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ORIGINAL ARTICLE

Red Cell Antibody Screening in Pregnancy: A Preliminary Insight?

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ABSTRAK

Aloimunisasi sel darah merah ditakrifkan sebagai pembentukan antibodi di dalam respons kepada antigen asing sel merah melalui proses transfusi atau mengandung. Di kalangan wanita hamil yang tidak mempunyai sejarah transfusi darah, mereka boleh mengalami aloimunisasi pada kehamilan yang lalu atau semasa disebabkan oleh kehadiran antigen sel merah bapa yang diwarisi kepada janin. Kajian ini bertujuan untuk menentukan prevalens aloimunisasi sel darah merah di kalangan wanita hamil tanpa sejarah transfusi darah di PPUKM dan juga untuk mengaitkan kelazimannya dengan nombor kehamilan dan sejarah komplikasi obstetrik. Ini adalah satu kajian keratan rentas di mana 150 wanita hamil telah dipilih secara rawak di klinik antenatal PPUKM. Sepuluh ml darah periferal telah diperolehi selepas persetujuan diambil dari setiap pesakit. Ujian yang dijalankan ke atas sampel-sampel darah tersebut termasuk ujian jenis kumpulan darah ABO dan Rh D dan saringan antibodi dengan menggunakan teknik ujian antiglobulin tidak langsung. Sampel positif terus dikenalpasti untuk pengkhususan antibodi. Dalam kajian kami, majoriti (37.3%) daripada wanita adalah primigravida. Aloantibodi sel darah merah telah dikesan pada dua daripada 150 (1.3%) pesakit yang kemudiannya dikenalpasti sebagai anti-C dan anti-D. Namun tiada seorang pun daripada primigravida yang mengalami aloimunisasi. Seorang wanita dari gravida 2 (2.9%) dan gravida 3 (3.6%) didapati positif bagi aloimunisasi. Salah seorang daripada mereka juga mempunyai sejarah obstetrik buruk. Kajian ini menunjukkan bahawa kelaziman aloimunisasi sel darah merah di kalangan wanita hamil adalah rendah di pusat ini. Walau bagaimanapun, ujian saringan bagi aloantibodi sel darah merah perlu disediakan untuk mengurangkan komplikasi kepada janin atau ibu yang mungkin timbul akibat aloimunisasi sel darah merah.

Kata kunci: antibodi sel merah, aloimunisasi, saringan antibodi.

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ABSTRACT

Red cell alloimmunisation is defined as the development of antibodies in response to foreign red cell antigens through transfusion or pregnancy. In pregnant women even without the history of previous blood transfusion, this is possible through previous or current pregnancy with the presence of paternal red cell antigen inherited by the fetus. This study was aimed to determine the prevalence of red cell alloimmunisation among pregnant women without previous history of blood transfusion and the association with number of pregnancy and history of obstetric complications. This was a cross-sectional study in which 150 pregnant women were randomly selected from the antenatal clinic. Ten mls of peripheral blood was obtained for antibody screening using indirect antiglobulin test besides the routine antenatal screening. In this study, the majority (37.3%) of the women were primigravidae. Red cell alloantibodies were detected in two out of 150 (1.3%) patients which were subsequently identified as anti-C and anti-D. However none of the primigravida was alloimmunised. One woman of gravida 2 (2.9%) and gravida 3 (3.6%) each were positive for alloimmunisation. One of them also had a bad obstetric history. This study showed that the prevalence of red cell alloimmunisation among pregnant women was low in this centre. Nevertheless, red cell alloantibody screening test should be made available to reduce possible complications of alloimmunisation in mothers and fetuses.

Key words: red cell antibody, alloimmunisation, screening, pregnancy

INTRODUCTION

Red cell alloimmunisation in pregnancy is an immune disorder due to an incompatibility between maternal and fetal red blood cell antigens (Roback et al. 2008). Haemorrhage of fetal red cells into the maternal circulation can occur spontaneously during pregnancy or with trauma, amniocentesis, cordocentesis, abortion, and other manipulations.

