of women had anti-E, 17% cases with anti-kell and anti-c, 10% cases reported with anti-s, 7% cases with anti-C and anti-Jk^b, 3% case with anti-s, anti-Js^b, anti-le, anti-M, anti-IH, anti-Jk^a, anti-Fy^a, and anti-Fy^b, and 14% had nonspecific antibodies. Note that some women presented with multiple antibodies [Figure 1 and Table 2].

Maternal demographics

Reviewing the maternal demographic characteristics of 29 alloimmunized pregnant women (four women did not follow-up in our hospital) the mean maternal age was 31.2 years (range 24-40) years, 26/29 of pregnant women were multiparous whereas the remaining 3/29 (10.3%) were primigravida. Gravidity ranged between 1 and 11 and parity ranged between 0 and 8. Out of 29 pregnant women, 13 had a history of previous miscarriage with an abortion frequency ranged between 1 and 8 times. There was history of blood transfusion in 18/29 (62%) women, with the most

common indication as lower segment cesarean section (LSCS) in 8/18 (44%) of them and sickle cell disease (SCD) in 7/18 (39%) pregnant patients. Maternal characteristics are shown in Table 3.

Fetuses with significant titers

Six (20.7%) cases were diagnosed with significant alloimmunization because they had high titers. These patients were followed up in the obstetric department with serial MCA-PSV Doppler every 2-3 weeks. Two of them had MCA Doppler suggestive of fetal anemia. First patient was alloimmunized with anti-Js^b with a titer of (1:128) at 9 weeks gestation, and MCA-PSV Doppler was suggestive of fetal anemia at 20 weeks of gestation. This patient required intrauterine blood transfusion, which was done at a nearby tertiary hospital. This fetus was transfused 4 times with exchange transfusion at 1st time and simple transfusion 3 times, but the fourth intrauterine blood transfusion was complicated with cord hematoma needing emergency cesarean delivery at 29 of gestation. The second patient was alloimmunized with multiple antibodies, which were anti-kell, anti-c and anti-E. She was followed up with regular MCA-PSV Doppler which showed evidence of fetal anemia at 32 weeks of gestation. Unfortunately, this patient had severe SCD with vaso-occlusive crisis and needed delivery at 33 weeks gestation by emergency cesarean section. Both fetuses required postnatal blood transfusion and neonatal intensive care unit (NICU) admission. The neonate of first patient stayed at

Table 1: The distribution of different blood groups

Blood groups	Number of pregnant women	Percentage
O positive	615	49.1
A positive	299	23.9
B positive	185	14.8
AB positive	61	4.9

Table 2: Type and frequency of minor RBC alloimmunization

Name of antibodies	Frequency (%)
Anti-E	38
Anti-kell and anti-c	17
Anti-s	10
anti-C and anti-Jk ^b	7
anti-s, anti-Js ^b , anti-le, anti-M, anti-IH, anti-Jk ^a , anti-Fy ^a , and anti-Fy ^b	3
Nonspecific antibodies	14

RBC: Red blood cell

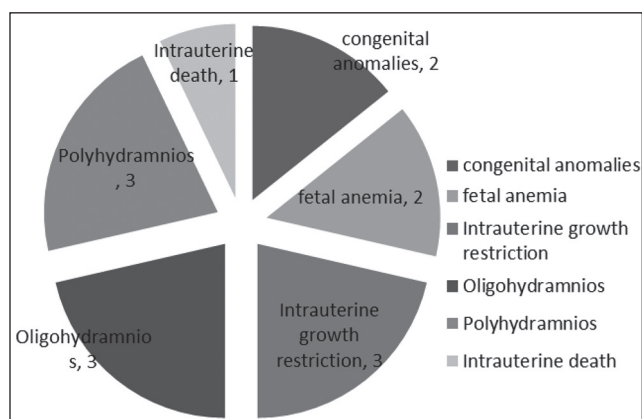


Figure 1: Fetal complications-some had more than one complication

NICU for 84 days while the neonate of the second patient stayed for 10 days. The other fetuses did well till near term.

Perinatal outcome

We evaluated the outcomes of 30 fetuses because one patient had twins. The perinatal outcomes in the study population was as follows: 2 fetuses out of 30 showed evidence of fetal anemia, 3 women had oligohydramnios, 3 polyhydramnios, 3 had intrauterine growth retardation, 1 congenital diaphragmatic hernia (CDH), 1 required intrauterine blood transfusion, and 1 died in utero due to congenital anomaly (omphalocele), (not included in further analysis), [Figure 2].

The mean gestational age at delivery was 36.5 weeks with a range of 29-40 weeks. 15/29 (51.7%) were delivered vaginally (spontaneous vaginal delivery [SVD]), while 14/29 (48.2%) had cesarean section. Table 4 provides information about gestational age at delivery and mode of delivery of these 29 patients.

