Original Article

Hemolytic disease of fetus and newborn due to maternal red blood cell alloantibodies in the Malay population

Mohd Nazri Hassan, Noor Haslina Mohd Noor, Shah Reza Johan Noor[1], Salamah Ahmad Sukri, Rapiaah Mustafa, Hans Van Rostenberghe Luc Aster[2]

Departments of Haematology, [1]Obstetrics and Gynaecology, [2]Paediatric, School of Medical Sciences, Health Campus, Universiti Sains, Kubang Kerian, Kelantan, Malaysia

Abstract:

Background: Maternal red blood cell (RBC) alloimmunization may lead to production of harmful antibodies that result in hemolytic disease of fetus and newborn (HDFN). There is insufficient data on the prevalence of HDFN due to RBC alloantibodies in the Malay neonatal population. **Aim:** The aim of this study was to determine the incidence of HDFN in the Malay neonatal population due to clinically significant RBC alloantibodies. **Subjects and Methods:** A cross sectional study was conducted in Transfusion Medicine Unit, Hospital Universitiy Sains Malaysia over one year period from January to December 2009. A total of 5163 Malay pregnant women who attended labor room for delivery were collected and analyzed prospectively. The blood samples were subjected to the standard immunohematological procedure for RBC antibody screening and identification using reagents of Diamed-ID Gel microtyping system. All the newborns with RBC alloantibody were investigated for the evidence of HDFN. **Results:** Thirty (0.58%) women were found to have clinically significant RBC alloantibodies. Most of the alloantibodies belonged to Rhesus (Rh) system (56.7%) where anti-E (33.3%) was the most common followed by anti-D (10.0%). Rh antibodies were the main cause of HDFN in fourteen (0.27%) neonates. Anti-D and anti-c were identified to cause moderate to very severe HDFN. **Conclusions:** With the low prevalence of clinically significant RBC alloantibodies and HDFN, routine antenatal antibody screening practice may not be advised as a routine practice at present, preferably reserved for those women of RhD negative or with history of HDFN, significantly of those attributed to anti-c.

Key words:

Clinically significant alloantibodies, HDFN, Malay

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Correspondence to:
Dr. Mohd Nazri Hassan,
Department of Haematology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
E-mail: nazrihas@yahoo.com

Introduction

Red blood cell (RBC) alloimmunization may develop during pregnancy or from previous blood transfusion.[1] During pregnancy, maternal alloimmunization may lead to production of antibodies that result in HDFN. The destruction of the fetus and newborn RBCs occur commonly due to ABO incompatibility or other RBC alloantibodies, which are clinically significant especially antibodies toward Rh blood group systems.[2] A clinically significant RBC alloantibody is defined as an antibody that is capable to accelerate destruction of RBCs bearing the corresponding antigen.[3]

Hemolytic disease of fetus and newborn is defined as a condition in which the lifespan of the fetal or neonatal RBCs is shortened due to maternal alloantibodies against RBC antigens inherited from the father.[2] and has been known for a long time as a major cause of neonatal morbidity and mortality. More than 50 different RBC antigens have been reported to be associated with HDFN.[4] However, these RBC antigens and alloantibodies are significantly different between different populations and ethnic groups.[1] The effect on the fetus or newborn infant also may vary according to the characteristics of the maternal RBC alloantibody.[2] Severe HDFN may cause fetal death or can result in hydrops and jaundice, leading to kernicterus and permanent cerebral damage or infant death, while the only clinical sign of mild HDFN is mild neonatal jaundice which is often treated with phototherapy alone.[5,6]

Guidelines for blood grouping, RBC antibody testing, and prevention and management of RBC alloantibodies during pregnancy are well-established in Caucasian populations.[7] Applicability of these guidelines to Malaysian population is unknown as a result of insufficient data on the prevalence of maternal RBC alloantibodies and HDFN due to maternal RBC alloantibodies in the Malay population. The aim of this study was to determine the incidence of HDFN in the Malay neonatal population due to clinically significant RBC alloantibodies.

We hope that this study will contribute to a better comprehension of the problem and will help in the

management of maternal alloimmunization during pregnancy and subsequently can predict and treat HDFN efficiently to reduce neonatal morbidity and mortality.

Subjects and Methods

This cross-sectional study was conducted over one year period from January to December 2009 at Transfusion Medicine Unit, Hospital Universiti Sains Malaysia (HUSM). The study was approved by the hospital's ethical committee, School of Medicine Science, Universiti Sains Malaysia (USM).

All Malay pregnant women who were admitted to HUSM labor room for delivery during study period were included in this study. The newborns of mothers with positive antibody screening were followed up for one week for jaundice and if present were included in the study. The Malay pregnant women with autoantibody were excluded from this study.

