Original Article

Anti-Mi^a and Anti-E: The Most Common Clinically Significant Red Cell Alloantibodies in Patients at Phramongkutklao Hospital

Songsak Srijinda[1], Sorratod Bosuwan[1], Chutsanat Nuanin[1] and Chamaiporn Suwanasophon[2]

[1]Division of Blood Bank, Department of Pathology, Phramongkutklao Hospital; [2]Division of Immunology, Department of Clinical Pathology, Army Institute of Pathology

Abstract:

Background: *Alloimmunization is an adverse effect of blood transfusion.* ***Objective:*** *To study the characterization and specificity of red cell antibodies in transfused patients at Phramongkutklao Hospital and to compare the data with previous reports that used column agglutination technology (CAT) as the technique for screening and investigating red cell antibodies.* ***Methods:*** *Study the characterization of red blood cell (RBC) alloantibodies in 1,178 immunized patients between 2005 to 2016.* ***Result:*** *The characterization of RBC alloantibodies consist of single alloantibody 83.19%, multiple alloantibodies 11.46%, alloantibodies plus autoantibodies 0.94%, autoantibodies 1.10% and antibody of undetermined specificity 3.31%. The most common single RBC alloantibody is anti-Mi^a 65.16% followed by anti-E 22.26%, whereas the common multiple RBC alloantibodies are anti-E + -Mi^a and anti-c + -E 28.77% and 14.38%, respectively. The high frequency of anti-Mi^a and anti-E were similar between this study and three previous reports from Songklanagarind Hospital, Maharaj Nakorn Chiangmai Hospital and Khon Kaen Hospital.* ***Conclusion:*** *Anti-Mi^a and anti-E are the most common RBC alloantibodies in Thai patients who received blood transfusions.*

Keywords: ● *Red cell alloantibodies* ● *Transfused patient* ● *Column agglutination technology*

RTA Med J 2017;70:65-71.

Received 27 April 2017 Accepted 31 May 2017

Correspondence should be addressed to Col. Songsak Srijinda, Division of Blood Bank, Department of Pathology, Phramongkutklao Hospital, Bangkok 10400 Email: songsaksrijinda@yahoo.com

นิพนธ์ต้นฉบับ

Anti-Mi^{a} และ Anti-E: แอนติบอดีต่อเม็ดเลือดแดงที่มีนัยสำคัญทางคลินิกที่พบได้บ่อยที่สุดในผู้ป่วยโรงพยาบาลพระมงกุฎเกล้า

ทรงศักดิ์ ศรีจินดา[1] สรทศ โบสุวรรณ[1] ชุษณัฐ นวลอินทร์[1] และ ชไมพร สุวรรณะโสภณ[2]
[1]แผนกธนาคารเลือด กองพยาธิวิทยา โรงพยาบาลพระมงกุฎเกล้า [2]แผนกอิมมูโนวิทยา กองพยาธิคลินิก สถาบันพยาธิวิทยา

บทคัดย่อ

ความเป็นมา *การกระตุ้นระบบภูมิคุ้มกันของร่างกายให้สร้างแอนติบอดีต่อแอนติเจนของเม็ดเลือดแดงเป็นสิ่งไม่พึงประสงค์อย่างหนึ่งของการให้เลือด* ***วัตถุประสงค์*** *เพื่อศึกษาลักษณะและความจำเพาะของแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยโรงพยาบาลพระมงกุฎเกล้าและเปรียบเทียบข้อมูลกับรายงานก่อนหน้านี้ที่ใช้เทคนิค column agglutination technology (CAT) ในการตรวจกรองและแยกชนิดแอนติบอดี* ***วิธีการวิจัย*** *ศึกษาย้อนหลังลักษณะและความจำเพาะของแอนติบอดีต่อเม็ดเลือดแดงในผู้ป่วยระหว่างปี พ.ศ. 2548 ถึง พ.ศ. 2559 ที่ถูกกระตุ้นให้สร้างแอนติบอดีจำนวน 1,178 รายและเปรียบเทียบข้อมูลกับรายงานก่อนหน้านี้* ***ผลการศึกษา*** *ลักษณะของแอนติบอดีที่พบประกอบด้วย alloantibody ชนิดเดียวร้อยละ 83.19 alloantibody หลายชนิดร้อยละ 11.46 alloantibody ร่วมกับ autoantibody ร้อยละ 0.94 autoantibody ร้อยละ 1.10 และแอนติบอดีที่ไม่สามารถแยกความจำเพาะได้ร้อยละ 3.31 alloantibody ชนิดเดียวที่พบบ่อยที่สุดเป็น anti-Mi^{a} ร้อยละ 65.16 รองลงมาเป็น anti-E ร้อยละ 22.26 ในขณะที่ alloantibody หลายชนิดที่พบบ่อยเป็น anti-E + -Mi^{a} ร้อยละ 28.77 และ anti-c + -E ร้อยละ 14.38 ตามลำดับ anti-Mi^{a} และ anti-E พบได้บ่อยมากในการศึกษานี้และจากรายงานก่อนหน้านี้จากโรงพยาบาลสงขลานครินทร์ โรงพยาบาลมหาราชนครเชียงใหม่และโรงพยาบาลขอนแก่น* ***สรุป*** *Anti-Mi^{a} และ anti-E เป็นแอนติบอดีต่อเม็ดเลือดแดงที่มีนัยสำคัญทางคลินิกที่พบได้บ่อยที่สุดในผู้ป่วยคนไทยที่ได้รับเลือด*

