Distribution of Blood Group Systems in Thai Blood Donors Determined by the Gel Test

Sasitorn Bejrachandra, M.D.*
Oytip Nathalang, Ph.D.**
Jariya Saipin, M.Sc.*
Sukunya Kuvanont, B.Sc.***
Krongthip Wichitchinda, B.E.*
Amporn Vongpattranon, B.Sc.*

Abstract : **The rapid availability of fully typed donor blood is of great advantage, especially for patients requiring repeated blood transfusions. Only limited blood group antigen typing has been carried out using the conventional tube technique. This study aimed to examine the distribution of blood group systems in Thai blood donors by the gel test. The ABO, Rh, MNSs, Duffy, Lewis, P, Kell, Lutheran and Kidd blood groups were examined in 500 Thai blood donors by the gel test. The distribution of blood group systems using the gel test was compared with other studies in the Thai population.**

Results : **For the ABO System, group O was the most common (42.6%) followed by group B (30.8%), group A (20.2%), and group AB (6.4%). The most common Rh gene complex was CCDee (53.8%) which was similar to other studies. The MMss and MNss gene complexes were the most common in the MNSs System. Fy^a was very common as in other Asians. In the Lewis System, the incidence of Le (a-b-) was 21.0%, which was consistent with other findings in the Thai population. One hundred and forty-five (29%) were positive for anti-P_1. For the Kell System, 1 out of 500 (0.2%) had the Kk type, 99.8% had the kk type and only Kp^b positive types were observed in this study, as well as Lu (a-b+) in the Lutheran System. The Jk (a-b-) was not found since it was a rare phenotype among Thai people.**

Discussion : **This study shows the blood group distribution in 500 Thai volunteers using the gel test. Because of its simplicity and efficacy, this test is practical in population studies. Additionally, it is useful for mass screening and can be applied in emergency situations.**

Key words : Blood group determination, gel test

เรื่องย่อ : การศึกษาการกระจายของหมู่เลือดระบบต่างๆ ด้วยวิธีเจลในผู้บริจาคโลหิต

ศศิธร เพชรจันทร พ.บ.*, อ้อยทิพย์ ณ ถลาง ปร.ด.**, จริยา สายพิณ วท.ม.*, สุกัญญา กุวานนท์ วท.บ.***, กรองทิพย์ วิจิตรจินดา ค.บ.*, อำพร วงศ์ภัทรนนท์ วท.บ.*

**ภาควิชาเวชศาสตร์การธนาคารเลือด, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, **ภาควิชาพยาธิวิทยา, วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า, ***กองธนาคารเลือด, สถาบันพยาธิวิทยา, ศูนย์อำนวยการแพทย์พระมงกุฎเกล้า.*

สารศิริราช 2545; 54: 403-409.

**Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University; **Department of Pathology, Phramongkutklao College of Medicine; ***Blood Bank, Army Institute of Pathology, Bangkok, Thailand.*

การที่ธนาคารเลือดสามารถจัดหาโลหิตที่ตรวจหาแอนติเจนของหมู่โลหิตระบบต่าง ๆ ไว้แล้ว ให้กับผู้ป่วยที่จำเป็นต้องได้รับโลหิตบ่อยครั้ง จะเป็นประโยชน์อย่างมากต่อการรักษา แต่การตรวจหมู่โลหิตด้วยวิธีหลอดทดลองนั้นมีข้อจำกัดหลายประการ การศึกษานี้มีวัตถุประสงค์เพื่อดูการกระจายของหมู่โลหิตระบบต่างๆ ด้วยวิธีเจล โดยตรวจหาแอนติเจนของหมู่โลหิตระบบ ABO, Rh, MNSs, Duffy, Lewis, P, Kell, Lutheran และ Kidd ในผู้บริจาคโลหิต 500 ราย พบว่าในระบบ ABO หมู่ O พบมากที่สุด (42.6%), รองลงมาคือหมู่ B (30.8%), หมู่ A (20.2%) และหมู่ AB (6.4%) ตามลำดับ สำหรับระบบ Rh พบ CCDee มากที่สุด (53.8%) ซึ่งใกล้เคียงกับการศึกษาที่ผ่านมา ระบบ MNSs พบว่าส่วนใหญ่เป็น MMss และ MNss. แอนติเจน Fy^a พบได้บ่อยซึ่งคล้ายคลึงกับการศึกษาในคนเอเชีย ในระบบ Lewis พบว่ามี Le (a- b-) 21.0% และระบบ P พบแอนติเจน P_1 145 ราย (29%) ซึ่งคล้ายคลึงกับการศึกษาในคนไทยที่เคยมีรายงานไว้ ระบบ K พบ Kk 0.2%, kk 99.8% และทุกรายมีแอนติเจน Kp^b สำหรับระบบ Lutheran ส่วนใหญ่เป็น Lu (a- b+) ส่วนในระบบ Kidd นั้นไม่พบ Jk (a- b-) เลย การตรวจหมู่โลหิตด้วยวิธีเจลนั้นมีข้อดีคือ มีวิธีการทดสอบที่ง่าย และสะดวก เหมาะสำหรับการศึกษาในกลุ่มประชากรต่าง ๆ นอกจากนี้ยังมีประโยชน์ในกรณีที่ต้องทดสอบตัวอย่างตรวจจำนวนมาก และในกรณีฉุกเฉิน