The venous whole blood in ethylene diamine tetra acetate (EDTA) container was taken from the mothers and analyzed for ABO and RhD grouping, and antibody screening. Those with positive antibody screen were analyzed further for antibody identification to determine the antibody specificity. Antibody screening was performed using three-cell screening panel at 37°C by saline indirect antiglobulin test (IAT) and the antibody identification was performed using the eleven-cell panel. The entire testing was done using commercial cell panel by microcolumn gel agglutination method from Diamed-ID micro typing system (Diamed AG, 1785 Cressier, S/Morat, Switzerland).

The venous whole blood sample from the newborns was tested for full blood picture (FBP), direct Coombs test (DCT) and ABO and RhD grouping and serum bilirubin. The severity of HDFN was defined according to criteria developed for HDFN caused by anti-D;[8]

1. Mild cases were positive for DCT and required no transfusion;
2. Moderate cases were DCT positive with cord blood Hb >11 g/dL and required transfusion;
3. Severe cases were DCT-positive with cord blood Hb <11 g/dL and required transfusion; and
4. Very severe cases were DCT-positive with cord blood Hb <7.5 g/dL, or hydropic.

All analysis was performed using Statistical Package for the Social Sciences software (SPSS) version 12.0.1 for Windows. Descriptive statistics and Pearson chi-square statistical test was performed and a *P* value of less than 0.05 was considered significant.

Results

A total of 5163 Malay pregnant women were recruited in this study. Fifty one (0.99%) patients were found to have positive antibody screening and on further characterization of specificity, 30 (0.58%) of them were found to possess single or multiple clinically significant alloantibodies, 12 had clinically insignificant antibodies (anti-Le^a, -Le^b or both) and remaining 9 had antibodies with no specificity. Most of the clinically significant alloantibodies belonged to Rhesus (Rh) system (56.7%). The most common antibody in this group was anti-E (33.33%) followed by anti-D (10.00%) and anti-c,anti-E (6.67%). Specificity of clinically significant RBC alloantibodies is summarized in Table 1.

Among 30 newborns of women who possess clinically significant RBC alloantibodies, 14 newborns were considered to have HDFN clinically,and one of them was hydrops fetalis [Table 2]. Only six newborns had positive DCT in which three cases were due to anti-D, one due to anti-c, and two cases due to multiple antibodies which were anti-E, anti-c and anti-K, anti-Jk^b. Majority of the newborns developed only mild jaundice. The other 16 newborns did not develop jaundice within seven days of life. There was no significant association between development of HDFN and type of clinically significant alloantibody (Rh and non-Rh).

All the 14 newborns were admitted and given phototherapy alone or combined with intravenous immunoglobulin (IVIg) and exchange transfusion (ET). In cases of HDFN due to anti-D and anti-K, anti-Jk^b, all the newborns required IVIg infusion and intensive phototherapy (double phototherapy). The bilirubin levels were controlled by treatment and ET was not required,except for one newborn with HDFN due to anti-D who required packed cells (PC) transfusion for anemia (Hb 11.6 g/dl). The newborn with anti-E, anti-c related HDFN required IVIg infusion, intensive phototherapy, and ET. One woman with anti-c, delivered a baby with hydrops fetalis and the baby expired on the same day due to severe anemia (Hb 2.2 g/dl) and heart failure.

Discussion

The data on the incidence of HDFN due to clinically significant RBC alloantibodies is virtually unknown in the Malaysian population especially Malays. From this study the prevalence of HDFN due to clinically significant RBC alloantibodies in Malay women was found to be only 0.27% and was considered low. This result was similar to the reported prevalence in Hong Kong and Dutch pregnant women, which accounted to approximately 0.2%

Table 1: Specificity of clinically significant antibody identified in the Malay pregnant women

Antigen group	Antibody specificity	Number of patients (%)	Neonatal outcomes	
			Neonatal jaundice	Hydrops fetalis
Rhesus	Anti-D	3 (10.00)	3	
	Anti-E	10 (33.33)	3	
	Anti-c	1 (3.33)		1
	Anti-C^w	1 (3.33)	0	
	Anti-c,-E	2 (6.67)	2	
	Total	17 (56.7)		
Non-Rhesus Kell	Anti-K	2 (6.7)	2	
Kidd	Anti-Jk^a	1 (3.33)	0	
	Anti-Jk^b	2 (6.67)	0	
	Total	3 (10.0)		
MNS	Anti-M	2 (6.67)	0	
	Anti-S	2 (6.67)	1	
	Total	4 (13.3)		
Duffy	Anti-Fy^b	1 (3.3)	0	
Lutheran	Anti-Lu^a	1 (3.3)	0	
Multiple	Anti-k,-Lu^b	1 (3.33)	1	
	Anti-K,-Jk^b	1 (3.33)	1	
	Total	2 (6.7)		
Total		30 (100)	13	1