คำสำคัญ: ● *Red cell alloantibodies* ● *Transfused patient* ● *Column agglutination technology*

เวชสารแพทย์ทหารบก 2560;70:65-71.

Introduction

Alloimmunization is a common problem in patients undergoing blood transfusion. Most studies have been carried out on patients who chronically received blood transfusions[1-5]. In patients affected with hemoglobinopathies, hematologic diseases, various types of cancer, receiving of organ transplantation, and patients with renal failure, the prevalence of alloimmunization has been reported to be up to 60%, while in hospitalized patients receiving transfusions alloimmunization has been seen in about 1% to 10%[1-5].

The aim of pretransfusion testing was performed for detection and investigation of clinically significant red cell alloantibodies, which may be formed following exposure to red cell antigens. In most cases, the alloimmunization may result from previous transfusion and pregnancy. In addition, some blood group antibody may also be naturally occurring. The frequency of red cell alloantibodies varies among different population demographics, ethnic groups, immunogenicity of the antigens and the sensitivity of techniques used, and they do have a clinical significance for their adverse immunological reactions. The development of alloantibodies can significantly complicate transfusion therapy and result in difficulties in crossmatching of blood. Clinically significant antibodies are capable of causing mild and severe adverse events following transfusions, and hemolytic disease of the fetus and newborn. Thus, knowledge of such alloantibodies is essential for selecting appropriate red cell products for transfusion.

The aim of this study was to analyze the characterization and specificity of red cell antibodies in transfused patients at Phramongkutklao Hospital and to compare the data with previous reports that used column agglutination technology (CAT) as the technique for screening and investigating red cell antibodies.

Materials and Methods

This is a retrospective study that utilized data of all patients at Phramongkutklao Hospital, Bangkok, Thailand during the year 2005 to 2016. The essential data sought were those of alloantibodies obtained during routine antibody screening test and immunological investigations and hold as part of the support of the transfusion service at the hospital. In total, there were data of antibody screening test positive and specific red cell antibodies were identified from 1,178 patients recruited in the study.

Antibody screening test was performed by analyzing additional alloantibody formation, against the Rh, P1PK, Lewis, MNS, Kidd, Kell, Duffy, and Diego blood group systems and using 2 group O screening cells (National Blood Centre, Thai Red Cross Society) and DiaMed ID LISS/Coombs microtyping cards (DiaMed AG, Murten, Switzerland). All pretransfusion plasma samples that had a positive antibody screen were investigated. The antibody specificity determined using panel cells (National Blood Centre, Thai Red Cross Society). The test was performed against the patient's plasma using the same technique following antibody screening. Selective red cell antigen typing of the patients for the corresponding blood group antibody was performed following antibody identification.

In case of warm-reactive autoantibodies may mask the presence of concomitant alloantibodies in the plasma. Adsorbing the plasma with autologous red blood cells could remove autoantibody from the plasma, permitting detection of underlying alloantibodies. The use of ZZAP reagent containing a mixture of 0.1 M dithiothreitol (DTT) plus 0.1% cysteine-activated papain, a mixture of a proteolytic enzyme and a thiol reagent was used to remove coating antibody.

Results

As described in Table 1, we found that the red cell antibody characteristics in 1,178 samples consisted of single alloantibody, 980 (83.19%), multiple alloantibodies, 135 (11.46%), alloantibodies and autoantibodies, 11 (0.94%), autoantibodies, 13 (1.10%) and antibody of undetermined specificity, 39 (3.31%), respectively.

