REPORT

Blood group antigen distribution in Lao blood donors

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Blood group antigens can be distributed differently within different nationalities. Therefore, information about the prevalence of blood group antigens in the Lao population will be useful for providing better blood transfusion services in the Lao People's Democratic Republic. The purpose of this study was to determine the prevalence of blood group antigens in Lao blood donors. Blood samples from 464 Lao national volunteer blood donors were typed for antigens in various blood group systems including ABO, MNS, P1PK, Rh, Kell, Lewis, Duffy, Kidd, and Diego. The results show similar antigen prevalence to that among Northeast Thais for ABO, MNS, P1PK, Rh, Kell, and Duffy systems. In the ABO system, O was the highest at 37.72 percent, followed by 35.56 percent B, 19.83 percent A_1, 6.47 percent A_1B, and 0.43 percent A_2B. The common phenotypes were D+C+E−c−e+ at 60.43 percent, M+N−S−s+ at 46.55 percent, Fy(a+b−) at 80.82 percent, Jk(a+b+) at 39.44 percent, and kk at 99.72 percent. Interestingly, Le(a−b−) was found at 50.43 percent, which was significantly higher than previous reports in Thais and Taiwanese. The P1 antigen was found in only 18.97 percent, which is much lower than in Whites and Blacks. Rare phenotypes were Fy(a−b+) and Jk(a−b−), found at 0.22 percent and 4.31 percent, respectively. In terms of negative antigens the study shows 0.22 percent Fy(a−), 35.34 percent Jk(a−), 29.53 percent Jk(b−), 3.04 percent C−, 2.39 percent e−, and 5.17 percent M−. The high prevalence of C, e, and Fy^a and immunogenicity of these antigens may induce alloimmunization in transfusion-dependent patients, creating difficulties providing blood from Lao donors. The information obtained from this study will be useful for improving transfusion therapy in the country, especially for estimation of the availability of compatible blood for patients who have produced antibodies. ***Immunohematology* 2012;28:132–6.**

Key Words: blood groups, RBC antigens, alloimmunization, transfusion therapy

Knowledge of blood group distribution is important for blood transfusion management. A search of published data clearly demonstrates that blood group antigens can be distributed differently within different nationalities.[1–4] Therefore, information about the antigen distribution of different blood group systems in the Lao population will be useful for providing better blood transfusion services in the Lao People's Democratic Republic (PDR), a country in Southeast Asia.

The Lao Red Cross National Blood Transfusion Centre is a government organization that controls policies and standards of blood transfusion services in the Lao PDR. Basically, the donated blood is typed for ABO and D and screened for four infectious markers: human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis. Therefore, except for ABO and D, there is little known information about blood group antigen distribution in the Lao population. Lack of knowledge about antigen prevalence makes it challenging to predict finding compatible blood for transfusion in sensitized patients. Information about the distribution of blood group antigens in blood donors is helpful for estimation of the availability of compatible blood for patients who have developed antibodies through previous transfusions or as the result of pregnancy. In this study, red blood cell (RBC) samples from volunteer Lao national blood donors of Lao Blood Centre were typed for A, A_1, B, M, N, S, s, Mi^a, P1, D, C, E, c, e, K, k, Le^a, Le^b, Fy^a, Fy^b, Jk^a, and Jk^b.

Materials and Methods

Sample Size

Calculation of sample size was based on the prevalence of common negative antigens reported in Northeast Thai blood donors.[2] The number of blood units collected at Lao Blood Centre was approximately 500 per month. The sample size estimation was calculated by the following formula[5]: $n = NZ^2_{\alpha/2}P(1-P)/e^2(N-1) + Z^2_{\alpha/2}P(1-P)$ where n is sample size, N is population size, Z is units of the standard normal distribution, P is population proportion, α is probability of type 1 error or significance level, and e is random error. The calculated sample sizes for donors negative for D, E, c, Le^a, P1, Mi^a, and Jk^a were 1324, 136, 142, 693, 107, 64, and 561, respectively. In considering budget and time limitations, this study proposed to perform antigen typing in 30 percent of blood donors available in a 3 month period, which was about 450 individual donors.

Specimens

RBC samples collected from Lao national (by interview) volunteer donors who donated between September 13 and November 12, 2010, and between January 2 and February 18, 2011, were used for blood group antigen typing.

Typing Antisera

Anti-A, -A1, -B, -A,B, -D, -C, -E, -c, -e, -M, -N, -S, -s, -Fy^a, -Fy^b, -Jk^a, -Jk^b, -K, and -k and anti-human globulin (AHG) were obtained from commercial sources (Thai Red Cross National Blood Center for Anti-A, -A1, -B, -A,B, -D, -E, -c, -M, -N and AHG; Ortho Clinical Diagnostics, Raritan, New Jersey, for Anti-C, -e, -S, -s, -Jk^a, -Jk^b, -Fy^a, -Fy^b, -K, and -k).