Neonatal outcome

In reviewing the neonatal outcomes of alloimmunized pregnancies, one baby died due to CDH and one baby was stillborn due to congenital abnormalities. The neonatal birth weight ranged between (1.2 and 4 kg), the average weight was 2.8 kg and 32.1% of them had low birth weight. The neonatal outcome is shown in Table 5.

Neonatal complications

The most common neonatal complications were jaundice 51.7% followed by respiratory distress 27.5%. Figure 3 shows the neonatal complications of (some neonates had more than one complication).

Discussion

Among the Rh-negative pregnant women anti-D remains the most common cause of alloimmunization. The widespread use of protective anti-D immunoglobulin program against Rh-D alloimmunization has an impact of reducing alloimmunization due to anti-D and unmasking the risk of minor RBC antibodies alloimmunization, making them a significant cause of hydrops fetalis and hemolytic disease of fetus and newborn. Hence,

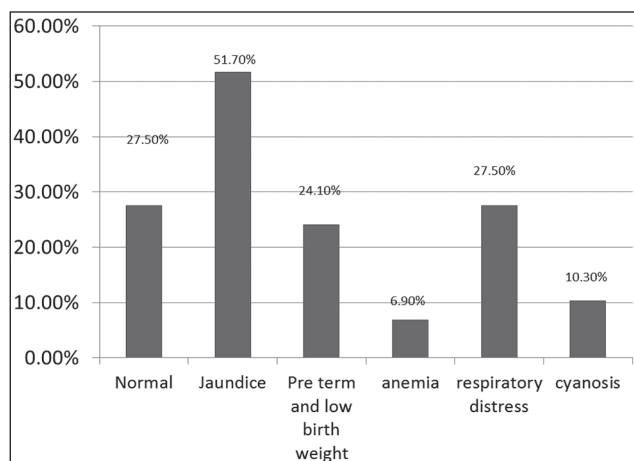


Figure 2: Neonatal complications of 29 (excluding the fetal death) with some neonates had more than one complication

determining the prevalence of minor antibodies to RBC antigens in Rh positive pregnant women is an important step in determining the magnitude of alloimmunization and the complications among Rh-positive pregnant women. The prevalence of Rh positive pregnant women in this study is similar to one in Saudi Arabia by Bondaji with a prevalence of 92.7%.^[12]

Table 3: Maternal characteristics of 29 pregnant patients who were included in this study

Mode of delivery	No (%)
Maternal age mean (range)	31.2 (24-40 years)
Gravidity, mean (range)	4 (1-11)
Multiparous women, n (%)	26 (89.7)
Primigravidae, n (%)	3 (10.3)
Parity mean (range)	2 (0-8)
Gestational age at first visit, mean (range) weeks	22+ (5-39)
History of previous miscarriage present, n (%)	13 (44.8)
No of miscarriages	
Once	7
Two times	2
Three times	3
Greater than three times	1
History of blood transfusion, n (%)	
Yes	18 (62.1)
No	4 (13.8)
Unknown	7 (24.13)
Indications for blood transfusion, n (%)	
Sickle cell disease	7 (38.9)
Beta thalassemia	1 (5.6)
Previous cesarean section (intra operative)	8 (44.4)
Postpartum anemia	2 (11.2)

Table 4: Mode of delivery of 29 pregnancies

Mode of delivery	No (%)
SVD	15 (51.7)
Elective LSCS*	9 (31)
Emergency LSCS*	5 (17.2)
Indications for elective cesarean section	
Breech presentation	1 (11.1)
Previous cesarean section	5 (55.6)
Intrauterine growth restriction	2 (22.2)
Placenta praevia	1 (11.1)
Indications for emergency cesarean section	
Non-reassuring fetal trace	2 (40)
Cord prolapse	1 (20)
Cord hematoma	1 (20)
Decrease fetal movement	1 (20)

