Original Article

The prevalence of red cell antibodies among patients and pregnant women in Pakchongnana Hospital

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Abstract:

Background: *The antibody screening test is an essential component of pretransfusion testing before blood transfusion. Antibody identification must be performed to determine antibody specificity in case of positive antibody screening. Limited data are available on the prevalence of red cell antibodies among patients and pregnant women from lower northeastern Thailand. This study aimed to determine the prevalence of red cell antibodies among patients and pregnant women in Pakchongnana Hospital.* ***Materials and Methods:*** *Blood samples from 601 pregnant women and 2,488 patients were tested using antibody screening by standard tube technique (STT). Positive samples were determined for antibody specificity and analyzed according to sex, age groups, ABO and Rh types. Moreover, red cell genotyping was performed in samples with positive autocontrol.* ***Results:*** *The prevalence of antibodies among pregnant women and patients was 1.33% and 1.89%, respectively. The frequencies of positive red cell antibodies were significantly higher among patients ≥ 40 years ($p = 0.036$), belonging to a single antibody (57.4%), multiple antibodies (12.8%) and unidentified antibodies (29.8%). Anti-Mi^a was the most common in the two groups, followed by anti-P1, anti-E, anti-Le^a and anti-Le^b. Additionally, for 2 patients having anti-Mi^a, the predicted phenotypes were confirmed to be Mi(a-) using multiplex PCR.* ***Conclusion:*** *This is the first report of the prevalence of red cell antibodies among patients and pregnant women in Pakchongnana Hospital, which attempted to provide red cell antigens that, have the potential for alloantibody formation. Hence, the prompt management of donor blood units corresponding to this data could be applied to ensure safer blood transfusions.*

Keywords : ● *Red cell antibodies* ● *Patients* ● *Pregnant women*

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นิพนธ์ต้นฉบับ

ความชุกของแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยและหญิงตั้งครรภ์ ณ โรงพยาบาลปากช่องนานา

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บทคัดย่อ

ความเป็นมา *การตรวจกรองแอนติบอดีเป็นส่วนประกอบสำคัญของการตรวจความเข้ากันได้ก่อนการให้เลือด เมื่อพบผลบวกจำเป็นต้องตรวจแยกชนิดแอนติบอดีเพื่อระบุชนิด ข้อมูลความชุกของแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยและหญิงตั้งครรภ์ในประชากรไทยภาคตะวันออกเฉียงเหนือตอนล่างยังมีจำกัด การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาความชุกของแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยและหญิงตั้งครรภ์ในโรงพยาบาลปากช่องนานา* ***วัสดุและวิธีการ*** *ตัวอย่างเลือดหญิงตั้งครรภ์ 601 รายและผู้ป่วย 2,488 ราย นำมาตรวจกรองแอนติบอดีด้วยวิธีมาตรฐานหลอดทดลอง ตัวอย่างที่ให้ผลบวกได้ตรวจหาชนิดของแอนติบอดีและวิเคราะห์ผลร่วมกับข้อมูลเพศ อายุ และหมู่เลือด ABO/Rh นอกจากนี้ตัวอย่างเลือดที่ autocontrol ให้ผลบวกนำมาตรวจจีโนไทป์หมู่เลือดเพิ่มเติม* ***ผลการศึกษา*** *ความชุกของแอนติบอดีต่อเม็ดเลือดแดงในหญิงตั้งครรภ์และผู้ป่วยเท่ากับ 1.33% และ 1.89% ตามลำดับ ความถี่ของการตรวจพบแอนติบอดีในผู้ป่วยอายุ ≥ 40 ปีสูงกว่าอายุอื่นอย่างมีนัยสำคัญ ($p = 0.036$) ส่วนใหญ่เป็นแอนติบอดีชนิดเดียว (57.4%) แบบหลายชนิด (12.8%) และไม่สามารถแยกชนิดได้ (29.8%) แอนติบอดีที่พบบ่อยทั้งสองกลุ่มตัวอย่าง คือ anti-Mi^{a} รองลงมาคือ anti-P1, anti-E, anti-Le^{a} และ anti-Le^{b} นอกจากนี้ผู้ป่วย 2 รายที่มี anti-Mi^{a} ผล predicted phenotypes ด้วยวิธี multiplex PCR ยืนยันว่าเป็น Mi(a-)* ***สรุป*** *การศึกษานี้เป็นครั้งแรกที่รายงานความชุกของแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยและหญิงตั้งครรภ์ที่โรงพยาบาลปากช่องนานา ซึ่งได้ข้อมูลของแอนติบอดีที่ตรวจพบได้บ่อย ดังนั้นการบริหารจัดการอย่างทันท่วงทีในการจัดหาเลือดผู้บริจาคที่สอดคล้องกับข้อมูลดังกล่าวสามารถประยุกต์ใช้เพื่อให้การให้เลือดมีความปลอดภัยยิ่งขึ้น*

คำสำคัญ : ● *แอนติบอดีต่อเม็ดเลือดแดง* ● *ผู้ป่วย* ● *หญิงตั้งครรภ์*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2562;29:317-24.