Anti-Le^{ab}, anti-Le^a, anti-Le^b, anti-Mi^a, and anti-P1 obtained from blood donors' plasma were kindly provided by Blood Transfusion Centre, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

Monoclonal typing antisera included Anti-A, -A1, -B, -A, B, -D, -C, -E, -c, -e, -M, -N, -Jk^a, and -Jk^b. Anti-S, -s, -Fy^a, -Fy^b, -K, and -k were manufactured from pooled human sera (Ortho Clinical Diagnostics). Anti-M and anti-N were rabbit polyclonal immunized antisera.

Methods

The antigen typing was performed by standard tube test following the providers' instructions. Antigens C, c, D, E, e, M, N, Le^a, Le^b, P1, Mi^a, Jk^a, and Jk^b were typed by saline room temperature phase. Antigens S, s, Fy^a, Fy^b, K, and k were typed by saline antiglobulin phase. For each test of antigen typing, positive and negative RBC controls were included. The positive control cells were donors with single dose antigen expression, selected from panel cells (products of Thai Red Cross National Blood Center).

Data Analysis

Data analysis for antigen frequencies and probability values was performed using STATA software version 10.0 (StataCorp LP, College Station, Texas). The prevalence among populations was compared using the *t* test. A probability value of less than 0.05 was considered significant.

This study was approved by the Lao National Ethics Committee for Health Research, dated September 10, 2010, No.325/NECHR and the Thai Human Research Ethics Committees, dated February 22, 2011, No.HE532464.

Results

There were 464 blood samples included in this study. As shown in Table 1, the O blood group was the highest prevalence at 37.72 percent, followed by B at 35.56 percent, A_1 at 19.83 percent, A_1B at 6.47 percent, and A_2B at 0.43 percent. The MNS system demonstrated two major phenotypes: M+N−S−s+, and M+N+S−s+ at 46.55 percent and 43.53 percent, respectively. Other phenotypes were found as follows: 5.17 percent M−N+S−s+, 2.59 percent M+N−S+s+, and 2.16 percent M+N+S+s+. In addition, Mi^a was also tested and found at 31.25 percent Mi(a+) and 68.75 percent Mi(a−). Regarding the P1PK system, it was discovered that most blood donors in this study were P1−, at 81.03 percent, and 18.97 percent were P1+. The phenotype prevalence in the Rh system was D+C+E−c−e+ at 60.43 percent, D+C+E+c+e+ at 19.13 percent, D+C+E−c+e+ at 10.22 percent, D+C+E+c−e+ at 6.09 percent, D+C−E+c+e+ at 1.30 percent, D+C+E+c+e− at 1.09 percent, D+C−E+c+e− at 1.30 percent, and D+C−E−c+e+ at 0.43 percent. There were only two phenotypes in the Kell system, kk and Kk, with 99.72 percent kk and only 0.28 percent Kk. Prevalence of the Lewis blood group system was 50.43 percent Le(a−b−), 28.45 percent Le(a+b−), and 21.55 percent Le(a−b+). There were three phenotypes in the Duffy blood group system: Fy(a+b−), Fy(a+b+), and Fy(a−b+), with prevalence of 80.82 percent, 19.18 percent, and 0.22 percent, respectively. Four phenotypes were found in the Kidd system, Jk(a+b+), Jk(a−b+), Jk(a+b−), and Jk(a−b−), at 39.44 percent, 31.03 percent, 25.22 percent, and 4.31 percent, respectively. Table 2 presents the results of the typing, demonstrating the distribution of blood group antigens in Lao blood donors. Phenotype comparison with Thais and Taiwanese is illustrated in Table 3. Table 4 presents the prevalence of individual antigens of the MNS and Rh systems in several additional populations for comparison.

