การคัดกรองและแยกชนิดแอนติบอดีในผู้เข้ารับการตรวจร่างกาย จากบริการวิชาการชุมชน คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่

Screening and identication of alloantibodies in blood samples from Community Medical Service Project, Faculty of Associated Medical Sciences, Chiang Mai University

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แขนงวิชาวิทยาศาสตร์การบริการโลหิตภาควิชาเทคนิคการแพทย์ คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่
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บทคัดย่อ

ผู้ที่เคยได้รับเลือด ปลูกถ่ายอวัยวะ หรือเคยตั้งครรภ์มาก่อน ร่างกายอาจมีการตอบสนองโดยการสร้าง alloantibody ที่จำเพาะต่อแอนติเจนของหมู่เลือดขึ้นได้ การศึกษาครั้งนี้เป็นตรวจกรองและจำแนกชนิดของแอนติบอดีต่อหมู่เลือดในผู้รับบริการตรวจสุขภาพในโครงการบริการวิชาการชุมชน คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่รวม 5 แห่ง จำนวน 1,518 ราย ระหว่างเดือนเมษายน-ธันวาคม พ.ศ. 2554 การศึกษาพบความถี่ของแอนติเจนหมู่เลือดระบบเอบีโอ ดังนี้ หมู่โอจำนวน 459 ราย (43.8%) หมู่บี 306 ราย (29.2%) หมู่เอ 228 ราย (21.8%) และหมู่เอบี 55 ราย (5.2%) และพบอาร์เอ็ซลบจำนวน 4 รายคิดเป็นร้อยละ 0.38 พบตัวอย่างซีรัมที่ให้ผลการคัดกรองแอนติบอดีเป็นบวกจำนวน 41 ราย จากตัวอย่างตรวจทั้งหมด 1,048 ราย (3.9%) มีเพียง 13 ราย (31.7%) เป็นชาย 3 คน หญิง 10 คน ที่มีตัวอย่างเลือดมากพอสำหรับตรวจแยกชนิดของแอนติบอดีได้ และผลการจำแนกชนิดพบเป็น anti-Mi^{a} จำนวน 8 ราย (61.5%) anti-P1 จำนวน 2 ราย (15.4%) anti-M จำนวน 2 ราย (15.4%) และ anti-D จำนวน 1 ราย (7.7%) เมื่อพิจารณาประวัติเพิ่มเติมจากจำนวนที่ตรวจแยกชนิดแอนติบอดีได้ทั้งหมด พบว่ามีประวัติการได้รับเลือด หรือการตั้งครรภ์ในกรณีเพศหญิงที่สอดคล้องกับผลการศึกษา ช่วยยืนยันที่มาของแอนติบอดีได้ นอกจากนี้ยังตรวจพบ anti-M ในตัวอย่างตรวจเพศชายที่ไม่มีประวัติรับเลือด ข้อมูลจากการศึกษาในครั้งนี้มีประโยชน์ในการเฝ้าระวังการให้เลือดเพื่อลดความเสี่ยงในการเกิดปฏิกิริยาในผู้ป่วยโดยรับเลือดที่มีความปลอดภัยสูงขึ้น

Abstract

Individuals with history of blood transfusion, transplantation or pregnancy might develop alloantibodies specific to red blood cell antigens. This work aimed to screen and identify alloantibodies in blood samples from the Community Medical Service Project, Faculty of Associated Medical Sciences, Chiang Mai University. Antibody detection was performed in 1,048 blood samples from 5 community sites during April-December 2011. The result showed that distribution of blood group O, B, A, and AB were 459 (43.8%), 306 (29.2%), 228 (21.8%), and 55 (5.2%), respectively. Four individuals were Rh negative (0.38%). There were 41 blood samples (3.9%) showed positive with antibody screening but only 13 cases (3 male and 10 female, 31.7%) were identified according to small volume of blood sample. Identified alloantibodies included anti-Mi^a (8 samples, 61.5%), anti-P1 (2 samples, 15.4%), anti-M (2 samples, 15.4%), and anti-D (1 sample, 7.7%). The additional data, including history of blood transfusion and/or pregnancy were reviewed. History of blood transfusion and/or pregnancy in women was reviewed and it was corresponded to those alloantibodies reported. One sample of anti-M was detected from male subject who showed no history of blood transfusion. Results from this finding provide enefit for preparing of appropriate blood components for those with alloantibodies in order to reduce risk of blood transfusion reaction.