INTRODUCTION

In most cases, red blood cell (RBC) transfusion, compatible with the ABO and Rh groups, is given to patients as a routine practice. If the patient is known to have an antibody that may be clinically significant, all units infused should be tested and proven negative for the offending antigen.[1,2] There is a high prevalence of thalassemia and hemoglobinopathies in Thailand, and most of these patients require repeated blood transfusions in order to maintain normal activity.[3] Although all patients receive group- and type-specific red cells that are compatible, there is an increase in the incidence of red cell alloantibodies and an autocontrol positive response in these patients.[4] For such cases. the rapid availability of fully phenotyped RBC antigens in donor blood is of great advantage. However, only limited blood group antigen typing in blood donors has been carried out because with conventional procedures, complete phenotyping is time consuming, cumbersome and expensive. Since 1993, the gel test has been introduced in Thailand and this test is widely used as a screening system to detect RBC antibodies. In addition to saving time, reducing laboratory procedures and costs, the gel test has been proven to be more sensitive than the conventional tube test.[5-10] Previously, 200 blood donors have been typed for different blood group systems using the gel test.[11] This study was undertaken to examine the ABO, Rh, MNSs, Duffy, Lewis, P, Kell, Lutheran and Kidd blood groups in Thai blood donors by the gel test and to provide more data concerning the blood types of blood donors.

MATERIALS AND METHODS

Subjects

Blood samples from 500 regular blood donors at the Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University and the Blood Bank of the Army Institute of Pathology, Bangkok, Thailand were typed. They were from 400 males and 100 females with ages ranging from 17 to 59 years.

Methods

Each sample was tested by the gel test (DiaMed, Cressier sur Morat, Switzerland) using five cards; the ABO-Rh card, Diaclon Rh subgroups + K card, Antigen profile I card (P_1, Le^a, Le^b, Lu^a, Lu^b), Antigen profile II card (k, Kp^a, Kp^b, Jk^a, Jk^b) and Antigen profile III card (M, N, S, s, Fy^a, Fy^b). ID-diluent 1 (bromelin) and ID-diluent 2 (modified LISS) were used to produce a red cell suspension as described by the manufacturer.[12] For the ABO and Rh (D) typing, the samples were tested in parallel with the tube test that was used routinely.

ABO-Rh (D) Typing

Fifty microliters of 1% RBC suspension in ID-diluent 2 was added to each microtube of the ABO-Rh card and then 25 µl of ID-diluent 1 was added to each microtube. After 10 min incubation at room temperature, the test card was centrifuged for 10 min using the preset cycle of the centrifuge supplied for the system (ID-centrifuge 24S, DiaMed, Switzerland).

Rh Subgroups and K Typing

Ten microliters of 5 % RBC suspension in ID-diluent 2 was added to each microtube of the Rh subgroups and K card. Then the ID card was centrifuged for 10 min.