The development of the alloantibody is due to the anamnestic response (Roback et al. 2008). With the first exposure to the foreign antigen, the B lymphocyte clones are invoked which will result in a moderate production of IgM or IgG antibodies. It is the secondary exposure that elicits a more rapid production of large quantities of significant IgG-class antibody which can cross the placenta. Destruction of the fetal red cells occurs when the maternal antibody binds to the fetal red cell antigen, causing attachment to the Fc receptor of the macrophages in the spleen of the fetus and leads to a condition called haemolytic disease of the fetus and newborn (HDFN) (Denise 2005 & Roback et al. 2008).The newborn infants with HDFN present with severe anaemia and jaundice. As an example, in a Rh-negative mother who is exposed to the fetal Rh-positive red cells, the maternal immune system will produce anti-D antibodies. In a

subsequent pregnancy, these anti-D antibodies will cross the placenta into the fetal circulation and result in haemolysis.

Approximately 1% of pregnant women are found to have clinically significant red cell antibodies and there are more than 50 red cell antigens that can elicit antibodies that have been found to cause HDFN (Howard et al. 1998 & Moise 2000). Of these, the commonest specificity is anti-D, although universal introduction of routine antenatal anti-D prophylaxis has reduced this sensitisation rate (Howard et al. 1998 & Moise 2000). However, with the introduction of antenatal anti-D prophylaxis, there has been a significant rise in positive antibody screening results, due to the detection of the prophylactic anti-D (Howard et al. 1998). Other alloimmune antibodies that can result in HDFN include other Rh (E, e, C, c), Kell (K and k), Duffy (Fya and Fyb), Kidd (Jka and Jkb), and MNSs (M, N, S and s) (Moise 2000 & Roback et al. 2008). Multiparous women and mothers with bad obstetric history (history of abortion, neonatal death and stillbirth) have been found to have higher chance of alloimmunisation due to multiple exposures to the incriminating foreign antigens (Patel et al. 2009 & Shanwell et al. 1999).

According to guidelines issued by the British Committee for Standards in Haematology, it has been suggested that ABO and Rh as well as the antibody screening be carried out during the antenatal follow up to detect the presence of maternal alloantibody. Therefore, this study aimed to look at the prevalence of development of significant alloantibodies in pregnant women in this centre and to identify the common alloantibodies encountered. The knowledge will help reduce the risk of complication of haemolytic disease of the fetus and newborn and further emphasise whether routine antibody screening during antenatal check-up is justified.

MATERIALS AND METHODS

This cross-sectional study was done on women who had antenatal follow-up at the antenatal clinic of a tertiary hospital. The study was approved by the Institutional Review Board and Ethics Committee. Women with previous history of blood transfusion, women with autoimmune hemolytic anaemia and those on antibiotics were excluded from the study. The patients were randomly selected during the period of study from January 2012 to April 2012. A total of 150 patients' blood samples were subjected to pre-transfusion testing which included ABO- and Rh-grouping and antibody screening. Data on age, gravida, parity, past medical and obstetrical history were taken. For each patient, 5 mls of blood was taken in two EDTA tubes. The pretransfusion testing were done following the American Association of Blood Bank standard which consists of identification of the recipient and blood sample collected, ABO and Rh D typing, and antibody screening. The antibody screening was done using indirect antiglobulin test, performed by gel agglutination technique using Diamed gel cards. For antibody screening, 3-cell screening panels (Diamed ID-Dia cell) were

used. The positive results were further identified for antibody specificity by the Diamed ID-Dia Panel (11 cell panel), including the use of enzyme treated cells (papain) at 37°C and anti human globulin (AHG) phase.

RESULTS

During the study period, only two patients were found to have positive antibody screening out of a total of 150 patients' samples, giving an incidence of 1.3%. The two positive samples were identified as anti-C and anti-D. Table 1 shows gravidity versus antibody formation. In our study, 56 samples were primigravidae (37.3%), 34 women were gravida 2 (22.6%), 28 women were gravida 3 (18.7%), 19 women were gravida 4 (12.7%) and 13 were of gravida 5 or more (8.7%). No alloimmunisation was found among primigravida, gravida 4 and gravida 5 or more. The two positive samples were from gravida 2 and gravida 3 with incidences of 2.9% and 3.6% respectively. The incidence of antibody production was less in normal cases (0.87%) than in bad obstetric history cases (2.86%) as expected (Table 2).