The frequency of detection of different red cell antibodies is presented in Table 2. The most common single red cell antibody is anti-Mi^a 65.16% (647/993) and anti-E 22.26% (221/993), respectively. In addition, the most commom multiple antibodies are anti-E + -Mia 28.77% (42/146) and anti-c + -E 14.38% (21/146), respectively.

Table 1 Frequency of red cell antibody characteristics in 1,178 patients

Red Cell Antibody Characteristics	N (%)
One alloantibody	980 (83.19)
Two alloantibodies	113 (9.59)
Three alloantibodies	18 (1.53)
Four alloantibodies	4 (0.34)
One alloantibody and autoantibody	9 (0.76)
Two alloantibodies and autoantibody	1 (0.09)
Three alloantibodies and autoantibody	1 (0.09)
Autoantibodies	13 (1.10)
Antibody of undetermined specificity	39 (3.31)
Total	**1,178 (100)**

Table 2 Screening and investigations for red cell antibody specificities in patients of Phramongkutklao hospital

Single antibody	N (%)	Multiple Antibodies	N (%)	Multiple Antibodies	N (%)
Anti-Mi^a	647 (65.16)	Anti-E+-Mi^a	42 (28.77)	Anti-E+-Le^b	1 (0.68)
Anti-E	221 (22.26)	Anti-c+-E	21 (14.38)	Anti-C+-Le^a	1 (0.68)
Anti-Le^a	16 (1.61)	Anti-c+-E+-Mi^a	10 (6.85)	Anti-P_1+-Jk^b	1 (0.68)
Anti-Di^a	15 (1.51)	Anti-Mi^a+-Jk^a	7 (4.79)	Anti-E+-M	1 (0.68)
Anti-Jk^a	14 (1.41)	Anti-E+-Jk^a	5 (3.42)	Anti-Mi^a+-Di^a	1 (0.68)
AutoAb	13 (1.31)	Anti-E+AutoAb	5 (3.42)	Anti-Le^a+-Le^b	1 (0.68)
Anti-D	11 (1.11)	Anti-c+-Mi^a	4 (2.74)	Anti-Le^a+-Di^a	1 (0.68)
Anti-c	10 (1.01)	Anti-Mi^a+AutoAb	4 (2.74)	Anti-Le^b+-Jk^a	1 (0.68)
Anti-e	10 (1.01)	Anti-c+-E+-Jk^b	3 (2.05)	Anti-C+-Jk^a	1 (0.68)
Anti-M	8 (0.80)	Anti-E+-Fy^b	3 (2.05)	Anti-E+-P_1	1 (0.68)
Anti-C	8 (0.80)	Anti-e+-Mi^a	3 (2.05)	Anti-c+-S	1 (0.68)
Anti-S	7 (0.70)	Anti-E+-Di^a	3 (2.05)	Anti-E+-S	1 (0.68)
Anti-Jk^b	3 (0.30)	Anti-c+-E+-Mi^a+-Fy^b	3 (2.05)	Anti-c+-Fy^b	1 (0.68)
Anti-P_1	3 (0.30)	Anti-E+-Fy^a	2 (1.37)	Anti-C+-e	1 (0.68)
Anti-Le^b	2 (0.20)	Anti-E+-Jk^b	2 (1.37)	Anti-E+-Mi^a+-Di^a	1 (0.68)
Anti-Fy^b	2 (0.20)	Anti-C+-Mi^a	2 (1.37)	Anti-E+-Mi^a+-Le^a	1 (0.68)
Anti-K	2 (0.20)	Anti-Mi^a+-Jk^b	2 (1.37)	Anti-E+-Mi^a+-Fy^b	1 (0.68)
Anti-Fy^a	1 (0.10)	Anti-Mi^a+-M	2 (1.37)	Anti-E+-Mi^a+AutoAb	1 (0.68)
		Anti-c+-E+-S	2 (1.37)	Anti-c+-E+-P_1+-Mi^a	1 (0.68)
		Anti-C+-Fy^b	1 (0.68)	Anti-N+-Mi^a+-Jk^a+AutoAb	1 (0.68)
Total	**993 (100)**			**Total**	**146 (100)**

The distribution of each red cell alloantibody among 1,126 immunized patients in this study and the previous report from Songklanagarind Hospital[6], Maharaj Nakorn Chiang Mai Hospital[7], and Khon Kean Hospital[8] were summarized in Table 3. The high frequency of anti-Mi[a] and anti-E were similar between four hospitals.

Discussions

A factor influencing the frequency and specificity of red cell alloantibodies is the different techniques using for screening and investigating of antibodies. Standard tube test technique at room temperature and enzyme technique would detect more cold alloantibodies such as anti-Le[a], anti-Le[b], and anti-P_1 with fewer warm alloantibodies, whereas using column agglutination technology (CAT) would detect more warm alloantibodies with fewer cold alloantibodies[6-8,10].