P_1, Le^a, Le^b, Lu^a, Lu^b, k, Kp^a, Kp^b, Jk^a, and Jk^b Typing

Five percent RBC suspension was prepared in ID-diluent 1 and the RBC suspension was incubated at room temperature for 10 min. Ten microliters of 5% RBC suspension was added to each microtube of the antigen profile I and antigen profile II cards. Then, the ID cards were centrifuged for 10 min.

M, N, S, s, Fy^a and Fy^b Typing

Fifty microliters of 1% RBC suspension in ID-diluent 2 was added to each microtube of the antigen profile III card. Fifty microliters of each test sera (anti-M, anti-N, anti-S, anti-s, anti-Fy^a and anti-Fy^b) were added to each microtube. After 10 min incubation at room temperature, the test card was centrifuged for 10 min.

The agglutination reactions were graded according to the distribution of the agglutinated particles throughout the gel matrix; negative reactions appeared as a discrete cell button at the base of the column, as indicated by the manufacturer.[12]

Statistical Analysis

The distribution of blood group systems was compared with other studies in the Thai population by chi-square test and standard P values.[13]

RESULTS

Five hundred blood samples were tested for red cell grouping by the gel test. For the ABO System; group O was the most common (42.6%) followed by group B (30.8%), group A (20.2%) and group AB (6.4%) (Table 1). When the ABO results were compared with other studies, group O and group B were significantly different ($P < 0.05$). For the Rh System, 99.2% of the samples were Rh (D) positive and CCDee was the most common Rh gene complex (53.8%) found. The Ccddee phenotype was not observed in this study (Table 2). For the MNSs System, MMss and MNss were the most common groups as shown in Table 3. Duffy system blood groups are presented in Table 4. Only four Fy^a negative phenotypes were found in the 500 tested. For the Lewis System, the results of typing for anti-Le^a and anti-Le^b are shown in Table 5 and the Le (a-b-) phenotype was found in 21.0%. The Le (a+ b-), Le (a- b+) and Le (a- b-) phenotypes were significantly different from other studies in the Thai population ($P < 0.001$). Regarding the P System, 145 or 29 % were positive for anti-P_1. For the Kell System, 1 out of 500 (0.2%) had the Kk types, 99.8% had the kk types and only Kp^b positive types were observed in this study. For the Lutheran System, 498 (99.6%) were Lu (a-b+) and only 2 (0.4%) were Lu (a-b-) (Table 6). Kidd system blood groups are presented in Table 7. Jk (a-b-) was not observed in this study.

DISCUSSION

Within eight years of its introduction for serological testing in the blood bank, the gel test has gained widespread usage throughout Thailand, especially for antibody screening, cross matching and direct antiglobulin testing. The gel test has been proven to be more sensitive than the standard tube test comparing sensitivity and specificity in detecting irregular RBC antibodies.[5-7] This study confirms and extends our previous observation to determine blood groups in Thai blood donors by the gel test.[11] From previous studies in 200 random blood donors, the distribution of ABO groups was similar to other studies.[11,14,15] When an additional 300 selected donors were included, the results were slightly different, especially the incidence of group O and group B; however, group O was still the most common, followed by group B, which was the usual pattern among populations in Southeast Asian countries.[16] Also, the low incidence of Rh (D) negative individuals (0.8%) and the high incidence of the CCDee gene complex was similar to other studies in South-

Table 1. ABO blood group distribution in 500 blood donors.

	Year	Number	Blood group (%)			
			O	A	B	AB
Present study	2001	500	213* (42.60)	101 (20.20)	154** (30.80)	32 (6.40)
Chandanayingyong D, *et al.*	1967	8,711	3,280 (37.65)	1,760 (20.19)	3,066 (35.19)	605 (6.97)

*p value = 0.03, **p value = 0.045

Table 2. Rh blood group distribution in 500 blood donors.

	Year	Number	Phenotype (%)									
			CCDee	CcDEe	CcDee	ccDEe	ccDEE	CCDEe	CcDEE	ccDee	ccddee	ccddEe	Ccddee
Present study	2001	500	269 (53.80)	129 (25.80)	56 (11.20)	10 (2.0)	15 (3.0)	9 (1.80)	5 (1.0)	3 (0.60)	3 (0.60)	1 (0.20)	0
Chandanayingyong D, *et al.*	1979	1,000	556 (55.60)	267 (26.7)	87 (8.7)	15 (1.50)	36 (3.60)	26 (2.60)	5 (0.50)	6 (0.60)	1 (0.10)	0	1 (0.10)

Table 3. MNSs blood group distribution in 500 blood donors.