DISCUSSION

Red cell alloimmunisation can result in significant consequences to pregnancy outcomes. The production of clinically significant anti-D in an Rh negative mother has been well established in causing severe haemolytic disease of the fetus and newborn. However, with the current practice of pretransfusion testing which includes ABO- and Rh-grouping, the possible clinical problems associated with anti-D can be anticipated and abated early with the use of Rh_0(D) immunoglobulin or better known as Rhogam. Nevertheless, there are more than 50 red cell antigens which are able to elicit antibody production in HDFN cases (Howard et al. 1998 & Moise 2000).

Red cell alloimmunisation may also pose a significant problem in blood transfusion practice. It may result in difficulty in finding compatible blood for transfusion and could result in grave consequences especially during emergency. The frequency of patients being alloimmunised to non-D antibodies has been rising in the past decades (Lobo et al. 2006 & Yousuf et al. 2012). Another study, Yousuf et al. (2012) found that the prevalence of positive antibody screening among transfusion recipients was 0.76% (184/24,263) of pretransfusion samples. In this study, the most frequent single alloantibody was anti-Mia (29.1%, 48/165), followed by anti-E (18.6%, 30/165). Antibody screening, which has been implemented in many developed countries, help identify and specify antibodies in potential recipients and thus antigen-negative blood can be prepared in the event of blood transfusion.

In pregnancy, however, the incidence of red cell alloimmunisation is 1% to 2% (Howard et al. 1998; Moise 2000; Solola et al. 1983 & Weinstein 1982). Lee et al. (2003) reported the prevalence of clinically significant antibodies among Chinese pregnant women to be 0.27%. Different ethnic backgrounds may be a confounding factor that influences the

Table 1: Gravidity versus Antibody Formation

	Gravida					
	1	**2**	**3**	**4**	**5**	**Total**
Total cases	56	34	28	19	13	150
Antibody screening positive	0	1	1	0	0	2
Prevalence of antibody formation (%)	0	2.9	3.6	0	0	1.3

prevalence of alloantibody production in pregnant women. Our study which was done on a multiracial population, demonstrated a prevalence of 1.3% (2/150, one of chinese and one of malay ethnicity). Nevertheless, due to the small sample size, a bigger study is needed to improve the power of the study and to differentiate between ethnic groups in this country.

Red cell alloantibodies detected in two (1.3%) patients were identified as anti-C and anti-D from a gravida 2 (2.9%) and gravida 3 (3.6%) respectively. None of the primigravidae was alloimmunised. Each pregnancy increases a woman's risk of fetomaternal circulation leak, and therefore increases the chance of alloimmunisation.

With the same understanding, there would be less chance of antibody production in women with normal pregnancy than in those with bad obstetric history. Our data shows the incidence for alloimmunisation of 0.87% (1/115) in women with normal obstetric history and 2.86% (1/35) in those with bad obstetric history. In this study, the gravida 2 had a history of an early neonatal death and she was positive for anti-C. The other patient, who is a gravida 3 at 11 weeks gestation, is a Rh negative mother with a previous normal obstetric history. She was found to be positive for anti-D,

Table 2: Incidence of Antibody Formation between Normal and Bad Obstetric History

	Normal	**Bad Obst H(x)**
Total case	115	35
Positive Alloantibody	1	1
%	0.87	2.86

with a titre of 1:32 (2IU/ml), most likely from the Rhogam which was administered to her a few days earlier due to threatened miscarriage. Passive anti-D can be detected for 12 weeks or more after anti-D prophylaxis has been given (BCSH 2006). Nevertheless, in this case active immunisation still could not be excluded as it is difficult to differentiate anti-D immunoglobulin from an immune anti-D.

From the relatively small sample the prevalence for alloimmunisation of 1.3% among pregnant women in our centre was in accordance with other studies (Howard et al. 1998; Moise 2000; Solola et al. 1983 & Weinstein 1982). The antibodies detected were anti-C and anti-D, which belong to the Rh group and the incidence of red cell alloimmunisation were higher in women with bad obstetric history. Nevertheless, a bigger sample size over a longer study period is needed for better demonstration of the true prevalence.

CONCLUSION

The prevalence of red cell alloimmunisation among pregnant women in this centre is low, similar to other population studies. Nevertheless, it is important that red cell antibody screening be included as part of the antenatal pretransfusion testing in accordance with the current international guidelines (BCSH 2006), so that pregnancies at risk of HDFN can be identified and appropriate management provided. Where laboratory facilities are limited or cost is a factor, selective screening of the patients especially those with previous bad obstetric history and history of blood transfusion should be implemented.

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