Table 2: Clinical data of 14 neonates with HDFN

Clinical data of mother				Clinical data of neonate						Severity of HDFN
Age (years)	Gravidity	ABO RhD	Antibody specificity	ABO RhD	Initial Hb level (g/dl)	Day of jaundice developed	Peak Br, mmol/l (D)	DCT	Treatment	
21	G_3P_2	B -ve	Anti-D	B+ve	15.6	D1	121 (D1)	IgG 3+	IVIg, Double photo	mild
29	G_2P_1	O -ve	Anti-D	O+ve	16.7	D1	250 (D2)	IgG 2+	IVIg, Double photo	mild
42	G_9P_{7+1}	O -ve	Anti-D	O+ve	11.6	D1	266 (D5)	IgG 3+	IVIg, Double photo, PC transfusion	moderate
21	G_1P_0	O+ve	Anti-K	A+ve	16.5	D2	106 (D2)	–ve	Single photo	mild
25	G_1P_0	O+ve	Anti-K	A+ve	12.6	D2	254 (D2)	–ve	Single photo	mild
33	G_9P_8	O+ve	Anti-E	O+ve	19.1	D2	214 (D2)	–ve	Single photo	mild
35	G_7P_{4+2}	B+ve	Anti-E	B+ve	17.0	D1	136 (D1)	–ve	Single photo	mild
34	G_5P_4	AB+ve	Anti-E	A+ve	17.8	D2	176 (D3)	–ve	Single photo	mild
42	$G_{10}P_9$	A+ve	Anti-S	AB+ve	16.4	D2	278 (D3)	–ve	Single photo	mild
39	$G_{10}P_{8+1}$	O+ve	Anti-c	B+ve	2.2	Hydrops fetalis	31(D1)	IgG 4+	Expired	very severe
46	$G_{17}P_{13+3}$	A+ve	Anti-E,-c	A+ve	20.1	D2	188 (D2)	–ve	Single photo	mild
32	G_2P_1	AB+ve	Anti-E,-c	B+ve	14.9	D1, prem	192 (D1)	IgG 2+	IVIg, Double photo, ET	moderate
29	G_2P_{0+1}	O+ve	Anti-k,-Lu^b	A+ve	16.6	D2	241 (D2)	–ve	Single photo	mild
31	G_4P_{2+1}	O+ve	Anti-K,-Jk^b	O+ve	16.7	D1	326 (D1)	IgG 1+	IVIg Double photo	mild

Br: Bilirubin; D: Day; Photo: Photothera; PC: Packed cell;+ve: Positive; –ve: Negative prem: Premature

and 0.25%, respectively.[9,10] However, our result was relatively higher when compared to the prevalence in Chinese population in Taiwan which was only 0.01%.[11]

We observed that the most common antibody that led to HDFN was anti-E, either alone (three newborns) or with other antibody (two newborns). Other Rh antibodies that were identified were anti-D and anti-c. Anti-E and anti-c were found mostly in women who were RhD positive and lack the c and E antigens.[12] Majority of Malay blood donors were found to express R_1R_1 (CDe/CDe) Rh genotypes[13] and thus the occurrence of anti-E and anti-c was expected. Majority of the mothers in this study developed anti-E, clinical manifestations of anti-E alloimmunization were found to be less severe, as the newborns only had mild HDFN and required single phototherapy. These findings support the evidence that E antigen is a less potent immunogen[6-14] and often being a naturally occurring antibody, it seldom causes HDFN.[15] The DCT of all the newborns was negative. Anti-E alloimmunization usually occur in a low titer and the low titer of antibody could explain negative DCT.[4]

Three HDFN cases caused by anti-D alloimmunization indicate that RhD alloimmunization still occurred despite application of both antenatal and postpartum anti-D Ig prophylaxis in Malay population. From this study, we observed that there was were four reasons for the failure to prevent RhD alloimmunization;

1. Already immunized women subsequently becoming pregnant,
2. Failure to administer an antenatal dose of anti-D Ig at 28-29 weeks of gestation,
3. Failure to recognize clinical events that placed patient at risk for alloimmunization and administer anti-D Ig appropriately, and
4. Failure to administer timely anti-D Ig postnatally.