	Year	Number	Phenotype (%)								
			MMss	MNss	NNss	MMSs	MNSs	NNSs	MMSS	MNSS	NNSS
Present study	2001	500	203 (40.60)	184 (36.80)	44 (8.80)	31 (6.20)	29 (5.80)	6 (1.20)	2 (0.40)	1 (0.20)	0
Chandanayingyong D, *et al.*	1979	456	164 (35.96)	184 (40.35)	33 (7.24)	37 (8.11)	33 (7.24)	4 (0.90)	0	0	1 (0.2)

Table 4. Duffy blood group distribution in 500 blood donors.

	Year	Number	Phenotype (%)			
			Fy (a+b-)	Fy (a+b+)	Fy (a-b+)	Fy (a-b-)
Present study	2001	500	406 (81.20)	90 (18.0)	3 (0.60)	1 (0.20)
Chandanayinyong D, *et al.*	1979	1,000	789 (78.90)	197 (19.70)	14 (1.40)	0

Table 5. Lewis blood group distribution in 500 blood donors.

	Year	Number	Phenotype (%)			
			Le (a+b-)	Le (a+b+)	Le (a-b+)	Le (a-b-)
Present study	2001	500	85* (17.0)	0	310* (62.0)	105* (21.0)
Chandanayingyong D, *et al.*	1979	1,668	475 (28.48)	0	687 (41.18)	506 (30.34)

*P < 0.001

Table 6. Lutheran blood group system distribution in 500 blood donors.

	Year	Number	Phenotype (%)			
			Le (a+b-)	Le (a+b+)	Le (a-b+)	Le (a-b-)
Present study	2001	500	0	0	498 (99.6)	2 (0.40)
Chandanayingyong D, *et al.*	1967	455	0	1 (0.22)	452 (99.34)	2 (0.44)

Table 7. Kidd blood group system distribution in 500 blood donors.

	Year	Number	Phenotype (%)			
			Jk (a+b-)	Jk (a+b+)	Jk (a-b+)	Jk (a-b-)
Present study	2001	500	133 (26.6)	230 (46.0)	137 (27.4)	0
Chandanayingyong D, *et al.*	1967	456	145 (31.80)	195 (42.76)	116 (25.44)	0

east Asia.[14,16,17] This is the reason why anti-E, instead of anti-D, is the most common Rh antibody found in multitransfused Thai patients.[18,19]

Regarding the MNSs System, the high frequencies of M and s and the low frequencies of N and S show the typical characteristics of people in Southeast Asian countries.[14,16,17] Fy^a is very common and Fy (a-b-) is exceedingly rare among Thai people.[14] In the Lewis System, the frequencies of all three phenotypes; Le (a+b-), Le (a-b+) and Le (a-b-) were significantly different from other studies[20] which might be due to the different testing procedures; however, 21.0% were found to be Le (a-b-), which also confirmed previous findings.[11] The numbers of P_1 positive (29%), kk (99.8 %) and Kp^b positive antigens (100%) were comparable with other studies in the Thai population.[14,17,20] The high frequency of Lu (a-b+) and the absence of Lu (a+b-) and Lu (a+b+) were shown among the 500 tested. Jk (a-b-), which is considered a rare phenotype among Thai people,[14] was not found in this study.

There are several advantages to the gel test; first, it has eliminated the RBC washing step during the antiglobulin phase for some blood group antigen typing. Therefore, it is less time consuming and requires less technical skill. Second, the gel test provides a clear-cut grading system. Grades 4+, 3+, 2+, 1+ and w+ can be distinguished by the naked eye and remain unchanged for 1 week at 4°C. Additionally, a photocopy of the typing results can be made and kept as firm laboratory data. With the conventional tube test, the grade 1+ and w+ need to be confirmed under the microscope. Furthermore, the gel test is simple and the exposure to blood bank personnel is low. Therefore, the risk of contracting blood-transmitted diseases, such as hepatitis or HIV infection, is decreased.