Introduction

Blood group antigens, molecules expressed on red blood cells mostly are protein but some are polysaccharides. However, a few are not yet identified.1 Blood group antigens with structurally polysaccharide are ABO, H, I, P and Lewis while proteins include Rh, MNSs, Kell, Kidd, Duffy and Lutheran.[1] These antigens are able to induce specific antibodies in an individual who has history of blood transfusion and/or pregnancy. However, some blood group antibodies are known to be non-red cell stimulated such as antibodies specific to antigens in ABH, Ii, MN, Lewis, Lutheran and P blood groups. Except ABH, the latter are IgM type which react well at room temperature and are known as non-clinical significance. The immune type antibodies mostly are IgG and activate complement system. The reaction causes both intravascular and/or extravascular hemolysis depending on the ability to bound complement. Individual who has history of blood transfusion and/or pregnancy, therefore, might be at risk of antigen exposure and subsequently produce specific antibody. To screen alloantibodies in donor processing and pre-transfusion process, thus, are important algorithm to prevent adverse effect according to the reaction of blood group antigen and antibody in the patients.

Antibody screening and identification are processes performed in blood bank under the principle of antibody detection test. The 3-step of the detected reaction includes room temperature, 37 ^{o}C and anti-human globulin. Negative result of antiglobulin test is confirmed by Coombs' Control Cells. In some cases, enhancement technique i.e., increasing serum-to-cell ratio, polyethylene glycol, enzyme treatment, may be required to promote the antigen-antibody reaction.[2]

One of the missions and aims of Faculty of Associated Medical Sciences, Chiang Mai University is academic service in which the Community Medical Service Project is included. Each month, staffs and the 4th year standing students volunteer to join the activity. Clinical services consist of blood chemistry analysis, hematological test, screening for thalassemia carrier, ABO and Rh phenotyping. To add the value of services, we then included antibody screening and identification. We proposed that it would be more advantages for those with positive antibody screening in order to reduce risk of blood transfusion reaction.

Objective of the study

To screen and identify the alloantibodies in blood samples collected from the Community Medical Service Project, Faculty of Associated Medical Sciences, Chiang Mai University during April-December 2011.

Table 1 Total blood samples and number of antibody screened

Community site	Total number	Number of antibody screened
Tambol Mae-Gaa, Amphur Sanpatong	365	198
Tambol San-Pong, Amphur Mae Rim	340	106
Tambol Ta-Wangtan, Amphur Sarapi	265	207
Tambol Mae-Rang, Amphur Pa-Sang	271	263
Tambol Ma-Kok, Amphur Pa-Sang	277	274
Total	**1,518**	**1,048**

Subjects

One thousand five hundred and eighteen samples from people in 5 community sites who joined the Community Medical Service Project, Faculty of Associated Medical Sciences, Chiang Mai University, during April-December 2011 (Table 1). No additional blood drawing was needed. Clotted blood samples were routinely chemistry analyzed within 2 days after site service. Prior to discarding, antibody screening was performed. Sera with positive antibody screening were continued to antibody identification while sera with too small volume were excluded.

Materials and methods

Anti-A anti-B anti-D human monoclonal anti-globulin (IgG/IgM) reagent screening O1 and O2, panel cells were purchased from The National Blood Centre, Thai Red Cross Society, Bangkok, Thailand.

Screening of ABO and Rh were performed by standard tube test. Sample showing negative with anti-D antibody at room temperature was further tested by incubating at 37 °C for 30 minutes following with anti-globulin test to confirm Rh negative or weak D.