In this study, the majority of the study subjects had single alloantibody (83.19%), whereas the remaining 11.46% had multiple alloantibodies, 0.94% had alloantibodies and autoantibodies, 1.10% had autoantibodies, and 3.31% had antibody of undetermined specificity. The anti-Mi[a] was the most common alloantibody found (56.7%) followed by the anti-E (25.5%). The distribution

Table 3 Distribution of red cell alloantibodies in 4 different hospitals

Blood group system	Types of antibodies	Phramongkutklao Hospital N (%)	Songkhlanagarind Hospital[6] N (%)	Maharaj Nakorn Chiangmai Hospital[7] N (%)	Khon Kaen Hospital[8] N (%)
Rh	Anti-c	56 (4.3)	39 (8.1)	2 (5.4)	3 (4.7)
	Anti-C	15 (1.2)	5 (1.0)	0	0
	Anti-D	11 (0.9)	19 (3.9)	0	0
	Anti-e	16 (1.2)	0	0	0
	Anti-E	329 (25.5)	83 (17.1)	13 (35.2)	11 (17.2)
P	Anti-P1	6 (0.5)	18 (3.7)	1 (2.7)	6 (9.4)
MNS	Anti-M	11 (0.9)	8 (1.7)	0	0
	Anti-N	1 (0.1)	0	0	0
	Anti-S	11 (0.9)	5 (1.0)	0	0
	Anti-s	0	7 (1.4)	0	0
	Anti-Mi[a]	732 (56.7)	159 (32.9)	14 (37.8)	31 (48.4)
Lewis	Anti-Le[a]	20 (1.6)	67 (13.8)	5 (13.5)	6 (9.4)
	Anti-Le[b]	5 (0.4)	37 (7.6)	0	4 (6.2)
Kell	Anti-K	2 (0.2)	0	0	0
Kidd	Anti-Jk[a]	29 (2.2)	14 (2.9)	2 (5.4)	2 (3.1)
	Anti-Jk[b]	14 (1.1)	5 (1.0)	0	0
Duffy	Anti-Fy[a]	3 (0.2)	3 (0.6)	0	0
	Anti-Fy[b]	8 (0.6)	8 (1.7)	0	1 (1.6)
Diego	Anti-Di[a]	21 (1.6)	7 (1.4)	0	0
Total		1,290 (100)	484 (100)	37 (100)	64 (100)
N (immunized patients)		1,126	360	30	54

of each red cell alloantibodies in this study and previous reports from Songklanagarind Hospital[6], Maharaj Nakorn Chiangmai Hospital[7], and Khon Kaen Hospital[8] were resemble, especially the high frequency of anti-Mi^a and anti-E. However, it is of great interest to note that anti-Mi^a and anti-E were the most common red cell alloantibodies detected by CAT in transfused Thai populations.

The GP. Mur phenotype of the miltenberger subsystem classified by Tippett et al[11] shows higher incidence among Asians than among Caucacians, with frequency of 7.3% in Taiwanese blood donors[12], 6.28% in Hong Kong Chinese blood donors[13], and 9.7% in Thai blood donors[14]. The term anti-Mi^a is used to describe antibodies in patients' sera that react with antibody screening cells of the MNS7 phenotype. It has been reported to cause hemolytic transfusion reactions and hemolytic disease of the fetus and newborn[15-18]. Therefore, it is warranted that the GP. Mur red cells be included in screening and panel cells for screening and identification of red cell alloantibodies in countries with a significant Asian population.

Antibodies of the Rh blood group system, in which anti-E was more common than anti-c, anti-C, anti-D and anti-e. In addition, anti-M, anti-N, anti-S, anti-Le^a, anti-Le^b, anti-P$_1$, anti-Jk^a, anti-Jk^b, anti-Fy^a, anti-Fy^b, anti-Di^a and rare antibody such as anti-K were detected in this study. In Conclusion, our study demonstrated alloantibodies against red blood cell antigens among occasionally transfused patient population, the most common clinically significant alloantibodies identified in these patients were anti-Mi^a and anti-E, respectively.

However, the relative prevalence of red blood cell alloantibodies in the general population has not been determined.