Introduction

Apart from ABO grouping and Rh(D) typing, the antibody screening test is an essential component of pretransfusion testing before blood transfusion among patients.[1] According to the National Blood Centre, Thai Red Cross Society (NBC-TRCS) guidelines, a patient with negative antibody screening and receiving compatible crossmatch donor units can assure ABO and Rh(D) compatibilities between donor and patient blood as well as detect most clinically significant red cell alloantibodies that react with antigens on donor red cells.[2] Among patients with a positive antibody screening having a crossmatch with either compatible or incompatible results, antibody identification must be performed to determine antibody specificity and to provide donor blood that lacks the corresponding antigen to the patients.[1-3]

According to guidelines for screening all pregnant women for unexpected red cell antibodies, they should be ABO and D-antigen typed and screened for the presence of red cell antibodies early in pregnancy and by the 28-week mark.[4,5] Alloimmunization among pregnant women has been reported in different populations, ranging from 0.4% to 2.7%.[6] A related study among Thai pregnant women at Taksin Hospital revealed that the prevalence of red cell antibodies was 2.3%.[7] Women who have developed clinically significant alloantibodies having the potential to cause hemolytic disease of the fetus and newborn (HDFN) are monitored during the gestation period. These procedures will facilitate the prompt provision of compatible blood when required for the woman and/or for the baby.[4]

In Thailand, a report of an external quality assessment scheme in red blood cell serology revealed that antibody screening test had been performed in all participating blood bank laboratories; however, antibody identification was not routinely performed in all laboratories. The samples with positive antibody screening test results were sent to the NBC-TRCS for investigation and crossmatching leading to a longer time for those patients to be transfused.[8] Pakchongnana Hospital is the secondary care hospital with the availability and occupancy of 300 beds located at Pakchong District, Nakhon Ratchasima Province, northeastern Thailand. Moreover, limited data are available concerning the prevalence of red cell antibodies among patients and pregnant women from this province and its nearby provinces to provide appropriate blood management of patients, pregnant women and child births. This study aimed to determine the prevalence of red cell antibodies among patients and pregnant women in Pakchongnana Hospital.

Materials and Methods

Samples

Blood samples were obtained from 2,488 patients requiring a transfusion and 601 pregnant women at the Blood Bank Section, Pakchongnana Hospital, Nakhon Ratchasima, Thailand from January 2019 to August 2019, with the approval of the Committee on Human Rights Related to Research Involving Human Subjects (COA No.108/2562), Thammasat University, Pathumtani, Thailand. All patients comprised 914 males and 1,574 females (age range from 1 day to 104 years). For pregnant women, their ages ranged from 13 to 47 years. Data on sex, age and ABO blood groups of both groups were also collected.

Antibody screening test

All samples underwent antibody screening test using saline indirect antiglobulin test (IAT) by standard tube test (STT). Two commercial screening cells (O1 and O2, NBC-TRCS, Bangkok, Thailand) were used, including D, C, E, c, e, M, N, S, s, Mi^a, P1, Le^a, Le^b, Jk^a, Jk^b, Fy^a, Fy^b, K, k, Di^a, Di^b and Xg^a antigens. Antibody screening test by STT was initially performed using the saline IAT.[1] Briefly, two drops of each plasma sample were mixed with one drop of the screening cells and centrifuged at 3,500 rpm for 15 seconds. A positive immediate spin phase for agglutination and/or hemolysis was observed. All reactions were read

macroscopically. The results were graded and recorded. Then the tubes were incubated at 37°C for 30 minutes, centrifuged and observed for agglutination. Next, the red cells were washed three times with normal saline solution at 3,500 rpm, 45 seconds and completely decanted in the final washing step. Two drops of anti-human globulin reagent (CE-Immunodiagnostika GmbH, Germany) were added, mixed, centrifuged and observed for agglutination. Negative or weak agglutination reactions were examined under a microscope (×10). The grading of agglutination reactions were 4+, 3+, 2+, 1+, w+ and negative, according to standard guidelines and the validity of a negative test was confirmed by adding IgG-coated red cells.