Antibody screening was tested. The reaction was performed in 3 phases including incubating at room temperature, 37 °C and anti-globulin. Negative in anti-human globulin phase was observed under microscopy before adding of Coombs' Control Cells to confirm true negative result. For antibody identification, 11 panel cells were used and reaction was followed as mentioned in the process of antibody screening. Past history of blood transfusion and pregnancy were reviewed by the sub-district medical volunteer in order to address the possible source of alloantibodies.

Results

There were 1,518 subjects from 5 community sites in Chiang Mai during April-December 2011 joined in this study (Table 1). However, only 1,048 samples (703 women [67%] and 345 men [33%]) were tested. The result showed that distribution of ABO blood group among these subjects were 459 (43.8%) O blood group, 306 (29.2%) B blood group, 228 (21.8%) A blood group, and 55 (5.2%) AB blood group as shown in Figure 1. Four subjects among 1,048 samples tested were Rh negative (0.38%) and 1,044 samples were Rh-positive.

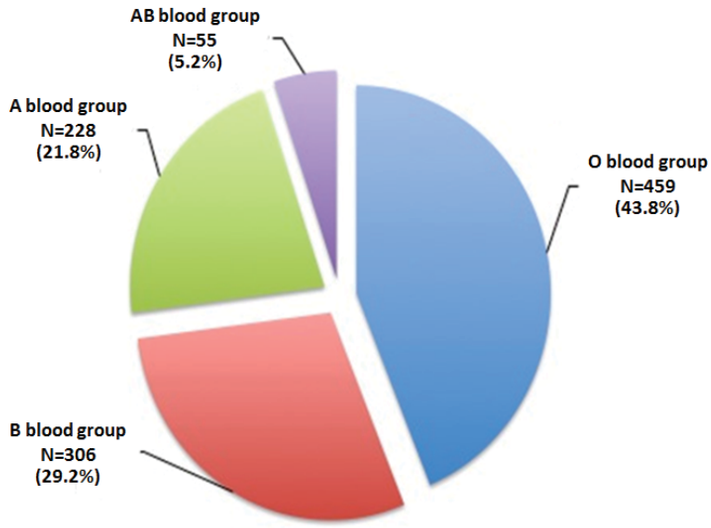

Figure 1 Distribution of ABO blood group among 1,048 blood samples tested

Table 2 Forty-one subjects with positive antibody screening from 1,048 blood samples

Community site	Positive screening	Gender	
		Male	Female
Tambol Mae-Gaa, Amphur Sanpatong	6	2	4
Tambol San-Pong, Amphur Mae Rim	6	3	3
Tambol Ta-Wangtan, Amphur Sarapi	10	3	7
Tambol Mae-Rang, Amphur Pa-Sang	14	5	9
Tambol Ma-Kok, Amphur Pa-Sang	5	2	3
Total	**41**	**15**	**26**