*LSCS: Lower segment cesarean section; SVD: Spontaneous vaginal delivery

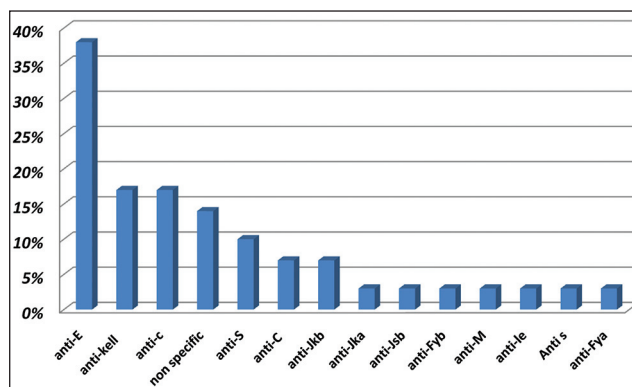


Figure 3: Frequency of various red cell antibodies

The prevalence of minor RBCs antibodies alloimmunization among Rh positive pregnant women is 2.8% in our study. This prevalence is high compared to a study done in Norway^[2] with a prevalence of 0.5% and a study done in India by Pahuja with a prevalence of 0.12%.^[13] The difference in prevalence in our study compared to other studies may be due to several factors, which are small sample size, all information obtained from one hospital mainly a tertiary hospital which receives high-risk population, a majority of our study population were SCD and thalassemics and all these factors increase the need for blood transfusion leading to an increase the risk of minor RBCs antibodies sensitization and alloimmunization. The distribution of minor red cell antibodies among study population is given in Table 2. These results are comparable to the literature.

Reviewing the history of the 29 pregnant women we found that 89.7% were multiparous while 10.3% were primigravidae and this was comparable to the results from Saudi Arabia by Bondagji^[12] History of previous miscarriage was found in 44.8% women, which is similar to that from Saudi Arabia,^[12] but less than one reported by Pahuja *et al.* from India.^[13] History of previous miscarriage has direct effects on the rate of alloimmunization either directly due to fetomaternal or indirectly by increasing the requirement of blood transfusion after miscarriage. From our analysis, we reported 18/29 (62%) pregnant women had a history of blood transfusion and the most frequent indication was previous LSCS followed by SCD, anemia in the postpartum period and thalassemia. From these results, we noticed that a blood transfusion was the most frequent cause for alloimmunization. Bondagji reported a history of blood transfusion in only 4.5% women, and this may be due to high-risk population received by SquH.^[12]

Description of high-risk cases

The high-risk patients' pregnancy course and the outcome are summarized in Table 6.

Table 5: Neonatal outcomes

Name of the parameter	Mean (range)
Birth weight (kg), mean (range)	2.8 (1.2-4)
Apgar score at 1 min, mean (range)	8 (1-9)
Apgar score at 5 min, mean (range)	9 (7-10)
Hb at birth, mean (range)	15 (9-19) g/dl
Hematocrit at birth, mean (range)	0.47 (0.28-0.58) l/l
Total duration in NICU, mean (range)	5 (0-84) days
Post-natal blood transfusion, n (%)	2 (6.7)
Congenital malformations, n (%)	2 (6.7)
IUFD	1 (3.3)
Neonatal death, n (%)	1 (3.3)

Hb: Hemoglobin; NICU: Neonatal intensive care unit; IUFD: Intrauterine fetal death

Table 6: Description of high risk cases

Type of antibody	Pregnancy course	Gestation at delivery	Neonatal outcome
Anti-Js ^b	Fetal anemia from 20 weeks pregnancy Mother sickle cell disease	29 weeks - cord hematoma	Alive and postnatal transfusion
Anti-C and anti-E	Uneventful	37 weeks - cesarean for previous cesarean	Alive and healthy
Are anti-E, anti-kell and anti-c	Fetal anemia 33 weeks Mother sickle cell disease	33 weeks - cesarean for fetal distress	Alive and healthy
Anti-kell, anti-Fy ^a , anti-Fy ^b and anti-E	Mother sickle cell disease	36 weeks emergency cesarean for fetal distress	Alive and healthy
Anti-s	Congenital diaphragmatic hernia of the fetus	37 weeks - cesarean for previous cesarean	Died after birth due to congenital anomalies
Anti-Jk ^b	Nil	39 weeks caesarean - for fetal distress	Alive and healthy

The first case was alloimmunized by anti-Js^b with a titer 1:128 at 9 weeks gestational age. The first MCA-PSV Doppler was performed at 16 weeks gestation, and at 20 weeks showed evidence of fetal anemia. Anti-Js^b belongs to kell antigen system and as described in literature these kell antibodies have the ability to suppress erythroid progenitor cells and cause RBC destruction. These two mechanisms make these antibodies a major contributor to HDFN and hydrops fetalis among minor RBC antibodies. In a study done in Columbus by McKenna, there were 9.3% cases reported with anti-kell alloimmunization of which 4/8 ended in fetal death, one of them died during intrauterine blood transfusion while others died secondary to alloimmunization complications.^[6] In the same study, 3/8 were reported with hydrops fetalis, one of them died at 25 weeks gestation, 1/8 fetus was delivered with hemoglobin of 11 g/dl and simple transfusion was done for him on the 1st day of life. In anti-kell immunization antibody titers are not necessarily correlated with fetal anemia. Our patient had a titer of 1:128 with evidence of fetal anemia, which started early in pregnancy. This patient also had severe SCD with a history of two previous pregnancies affected with anti-kell alloimmunization. Both these pregnancies were complicated by hydrops fetalis at 23 and 24 weeks gestation respectively. Our finding in this case was similar to McKenna *et al.*^[6] and it was observed from this case that the severity of the disease increased with increasing parity.