Antibody identification

In the case of a positive antibody screening test result, antibody identification was performed using 11 panel cells (NBC-TRCS, Bangkok, Thailand) together with autocontrol. The antibody specificities were identified using STT according to the above mentioned procedures. Additionally, other extra panel cells from commercial panels (ID-DiaPanel and ID-DiaPanel-P, Bio-Rad Laboratories, Cressier sur Morat, Switzerland) were used when the antibody specificity was inconclusive. The presence of identified alloantibodies was confirmed by antigen typing to determine all antigen negative status on corresponding donor's red cells.

In the case of positive autocontrol and/or positive for direct antiglobulin test (DAT), the red cell genotyping using in house multiplex PCR, as previously described[9] was then performed to determine predicted red cell antigens including E, e, Jk^a, Jk^b, Fy^a, Fy^b, Di^a and MNS7 (Mi^a). Genomic DNA was extracted from those samples using the Genomic DNA extraction kit (REAL Genomics, RBCBioscience, Taipei, Taiwan) was then stored at -20°C until used for genotyping. Briefly, the multiplex PCR contained two reaction mixes (A and B) of different amplification target for each mixed number. Each reaction mix contained four-specific primer pairs and one HGH-specific primer pairs was used as the internal control. The PCR reaction mixtures consisted of 12.5 μL of the 2× PCR reaction mixture (Green Hot Start PCR Master Mix, Biotechrabbit GmbH, Hennigsdorf, Germany), 1 μL (50-150 ng) of genomic DNA, 1 μL of each blood group specific primer (MIX A: FYA, JKA, RHCEe and DIA-forward and -reverse; MIX B: FYB, JKB, RHCEE and DIB-forward and -reverse), 1 μL of HGH primers (HGH-forward and -reverse) and 1.5 μL of PCR grade water in a final volume of 25 μL. PCR amplification of two multiplex sets was performed in a T100 Thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The cycling parameters for the PCR program consisted of 1 cycle at 95°C for 5 minutes, followed by 30 cycles at 95°C for 30 seconds, 61°C for 40 seconds, 72°C for 30 seconds with a final extension at 72°C for 5 minutes. Thereafter, PCR products were electrophoresed at 100 volts with 1.5% agarose gel using 1× Tris borate ethylenediaminetetraacetate (TBE) buffer for 40 minutes. The gel stained with SYBR® safe stain was visualized under a blue-light box transilluminator.

Statistical analysis

Descriptive analysis of antibody screening results obtained from testing by STT was performed according to sex, age groups and ABO blood groups. The Chi-square (χ^2) test was used to compare categorical variables. The results were analyzed using SPSS 16.0 Software (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant.

Results

Blood samples of 601 pregnant women and 2,488 patients were screened for the presence of red cell antibodies and their prevalences were 1.33% (8/601) and 1.89% (47/2,488), respectively. The distribution of the antibodies was found to be independent of the ABO blood groups among both groups. Group B showed the highest percentage, followed by O, A and AB, respectively, as shown in Table 1. For the patients' group, antibody screening test results were categorized and compared according to different sex and age groups (Table 2).

Table 1 Distribution of ABO groups among 601 pregnant women and 2,488 patients

ABO groups	Pregnant Women (%)	Patients (%)
A	96 (16.0)	521 (20.9)
B	224 (37.3)	922 (37.1)
O	218 (36.3)	869 (34.9)
AB	63 (10.5)	176 (7.1)
Total	**601 (100.0)**	**2,488 (100.0)**

Table 2 Distribution of antibody screening test results of 2,488 patients according to sex and age groups

Antibody screening	Sex	Numbers of patients in different age groups, year (%)					Total
		<1	1 - 29	30 - 39	40 - 49*	≥ 50	
Positive	Male	0	5	1	3	10	**19**
		(0.00)	(0.20)	(0.04)	(0.12)	(0.40)	**(0.76)**
	Female	0	6	3	1	18	**28**
		(0.00)	(0.24)	(0.12)	(0.04)	(0.72)	**(1.13)**
Negative	Male	10	160	112	167	446	**895**
		(0.40)	(6.43)	(4.50)	(6.71)	(17.93)	**(35.97)**
	Female	11**	548	315	152	520	**1,546**
		(0.44)	(22.03)	(12.66)	(6.11)	(20.90)	**(62.14)**
Total		**21**	**719**	**431**	**323**	**994**	**2,488**
		(0.84)	**(28.90)**	**(17.32)**	**(12.98)**	**(39.95)**	**(100.00)**