All the newborns with anti-D alloimmunization were RhD positive and born alive; however, required intensive treatment including IVIg and intensive phototherapy although were graded as mild to moderate HDFN. One of them required packed cells (PC) transfusion because of anemia (Hb 11.6 g/dl). These findings were consistent with the known clinical manifestations of anti-D alloimmunization where it can range from asymptomatic mild anemia to hydrops fetalis or stillbirth associated with severe anemia and jaundice.[2-16] It is because most of the antigens especially D is highly immunogenic if compared to other RBC antigens.[2-16] All the newborns had positive DCT with IgG monospecificity with variable strength of reaction. However, the strength of the DCT reaction did not correlate well with the severity of the HDFN.[2-17] Previous study reported that a positive DCT might develop by 8th week of gestation, and severe anemia and death in utero might occur as early as about the 18th week of gestation.[15] In this study, all three newborns with anti-D related HDFN were successfully treated with IVIg and intensive phototherapy with no ET required. As bilirubin levels can rise sharply after birth, prompt and intensive phototherapy was suggested to be started immediately after birth, which thus might prevent the need for ET.[18] IVIg also had been reported to be used as an alternative treatment for HDFN and found successful to reduce the need for ET, as well as the length of phototherapy and hospitalization.[16-19] We found that the frequency of anti-c with or without anti-E among Malay pregnant women was very low. Similar result had been shown in previous study where only 17 out of 21730 pregnant women in Yugoslavia developed anti-c[2] and whereas only five out of 21327 Chinese pregnant women possessed anti-c.[20]

We reported that newborns of mother with anti-c alone and with a combination of anti-c,-E developed HDFN. It was reported that anti-c associated HDFN was infrequent, because majority of infants were relatively often c-negative, however, if occurred could cause severe HDFN.[4-21] It was shown in this study where the newborn from anti-c alloimmunized woman delivered a hydropic infant that expired due to heart failure and severe anemia (Hb 2.2 g/dl), and one of newborn of anti-c,-E alloimmunized women delivered prematurely and required intensive treatment including IVIg,

intensive phototherapy and ET. Both of the newborns had positive DCT. These findings supported the fact that anti-c can cause severe HDFN which might lead to neonatal death as reported in Chinese population that all four of HDFN due to anti-c required ET but they all are alive.[10] While in USA, it was reported that 46 out of 655 pregnancies with anti-c had a positive DCT and eight of the affected newborns had HDFN requiring fetal transfusion.[19]

The clinically significant alloantibodies other than Rh that were identified were antibodies against Kell, Kidd, Duffy, and MNS antigen system. Anti-K alone or with other antibody was identified in three mothers. We found that all newborns from the K-alloimmunized mothers developed mild jaundice. One of the newborns had positive DCT, required IVIg and intensive phototherapy. Another two newborns had negative DCT and only required single phototherapy. We did not observe fetal anemia in anti-K HDFN, except in one newborn who had anemia with Hb level of 12.6 g/dl but did not require PC transfusion. Anti-K has been a known cause of severe HDFN and it differs from Rh, in that anti-K appears to cause fetal anemia by suppression of erythropoiesis, rather than immune destruction of mature fetal RBC.[22,23] Irrespective of the titer, it is enough for the anti-K to be present to allow close observation of the mother and newborn, unlike the other RBC alloantibodies.[8]

Anti-k is a very rare antibody and it is always immune and has been incriminated in some cases of mild HDFN.[22] In Malay population, 99.5% blood donors' RBC had kk phenotype.[13] It supported that probably majority of pregnant women were also kk phenotype in which k alloimmunization would not occur since they possess that antigen. It was consistent with this study where we found only one case of mother with anti-k where her newborn developed mild jaundice and was treated with single phototherapy alone.

Anti-S, are usually immune, IgG and can cause HDFN.[2] We found only one pregnant woman with anti-S. The newborn developed jaundice but with negative DCT and required only single phototherapy. This result was consistent with a reported case in US that anti-S antibody causes mild HDFN.[24]

Conclusion

It is important to know that not all potentially clinically significant alloantibodies seen during pregnancy will cause HDFN, especially when the antibody stimulus is unrelated to the current pregnancy. For this reason, the antibody screen test should be restricted to women who are RhD negative, or who have past history of HDFN especially due to anti-c which might result in moderate to very severe HDFN. Hence, it is recommended that blood to be transfused to women planning for future pregnancies should be compatible not only with the D antigen status of the patient but also with other Rh antigens especially Rhc. The occurrence of anti-D alloimmunization is still seen nowadays despite of routine practice of giving prophylactic anti-D Ig antenatally and postnatally to all RhD negative women. Hence, prevention programme of RhD alloimmunization may be further enhanced by proper detection of RhD negative women and strict compliance to guidelines concerning determination of fetomaternal hemorrhage and accordingly by delivering adequate doses of anti-D Ig prophylaxis.

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