Anti-P_1 : The Most Common Unexpected Antibodies in Northeastern-Thais

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Abstract

The prevalence of unexpected antibodies in the Northeastern-Thai population was studied. Sera were collected from 25,673 blood donors including 18,209 males and 7,464 females. The sera were screened for unexpected antibodies by saline and enzyme techniques. The sera which gave a positive antibody screening test were identified for specificity of antibody. The result demonstrated that 3,928 from 25,673 samples (15.30%) were positive for the antibody screening test and only 3,883 samples could be identified for specificity of antibody. The most common unexpected antibodies were anti- P_1, anti- lewis and anti- P_1 + anti- lewis with the frequency of 70.8, 18.6 and 10.1 per cent, respectively. The prevalence of anti-P_1 in this study was higher than that reported in Central Thailand and Southeast Asia which may due to the high prevalence of liver fluke infection in the Northeastern-Thai population.

Key word : Anti-P_1, Unexpected Antibody

Unexpected antibodies can occur naturally as a result of antigen-like substances (in some bacteria etc.) and can be as a result of acquired immunization by blood transfusion or pregnancy. Such antibodies are found in some 0.3-2 per cent of the various populations and 0.17-18 per cent of the patients, depending upon the group of studies and the sensitivity of the tests[1-10]. Screening for these antibodies in blood donors is helpful in preparing blood components for patients. On occasion, the unexpected antibody may be potent enough to be suitable for use as a standard typing antisera. This study was to determine the prevalence of unexpected antibodies in Northeastern-Thai blood donors.

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MATERIAL AND METHOD

Samples: Blood samples were obtained from donors in the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Thailand, from January 1995 to December 1996. These 25,673 samples were selected only from blood donors who lived in the northeast of Thailand and no repeat donors were used. Donor sera were separated from clotted blood and frozen at -30°C until tested.

Screening cells: Reagent red cells for antibody screening by saline technique (SC) were a pool of two cells of equal volume. They were both P_2 and Le(a-b-), but inherited the common antigens (C, D, E, c, e, M, N, S, s, Mi^a, Fy^a, Fy^b, Jk^a, Jk^b). Screening cells for enzyme technique (SP) were selected, P_1, Le(a+b-) and P_1, Le(a-b+) cells were pooled in equal volume, testing only for P_1 and Lewis antibodies. The SP cells were prepared to 3 per cent cell suspension in normal saline (NSS).

Panel cells: Two sets of panel cells were used : 1) Panel cells for anti-P_1 and anti-Lewis identification were treated with papain and prepared as SP cells. 2) Panel cells for the other unexpected antibodies were prepared as 3 per cent cell suspensions in low ionic strength saline (LISS).

Antisera: Known antisera for red cell antigen typing including anti-P_1, anti-Le^a, anti-Le^b and anti-Mi^a were prepared in the Blood Transfusion Center, Khon Kaen University. Anti-H and polyspecific antihuman globulin were purchased from the National Blood Center, the Thai Red Cross Society, but anti-E was purchased from Ortho Diagnostic, U.S.A.

Antibody screening test

The standard techniques[11] were used: (i) Saline technique. Two drops of serum and 1 drop of 3 per cent SC cells were incubated at room temperature for 5 mins. Agglutination or hemolysis were macroscopically observed as a positive result after centrifugation at 1000xg for 15 secs. The mixture was incubated at 37°C for 30 mins. The result was observed as above. The red cells were washed three times in NSS and 1 drop of polyspecific antihuman globulin added as antiglobulin testing phase. The result was macro and microscopically examined for red cell agglutination. (ii) Two-stage enzyme technique. Two drops of prewarmed serum and 1 drop of prewarmed SP cells were incubated at 37°C for 30 mins, centrifuged and read for hemolysis. After that the red cells were tested for antiglobulin phase as above. The screening antibody test was positive when the test recorded positive in any phase of both techniques.

Antibody identification

Positive antibody screening sera with SP cells were identified with panel cells by the two-stage enzyme technique[11]. The sera that gave positive results with the SC cell were identified with panel cells by LISS technique[12], and in some cases an enzyme technique was used as well.

Red cell antigen typing

The donor's cells were tested with known antisera for a corresponding antigen to confirm the result of antibody identification.

RESULTS

As shown in Table 1, sera were collected from 25,673 blood donors including 9,646 group O (37.57%), 5,289 group A (20.60%), 8,917 group B (34.73%) and 1,821 group AB (7.09%). Table 2 shows that the antibody screening test gave 3,928 positive results in 25,673 (15.30%). The results show significant difference between male (14.96%) and female (16.13%) (p=0.021). Only 3,883 sera which gave a positive antibody screening test could be identified for specificity of antibodies as shown in Table 3. Some sera demonstrated two or three specific antibodies. The most common unexpected antibodies were 2,751 anti- P_1 (70.8%), 723 anti-lewis (18.6%) and 394 anti- P_1 + anti- lewis (10.1%). We also found anti-Mi^a, anti-H and anti-E in 12, 2 and 2 sera, respectively.