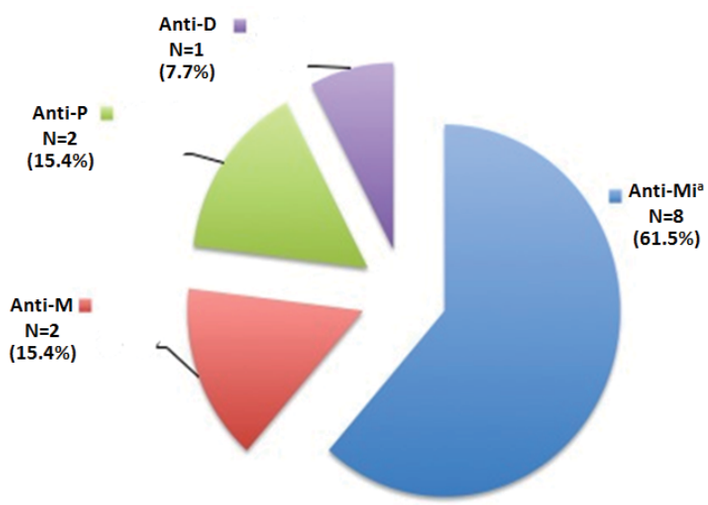

Figure 2 Alloantibodies identified in 13 subjects

Table 3 Information of history related to alloantibodies found

Specificity of antibody	Gender*	Age	History of blood transfusion	History of Pregnancy / number of child	ABO, Rh
Anti-Mi^a	F	64	No	Yes / 2	O, Rh pos
Anti-Mi^a	F	42	No	Yes / 2	B, Rh pos
Anti-Mi^a	M	48	Yes	NA	B, Rh pos
Anti-Mi^a	F	65	Yes	Yes / 2	A, Rh pos
Anti-Mi^a	F	62	No	Yes / 2	O, Rh pos
Anti-Mi^a	F	50	No	Yes / 2	O, Rh pos
Anti-Mi^a	M	56	Yes	NA	B, Rh pos
Anti-Mi^a	M	49	Yes	NA	A, Rh pos
Anti-P1	F	67	No	Yes / 3	O, Rh pos
Anti-P1	F	46	No	Yes / 3	A, Rh pos
Anti-M	F	42	No	Yes / 2	B, Rh pos
Anti-M	M	40	No	NA	O, Rh pos
Anti-D	F	46	No	Yes / 3	B, Rh neg

**M: male, F: female, NA: not available*

Forty-one serum samples (3.9%, 26 women and 15 men) were positive with antibody screening test (Table 2). Thirteen serum samples (3 men and 10 women) with aged ranged from 40-67 years (mean 54) were antibody identified. Four types of alloantibodies were identified. Three IgM alloantibodies were specific to blood group antigen Mi^a (N=8, 61.5%), P1 (N=2, 15.4%) and M antigen (N=2, 15.4%). One clinically significant antibody was identified as anti-D (N=1, 7.7%) as shown in Figure 2. The history of blood transfusion was interviewed from these subjects by village health volunteer to address possible source of antibodies. History of pregnancy was also interviewed in case of female subjects. Data revealed that all 10 women were married and gave birth with at least one child. Moreover, one woman was once blood transfused. One man with anti-M had no history of blood transfusion (Table 3).

Discussion and conclusion

Community Medical Service Project, Faculty of Associated Science, Chiang Mai University is the activity that serve people's healthcare. Program includes hematology and blood chemistry analysis for people age over 30 years. This study focused on value adding in blood samples from Community Medical Service Project. We added antibody screening and identification in blood samples after routine blood chemistry analysis, ABO and Rh typing, in order not to ask for more blood drawing. We proposed that more information in finding alloantibodies might be more helpful for person at risk of blood transfusion and/or pregnancy. We tested 1,518 blood samples collected from people who joined the project during April-December 2011. All blood samples were separated, antibody screened and identified tested as soon as possible after routine blood chemistry analysis. No more blood sample was taken for this purpose. From our study we found that ABO distribution was not changed from previous reported.[3-4] Blood group O was the most incidence where blood group AB was the least population. As there were some problems during process such as

transportation that caused hemolysis and some samples were not enough to complete the protocol, therefore, only 1,048 samples were screened and very few blood samples were identified for alloantibody. Forty-one samples (3.9%) were positive screened and only 13 samples (10 women and 3 men) were completely identified. Four specific antibodies were demonstrated with both IgG and IgM. The IgM antibodies included anti-Mi^{a}, anti-P1 and anti-M antibodies. Anti-D which was IgG and clinically significance was found in one sample. History of blood transfusion and pregnancy was reviewed by health volunteer. It was confirmed that all 10 women subjects had history of pregnancy and one with anti-D was typed as Rh negative. Two men has been blood transfused. Only one man with anti-M had no history of blood transfusion and non-red cell stimulating antibody could be considered.

Taken together we concluded that adding of antibody screening and identification in blood samples for community medical service to people would be more benefit since no more blood collection was needed. Moreover, the important information obtained would be helpful for person at risk of blood transfusion and/or pregnancy. We also suggested that by adding a question concerning history of blood transfusion and pregnancy in subjects joining in Community Medical Service program before blood collecting, antibody screening and identification would be more helpful since only IgG antibody was interested.

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