*χ^2 = 4.414; DF = 1; p = 0.036

**The maternal serum samples were used to screen for red cell alloantibodies in 11 newborn cases (age < 1 month).

Moreover, the patients were divided into two groups: less than 40 years *vs.* 40 and older. The frequencies of positive red cell antibodies were significantly higher among patients ≥ 40 years (χ^2 = 4.414, DF = 1, p = 0.036) and no significant difference was found between male and female patients.

The positive antibody screening test results of 8 pregnant women belonged to a single antibody for 6 samples (75.0%) and multiple antibodies for 2 samples (25.0%). Antibody specificities found in all samples were naturally occurring antibodies including anti-Mi^a, anti-P1 and antibodies in the Lewis system (Table 3).

According to the antibody specificities detected in 47 patients with positive antibody screening test (Table 3), 27 (57.4%) patients had a single antibody, 6 (12.8%) patients had multiple antibodies and 14 (29.8%) patients had unidentified antibodies. For single antibody cases, anti-Mi^a was the most common (25.6%), followed by anti-E (8.5%), anti-Le^a (8.5%) and anti-Le^b (8.5%). Moreover, anti-M, anti-Di^a and anti-Jk^b were found in 3 patients. Regarding multiple antibodies, anti-Le^a and -Le^b were detected in 2 patients, and anti-E + -c and anti-E + -Mi^a were observed in another two patients. All identified antibodies in these patients were confirmed by antigen typing and the results showed negative status on corresponding antibodies. Unfortunately, 7 samples showed positive results of autocontrol and DAT. Four samples had unidentified antibodies and 3 samples had anti-Mi^a, anti-P1 + -Le^b and anti-P1 + -Mi^a. Those samples were subsequently genotyped by multiplex PCR and the observed genotypes and predicted phenotypes of 7 patients are presented in Table 4. For two patients who had anti-Mi^a, the predicted phenotypes were confirmed to be Mi(a-).

Table 3 Frequencies of red cell antibody specificities detected among 8 pregnant women and 47 patients with positive antibody screening test

Antibody specificity	Pregnant Women (%)	Patients (%)
Single antibody	**6 (75.0)**	**27 (57.4)**
Anti-Mi[a]	3 (37.5)	12 (25.6)
Anti-E	0 (0.0)	4 (8.5)
Anti-Le[a]	0 (0.0)	4 (8.5)
Anti-Le[b]	0 (0.0)	4 (8.5)
Anti-M	0 (0.0)	1 (2.1)
Anti-Di[a]	0 (0.0)	1 (2.1)
Anti-Jk[b]	0 (0.0)	1 (2.1)
Anti-P1	3 (37.5)	0 (0.0)
Multiple antibodies	**2 (25.0)**	**6 (12.8)**
Anti-Le[a] + -Le[b]	1 (12.5)	2 (4.3)
Anti-E+ -c	0 (0.0)	1 (2.1)
Anti-E +-Mi[a]	0 (0.0)	1 (2.1)
Anti-P1 + -Le[b]	1 (12.5)	1 (2.1)
Anti-P1 + -Mi[a]	0 (0.0)	1 (2.1)
Unidentified	**0 (0.0)**	**14 (29.8)**
Total	**8 (100.0)**	**47 (100.0)**

Table 4 Red cell genotypes and their predicted phenotypes of 7 patients with positive autocontrol and DAT