DISCUSSION

Several reports have described the incidence of alloantibodies in central Thai patients.

Table 1. ABO phenotype frequencies in 25,673 Northeastern-Thai blood donors.

Blood group	Number	Frequencies (%)
O	9,646	37.57
A	5,289	20.60
B	8,917	34.73
AB	1,821	7.09

Table 2. Antibody screening test in male and female Northeastern-Thai blood donors.

Sex	Number	Antibody Screening test		
		+	-	% positive
Male	18,209	2,724	15,485	14.96
Female	7,464	1,204	6,260	16.13
Total	25,673	3,928	21,745	15.30

Table 3. Type and number of unexpected antibodies in Northeastern-Thai blood donors.

Type of antibody	Number
anti-P_1	2,751
anti-P_1 + anti-Le^a	108
anti-P_1 + anti-Le^b	109
anti-P_1 + anti-Le^a + anti-Le^b	174
anti-P_1 + anti-Mi^a	3
anti-Le^a	286
anti-Le^b	140
anti-Le^a + anti-Le^b	297
anti-Mi^a	11
anti-Mi^a+ anti-E	1
anti-H	2
anti-E	1
not available for identification	45
total	3,928

These reports showed 0.17 per cent in a routine crossmatch procedure, 6 per cent in transfused patients and 14.97 per cent in pregnant women. Most of them were anti-Lewis (43.17 per cent[7] and 86 per cent[8]) and in some of them no anti-P_1 was found. In our previous report[13], a high prevalence of unexpected antibodies (10.08%) has been described in healthy Northeastern-Thais, anti-P_1 (51.59%) and anti-Lewis (54.3%) were common. The prevalence in the present study showed 15.3 per cent and anti-P_1 was the most common (70.8%). Compared to a previous study[13], the high prevalence may be due to the different screening cells used in the enzyme technique. The strength of P_1 antigen in SP cells in this study gave a stronger reaction. Anti-Mi^a and anti-P_1 were rarely found compared to the central Thai population.

P_1 antigen, first isolated from human blood cells, has been recognized by Cameron and Staveley[14] as a soluble glycoprotein present in *Echinococus granulosus* cyst fluid and was also found in molluscs, crustaceans, annelids and *Ascaris* suum[15]. Strong anti-P_1 in P_2 people has been associated with *Echinococus* granulosus[14,16,17], liver fluke[18], *Clonorchis sinensis*[18] and *Opisthorchis viverrini*[19]. Zhang demonstrated that human anti-P_1 could bind to a cyst fluid protein component of approximately 38kD[20]. The northeastern part of Thailand is an endemic area of *Opisthorchis viverrini*[21,22]. Thus, the high prevalence of anti-P_1 in this study may be due to the prevalence of liver fluke infection in the Northeastern-Thai population. Further study would be of interest in confirming the correlation between liver fluke infected people and the finding of anti-P_1 in their sera.

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แอนติพี$_1$: แอนติบอดีย์ที่พบได้บ่อยในชาวไทยภาคตะวันออกเฉียงเหนือ

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การศึกษาอุบัติการของแอนติบอดีต่อแอนติเจนของหมู่เลือดชนิดอื่น ๆ นอกจากระบบเอบีโอ (unexpected antibody) ในกลุ่มประชากรภาคตะวันออกเฉียงเหนือของประเทศไทย โดยเก็บตัวอย่างจากผู้บริจาคโลหิตทั้งสิ้น 25,673 ราย แยกเป็นเพศชาย 18,209 ราย และเพศหญิง 7,464 ราย ทำการตรวจกรองแอนติบอดีในซีรัมทั้งหมดด้วยเทคนิค saline และเอนไซม์ พบซีรัมที่ให้ผลบวกกับการตรวจกรองแอนติบอดีสูงถึง 15.30% (3,928 ราย) เมื่อนำซีรัมดังกล่าวไปตรวจแยกเพื่อหาชนิดของแอนติบอดี สามารถพบ anti-P_1 สูงที่สุด 2,751 ราย (70.8%) รองลงมาคือ anti-Lewis 723 ราย (18.6%) และ anti-P_1+ anti-Lewis 394 ราย (10.1%) จะเห็นว่าอุบัติการณ์ของ anti-P_1 ในประชากรกลุ่มนี้ สูงกว่าประชากรไทยในภาคกลางมาก ซึ่งอาจจะมีความสัมพันธ์กับการเป็นโรคพยาธิใบไม้ในตับที่พบได้สูงในประชากรภาคตะวันออกเฉียงเหนือของไทย

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