In conclusion, this study shows blood group distribution in 500 Thai volunteers using the gel test, which is beneficial for the selection of appropriate red cells for multitransfused patients. The test itself is simple, less time-consuming and practical for population screening and application in emergency situations.

REFERENCES

1. Vengelen-Tyler V. Technical Manual. 12th ed. Bethesda: American Association of Blood Banks, 1999: 269-337.
2. Larison PJ, Cook LO. Adverse effects of blood transfusion. In: Harmening DM, ed. Modern Blood Banking and Transfusion Practices. 3rd ed. Philadelphia, F.A. Davis Company, 1994: 351-74.
3. Wasi P, Pootrakul S, Pootrakul P, Pravatmuang P, Winichagoon P, Fucharoen S. Thalassemia in Thailand. Ann NY Acad Sci 1980; **344:** 352-60.
4. Srijinda S, Nathalang O, Suwanasit T, Torcharus K, Krutvacho T, Sriphaisal T. Red cell alloantibodies in thalassemic patients by the gel test. Thai J Hematol Transfus Med 1996; **6:** 32-38.
5. Nathalang O, O-Charoen R, Sandondu N, Meesoonthorn R. Application of the gel test for antibody screening and identification. Thai J Hematol Transfus Med 1993; **3:** 299-306.
6. Nathalang O, Kuvanont S, Suwanasit T, Yensuang K, Sriphaisal T, Krutvacho T. A preliminary study of the gel test for crossmatching in Thailand. J Med Tech Assoc Thai 1993; **21:** 101-6.
7. Nathalang O, Chuansumrit A, Prayoonwiwat W, Siripoonya P, Sriphaisal T. Comparison between the conventional tube technique and the gel technique in direct antiglobulin tests. Vox Sang 1997; **72:** 169-71.
8. Lapierre Y, Rigel D, Adam J, et al. The gel test: A new way to detect red cell antigen-antibody reactions. Transfusion 1990; **30:** 109-13.
9. Bromilow IM. Gel technique in blood group serology. Med Lab Sci 1993; **49:** 129-32.
10. Malyska H, Weiland D. The gel test. Lab Med 1994; **25:** 81-85.
11. Nathalang O, Kuvanont S, Punyaprasiddhi P, Tasaniyanonda C, Sriphaisal T. A preliminary study of the distribution of blood group systems in Thai blood donors determined by the gel test. Southeast Asian J Trop Med Public Health 2001; **32:** 201-4.
12. ID Micro Typing System; Directions for use. DiaMed AG, CH-1785 Cressier sur Morat, Schwiz/Switzerland, 1998.
13. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. Biostatistics in Clinical Medicine. 3 rd ed. New York: McGraw-Hill, Inc, 1994: 174-92.

14. Chandanayingyong D, Sasaki TT, Greenwalt TJ. Blood groups of the Thais. Transfusion 1967; **7:** 267-76.
15. Sringarm S, Chupungart C, Giles CM. The use of Urex europaeus and Dolichos biforus in routine ABO groupings of blood donors in Thailand. Vox Sang 1972; **23:** 537-45.
16. Mollison PL, Engelfriet CP, Contreras M. Blood Transfusion in Clinical Medicine. 9th ed. Oxford: Blackwell Scientific Publications,1993.
17. Phansomboon S, Ikin EW, Mourant AE. The ABO, Rh and MN blood groups of the Siamese. Am J Phys Anthrop 1949; **7:** 562-68.
18. Chandanayingyong D. Common problems in routine pre-transfusion testing. Southeast Asian J Trop Med Public Health 1979; **10:** 193-95.
19. Bejrachandra S, Chandanayingyong D. Unexpected red cell antibodies in donors and patients at Siriraj Hospital. Southeast Asian J Trop Med Pub Health 1979; **10:** 204-6.
20. Chandanayingyong C, Bejrachandra S, Metaseta P, Pongsataporn S. Further study of Rh, Kell, Duffy, P, MN, Lewis and Gerbiech blood groups of the Thais. Southeast Asian J Trop Med Pub Health 1979; **10:** 209-11.