Table 1. Prevalence of blood group phenotypes in Lao blood donors

Blood group system	Number tested	Phenotype prevalence (%)
ABO	464	A_1 19.83, B 35.56, O 37.72, A_1B 6.47, A_2B 0.43
MNS	464	M+N−S−s+ 46.55, M+N+S−s+ 43.53, M−N+S−s+ 5.17, M+N−S+s+ 2.59, M+N+S+s+ 2.16 Mi(a+) 31.25, Mi(a−) 68.75
P1PK	464	P1+ 18.97, P1− 81.03
Rh	460	D+C+E−c−e+ 60.43, D+C+E+c+e+ 19.13, D+C+E−c+e+ 10.22, D+C+E+c−e+ 6.09, D+C−E+c+e+ 1.30, D+C+E+c+e− 1.09, D+C−E+c+e− 1.30, D+C−E−c+e+ 0.43
Kell	358	K−k+ 99.72, K+k+ 0.28
Lewis	464	Le(a+b−) 28.45, Le(a−b+) 21.55, Le(a−b−) 50.43
Duffy	464	Fy(a+b−) 80.82, Fy(a+b+) 19.18, Fy(a−b+) 0.22
Kidd	464	Jk(a+b−) 25.22, Jk(a−b+) 31.03, Jk(a+b+) 39.44, Jk(a−b−) 4.31

Discussion

In the ABO system, the O blood group had the highest prevalence at 37.72 percent followed by 35.56 percent B, 19.83

Table 2. Blood group antigen distribution in Lao blood donors

Antigen	Negative		Positive	
	Number	%	Number	%
M	24/464	5.17	440/464	94.83
N	228/464	49.14	236/464	50.86
S	442/464	95.26	22/464	4.74
s	0/464	0.00	464/464	100.00
Mi[a]	319/464	68.75	145/464	31.25
P1	376/464	81.03	88/464	18.97
D	0/460	0	460/460	100
C	14/460	3.04	446/460	96.96
E	327/460	71.19	133/460	28.91
c	306/460	66.52	154/460	33.48
e	11/460	2.39	449/460	97.61
K	357/358	99.72	1/358	0.28
k	0/358	0.00	358/358	100.00
Le[a]	332/464	71.55	132/464	28.45
Le[b]	364/464	78.45	100/464	21.55
Fy[a]	1/464	0.22	463/464	99.78
Fy[b]	375/464	80.82	89/464	19.18
Jk[a]	164/464	35.34	300/464	64.66
Jk[b]	137/464	29.53	327/464	70.47

percent A_1, 6.47 percent A_1B, and 0.43 percent A_2B. The data from this study confirmed the findings of previous studies in Thai blood donors.[2,3] However, these findings are quite different from data on Taiwanese, in whom the A and B blood groups were almost equal, 25.6 percent and 25.8 percent, respectively.[4] This study also confirmed that the second highest ABO blood group among Asians is B,[2,6] whereas in Whites it is A[7] (Table 3). The rare A_2B subgroup was also found at 0.43 percent, confirming previously published data by Dean,[8] who stated that it is rare to find this phenotype in Asians. The presence of the A_2B subgroup indicates that the A_2 gene is present among Lao, but this study did not find any A_2 blood group, which might be owing to the small sample size.

Regarding the MNS system, there were five phenotypes found: M+N−S−s+, M+N+S−s+, M−N+S−s+, M+N+S+s+, and M+N−S+s+. This is different from the study in Thai blood donors reported by Fongsarun et al.,[3] who discovered four more phenotypes: M−N+S+s+, M+N−S+s−, M+N+S+s−, and M−N+S+s−, which were also found in other Thai studies.[2,9] In addition, when comparing Lao and Northeast Thais, the percentages of the five phenotypes were close to each other. The difference was that whereas there were 1.87 percent M+N−S+s− and 3.27 percent M+N+S+s− found in Northeast Thais, these phenotypes were not found in Lao (Table 3).[2]