The second patient was alloimmunized with anti-C with a titer of 1:16 and anti-E with a titer of 1:64 at a gestational age of 36 weeks. Anti-C rarely causes serious clinical manifestations whereas anti-E is the third frequent antibody after anti-kell and anti-c in causing severe HDFN and hydrops fetalis. According to a study done in Columbus by Joy only 11.3% cases were reported with anti-E alloimmunization.^[14] Five of 32 (15%) fetuses had Hb <10 g/dL and one fetus had hydrops fetalis due to anti-E alloimmunization. The pregnancy course of our patient was uneventful with no obvious complications, and she was delivered at 37 weeks gestation by elective caesarean section due to previous caesarean section. The baby had a hemoglobin level of 13.9 g/dl with mild respiratory distress.

The third case was alloimmunized with multiple antibodies, which are anti-E, anti-kell and anti-c. Hackney *et al.* reported 8/46 pregnancies complicated with anti-C were treated with intrauterine blood transfusion with a frequency that ranged between 2-8 times.^[5] In our case, the mother was a primigravida with SCD. Evidence of fetal anemia was detected by MCA-PSV Doppler at 33 weeks gestation and due to critical maternal and fetal conditions the intrauterine blood transfusion was not done. Emergency cesarean section was done at 33 weeks due to nonreassuring fetal heart trace.

The fourth patient was a primigravida with SCD. She was alloimmunized with anti-kell with a titer of 1:1024, anti-Fy^a with a titer of 1:8, anti-Fy^b with a titer of 1:4 and finally anti-E with a titer of 1:4. Anti-Fy^b antibody is not associated with HDFN, while anti-Fy^a can end in severe HDFN and the severity correlates with antibody titer. In the study by Moise 18% of 19 pregnancies complicated with anti-Fy^a alloimmunization ended with fetal death and two-third of cases required exchange transfusion.^[8] This fetus was delivered at 36 weeks gestational age with emergency caesarean section due to decreased fetal movements and with a hemoglobin level of 14.4 g/dl at birth.

The fifth case was alloimmunized by anti-s with a titer of 1:32 at 17 weeks gestation. MCA-PSV Doppler was done for this patient at 33 weeks gestation and showed negative results for fetal anemia. According to a study done by Kenneth and Moise in Oxford region in England about 22/175,000 cases were reported with anti-s and only one case required exchange transfusion while the remaining 21/175,000 had only mild neonatal disease.^[8]

The last pregnancy was alloimmunized with anti-Jk^b with a titer of 1:128 at 39 weeks gestation. Anti-Jk^b related to Kidd antigen system, and it is rarely associated with hemolytic disease and severe complications as described in the literature. From these pregnancies, we reported 5/6 pregnancies delivered with emergency caesarean section, but only one of them due to alloimmunization complications while others due to other pregnancy issues not related to alloimmunization.

Among maternal complications one woman had preeclampsia, one had parvovirus infection, and the third had acute chest syndrome due to SCD. There were two fetuses with congenital anomalies, omphalocele, and CDH, the former died in utero at 24 weeks gestation. About 10% pregnancies complicated by polyhydramnios, 10% complicated by oligohydramnios, and another 10% had intrauterine growth restriction. None of these complications were seen among the six fetuses who were significantly alloimmunized.

Neonatal outcomes and complications

The most common neonatal complication was jaundice with a rate of 51.7%, the rate of jaundice among significantly alloimmunized pregnancies was 66.7%, which was higher than nonsignificantly alloimmunized pregnancies, 47.8%. Karagol *et al.* reported hyperbilirubinemia in 106 infants (about 1.5%) of neonates admitted to NICU in 6 years due to anti-kell, C, c, E and e antibodies.^[15]

There were 2 (7%) neonates with anemia in our study, with a hemoglobin and hematocrit level of 9 g/dl (0.355 l/l) and 10 g/dl (0.460 l/l) respectively. The first neonate was delivered from a pregnancy complicated by anti-Js^b antibody alloimmunization, while the second from a pregnancy complicated by multiple antibodies alloimmunization, which are anti-E, anti-Kell and anti-c. They both were preterm deliveries needing a postnatal transfusion and prolonged stay in the neonatal unit. This is much less compared to 22 (20.8) infants in the study by Karagol *et al.*

Conclusion

The prevalence of Rh-positive blood group among pregnant women in our study was 92.7%. The prevalence of minor RBCs

antibodies alloimmunization, in this study was 2.8% and it is higher than that described in some studies. Minor red cell antibodies were a significant cause of perinatal and neonatal morbidity.

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