Patients	Antibody specificity	Observed genotypes	Predicted phenotypes
PNT007	Unidentified	*RHCE*E/*E*; *JK*B/*B*; *FY*A/*A*	E+e-; Jk(a-b+); Fy(a+b-); Di(a-); Mi(a-)
PNT010	Unidentified	*RHCE*e/*e*; *JK*A/*B*; *FY*A/*A*	E-e+; Jk(a+b+); Fy(a+b-); Di(a-); Mi(a-)
PNT014	Unidentified	*RHCE*e/*e*; *JK*A/*A*; *FY*A/*A*	E-e+; Jk(a+b-); Fy(a+b-); Di(a-); Mi(a-)
PNT015	Unidentified	*RHCE*e/*e*; *JK*B/*B*; *FY*A/*A*	E-e+; Jk(a-b+); Fy(a+b-); Di(a-); Mi(a-)
PNT024	Anti-Mi[a]	*RHCE*e/*e*; *JK*A/*B*; *FY*A/*A*	E-e+; Jk(a+b+); Fy(a+b-); Di(a-); Mi(a-)
PNT026	Anti-P1 + -Le[b]	*RHCE*E/*e*; *JK*B/*B*; *FY*A/*A*	E+e+; Jk(a-b+); Fy(a+b-); Di(a-); Mi(a-)
PNT028	Anti-P1 + -Mi[a]	*RHCE*E/*e*; *JK*A/*B*; *FY*A/*A*	E+e+; Jk(a+b+); Fy(a+b-); Di(a-); Mi(a-)

Discussion

Clinically significant red cell antibodies are implicated in hemolytic transfusion reactions (HTRs) and HDFN. Screening for unexpected red cell antibodies is useful to prevent alloimmunization and reduce the risks of HTRs and HDFN.[1] Related reports of the prevalence of red cell antibodies in populations revealed that unexpected antibodies were observed in up to 0.8% of blood donors; 2.9% among patients with a history of blood transfusions and 9 to 30% among patients receiving chronic transfusion therapy.[1,10,11] In this study, the prevalence of red cell antibodies among patients at Pakchongnana Hospital (lower northeastern Thailand) was 1.89%, which was lower than a related study in Taksin Hospital, Bangkok (2.62%) but its prevalence was higher than that of patients in lower northern Thailand (0.54%).[7,12] Additionally, the frequency of red cell antibodies among pregnant women was 1.33%, which was lower than that in a previous report;[7] however, its prevalence and importance were similar to other Asian populations.[6,13]

In this prospective study, the ABO blood groups were analyzed among pregnant women and patients.

The results differed slightly in the prevalence of group O and group B when compared with related studies in Thailand.[14-17] This finding confirmed that the sampling distribution of both groups was drawn at random from a population. When we compared the association of positive antibodies among patients and different sex and age groups, the presence of red cell antibodies increased significantly among those patients ≥ 40 years old but no significant difference was found in both sexes. On the contrary, female Brazilian patients with sickle cell disease, who were older than 14 years, showed an increased risk of alloimmunization.[18]

Regarding the specificity of red cell antibodies, the majority of the study groups had a single antibody rather than multiple antibodies of which anti-Mi^a was the most common in the two groups, followed by anti-P1, anti-E and anti-Le^a and -Le^b, which may be determined genetically. Even though only cold alloantibodies were found in pregnant women in this study, those antibodies reacted optimally at 4 °C showing little or no clinical significance but may occasionally be detected at 37 °C and IAT. Antigen-negative red cell units should be given in this situation.[1] For the patient group, anti-Mi^a was generally detected in almost other studies in Thai populations at relatively high levels. Other alloantibodies were anti-Le^a and anti-Le^b. This result suggested that anti-Mi^a and the antibodies in the Lewis system were the most common alloantibodies in Thai populations,[7,12,19-21] which may cause mild to severe HTRs.[13,22] Other clinically significant antibodies such as anti-E was detected in a single antibody and combined with anti-c and anti-Mi^a. Anti-Di^a and anti-Jk^b were also found. Moreover, a high prevalence rate of anti-E combined with Lewis antibodies were also reported in Thai patients.[19] Performing red cell phenotyping among patients and donor units is required to avoid unwanted clinical consequences.[1]

In addition to the above mentioned findings, red cell phenotyping could not be performed among 2 patients with known IgG antibody specificities because commercial anti-Mi^a required IAT testing as well as 7 patients with unidentified antibodies because of positive autocontrol and DAT results. Two patients with anti-Mi^a could be confirmed to be the Mi(a-) predicted phenotype using red cell genotyping, while other patients should be transfused with predicted phenotype-matched donors to reduce alloimmunization risk. Furthermore, one limitation using multiplex PCR for genotyping was only genotyped-based results concerning frequencies of alloantibodies in Thai populations including 7 alleles and 1 hybrid GP group, so the other clinical importance of red cell antigens must be considered.

Conclusion

This study was the first to report the prevalence of red cell antibodies among patients and pregnant women in Pakchongnana Hospital, which attempted to provide red cell antigens that have the potential for alloantibody formation. Hence, the prompt management of donor blood units corresponding to this data could be applied to ensure safer blood transfusions.

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