Reference

1. *Schonnewille H, van de Watering LMG, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures. Transfusion. 2006;46:630-45.*
2. *Redman M, Regan F, Contreras M. A prospective study of the incidence of red cell allo-immunisation following transfusion. Vox Sang. 1996;71:216-20.*
3. *Azarkeivan A, Ahmadi MH, Hajibeigy B, Gharehbaghian A, Shabeh pour Z, Maghsudlu M, et al. Evaluation of transfusion reaction in Thalassemic patients referred to the Thehran adult thalassemia clinic. ZUMS J. 2008;16:57-66.*
4. *Azarkeivan A, Ahmadi MH, Gharehbaghian A, Zolfaghari S, Nasizadeh S, Maghsudlu M, et al. Antibody screening and identification by gel method in thalassemic patients. Sci J Iran Blood Transfus Org. 2008;5:99-105.*
5. *Natukunda B, Schonewille H, van de Watering L, Brand A. Prevalence and specificities of red blood cell alloantibodies in transfused Ugandans with different diseases. Vox Sang. 2010;98: 167-71.*
6. *Promwong C, Siammai S, Hassarin S, Buakaew J, Yeela T, Soisangwan P, et al. Frequencies and specificities of red cell alloantibodies in Southern Thai population. Asain J Transfus Sci 2013;7:16-20.*
7. *Fongsatitkul L, Limtipsoontorn A, Pisaipong P, Kamtorn N. Screening and identification of unexpected red cell antibody by the gel test (abstract). Thai J Hematol Transf Med. 2003;3:245.*
8. *Daowsee K, Urwijitaroon Y. Red cell alloantibodies in transfused patients detected by standard tube test and column gel agglutination: A study for improving transfusion service. Thai J Hematol Transf Med. 2013;23:269-76.*
9. *Pisaipong P, Fongsatitkul L, Chainuan P, Kamtorn N. Prevalence and specificities of red cell antibodies in the North-Thai population (abstract). Thai J Hematol Transf Med. 2003;3:254.*
10. *Bejrachandra S, Chandanayingyong D. Unexpected red cell antibodies in donors and patients at Siriraj Hospital. Southeast Asain Trop Med Public Health. 1979;10:204-6.*
11. *Tippett P, Reid ME, Poole J, Green CA, Daniels GL, Anstee DJ. The Miltenberger subsystem: is it obsolescent? Transfus Med Rev. 1992;6:170-82.*
12. *Lin-Chu M, Broadberry RE, Chang FJ. The distribution of blood group antigens and alloantibodies among Chinese in Taiwan. Transfusion. 1988;28:350-2.*
13. *Poole J, King MJ, Mak KH, Liew YW, Leong S, Chua KM. The MiIII phenotype among Chinese donors in Hong Kong: immunochemical and serological studies. Transfus Med. 1991;1:169-75.*

14. *Chandanayingyong D, Bejrachandra S. Studies on the Miltenberger complex frequency in Thailand and family studies. Vox Sang 1975;28:152-5.*
15. *Bejrachandra S. Clinically significant of blood group antibody. Thai J Hematol Transf Med. 2005;4:211-5.*
16. *Broadberry RE, Lin M. The incidence and significance of anti-Mi[a] in Taiwan. Transfusion. 1994;34:349-52.*
17. *Lin CK, Mak KH, Cheng G, Lao TT, Tang MH, Yuen CM, et al. Serologic characteristics and clinical significance of Miltenberger antibodies among Chinese patients in Hong Kong. Vox Sang 1998;74:59-60.*
18. *Chiewsilp P, Wiratkasem Y, Sae-huan C. Delayed haemolytic transfusion reaction due to anti-Mi[a]. Thai J Hematol Transfus Med. 1996;6:289-90.*
19. *Fluit CR, Kunst VA, Drenthe-Schonk AM. Incidence of red cell antibodies after multiple blood transfusion. Transfusion 1990;30: 532-5.*
20. *Schonnewille H, Haak HL, van Zijl AM. Alloimmunization after blood transfusion in patients with hematologic and oncologic diseases. Transfusion. 1999;39:763-71.*
21. *Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical Significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusion. Transfusion. 2002;42: 37-43.*
22. *Schonnewille H, de Vries RR, Brand A. alloimmune response after additional red blood cell antigen challenge in immunized hematooncology patients. Transfusion. 2009;49:453-7.*
23. *Reyhanch K, Ahmad G, Gharib K, Vida V, Raheleh K, Mehdi N. Frequency & specificity of RBC alloantibodies in patients due for surgery in Iran. Indian J Med Res. 2013;138:252-6.*
24. *Nathalang O, Kuvanont S, Punyaprasiddhi P, Tassaniyanonda C, Sriphaisal T. A preliminary study of the distribution of blood group systems in Thai blood donors determined by the gel test. Southeast Asian Trop Med Public Health. 2001;32:204-7.*