Table 3. Blood group phenotype comparison with Thais and Taiwanese

System	Phenotype	This study	Northeast Thais[2] (%)	Bangkok Thais[3] (%)	Bangkok Thais[9] (%)	Taiwanese[4] (%)
ABO	O	37.72	35.50	37.70	37.60	42.40
	B	35.56	34.33	33.40	35.20	25.80
	A	19.83	22.40	21.40	20.10	25.60
	AB	6.90	7.68	7.30	7.00	6.20
MNS	M+N−S−s+	46.55	42.06	29.90	36.00	28.50
	M+N+S−s+	43.53	47.19	38.50	40.00	43.70
	M−N+S−s+	5.17	5.61	14.50	7.20	19.10
	M+N−S+s+	2.59	0	7.30	8.00	4.10
	M+N+S+s+	2.16	3.27	6.30	7.20	3.40
	M−N+S+s+	0	0	1.60	0.90	1.20
	M+N−S+s−	0	1.87	0.70	0	0
	M+N+S+s−	0	3.27	0.90	0	0.20
	M−N+S+s−	0	0	0.30	0.20	0
	Mi(a+)	31.25	13.92	9.10	9.70	No data
	Mi(a−)	68.75	86.08	90.90	90.30	No data
P1PK	P1+	18.97	22.65	31.00	No data	32.40
	P1−	81.03	77.35	69.00	No data	67.60
Rh	D+C+E−c−e+	60.43	61.00	49.38	55.60	47.50
	D+C+E+c+e+	19.13	14.67	19.91	26.70	34.40
	D+C+E−c+e+	10.22	11.00	10.89	8.70	5.90
	D+C+E+c−e+	6.09	7.33	2.15	2.60	0.90
	D+C−E+c+e+	1.30	0	3.90	1.50	0.30
	D+C+E+c+e−	1.09	1.67	1.21	0.50	0.30
	D+C−E+c+e−	1.30	1.33	11.21	3.60	2.00
	D+C−E−c+e+	0.43	0	1.13	0.60	0.20
	D+C+E−c+e-	0	0.33	No data	0.10	0
Kell	K−k+	99.72	99.68	98.16	No data	100.00
	K+k+	0.28	0.32	1.78	No data	0
	K+k−	0	0	0.07	No data	0
Lewis	Le(a+b−)	28.45	No data	No data	28.48	10.30
	Le(a−b+)	21.55	No data	No data	40.65	67.30
	Le (a−b−)	50.43	No data	No data	30.87	9.40
Duffy	Fy(a+b−)	80.82	85.33	76.70	78.90	90.80
	Fy(a+b+)	19.18	14.00	19.50	19.70	8.90
	Fy(a−b+)	0.22	0.67	3.70	1.40	0.30
	Fy(a−b−)	0	0	0.06	0	0
Kidd	Jk(a+b−)	25.22	No data	26.50	31.80	23.00
	Jk(a+b+)	39.44	No data	50.30	42.80	48.00
	Jk(a−b+)	31.03	No data	23.20	25.40	29.00
	Jk(a−b−)	4.31	No data	0.06	0	0

The single antigens in the MNS system and Mi[a] also showed similar hierarchy for prevalence to that in Thai studies (Table 4).[2,3,6,9,10] The M antigen was the most common (84%–95%) followed by N (51%–63%). The s antigen was found in 100 percent, whereas antigen S was found in only 4.74 percent of Lao compared with Northeast Thais at 10.75 percent,[2] and 15.5 percent in Bangkok Thais.[3] In addition, when considering antigen Mi[a], it is interesting to note that it was found at a higher prevalence in Lao than in Thais, 31.25 percent in Lao, but only 13.92 percent in Northeast Thais[2] and only 9.1 percent in Bangkok Thais (Table 3).[3,10] All three studies used anti-Mi[a] typing antisera from blood donor plasma. We also performed Mi[a] antigen typing in another 100 random samples and found 23 percent Mi(a+). The data from this study indicate that there

Table 4. Comparison of prevalence of MNS and Rh blood group antigens in different populations

Antigen	This study (%)	Northeast Thais[2] (%)	Bangkok Thais[3] (%)	Taiwan Chinese[4] (%)	Whites[1] (%)	Blacks[1] (%)
M	94.83	94.39	83.90	79.70	79.00	74.00
N	50.86	56.06	62.80	67.40	70.00	75.00
S	4.74	10.75	15.50	8.70	52.00	30.00
s	100.00	98.13	100.00	100.00	90.00	92.00
D	100.00	99.67	99.78	99.40	85.00	92.00
C	96.96	98.67	83.76	91.60	68.00	27.00
E	28.91	28.00	38.60	43.50	29.00	22.00
c	33.48	31.67	48.47	51.60	80.00	96.00
e	97.61	97.00	87.58	93.80	85.00	89.00

is a high probability that anti-Mi^a may be stimulated in Mi(a–) multiply-transfused patients receiving blood from Lao donors compared with those receiving blood from Whites and Blacks because the prevalence of Mi(a+) in those populations is only 0.01 percent.[11] This was confirmed by the Taiwan report, which indicated anti-Mi^a caused transfusion reaction and also hemolytic disease of the fetus and newborn.[12] Therefore, antibody screening cells routinely used in Lao must include Mi(a+) cells.

In the P1PK system, P1+ was less frequently found at 18.97 percent, given the high prevalence of P1– at 81.03 percent. This prevalence is opposite to the prevalence of these antigens in Whites and Blacks, which are mostly P1, 79 percent in Whites and 94 percent in Blacks.[1] The prevalence of the P1 antigen in the Lao population is not different from the prevalence found in Northeast Thais[2] ($p > 0.05$), but it is significantly different from the prevalence among Bangkok donors[3] and Taiwanese[4] ($p < 0.0001$; Table 3). With the high prevalence of P1–, anti-P1 would be commonly detected and can be clinically significant if reacting at 37°C,[13] but it would not be a problem for providing negative blood for transfusion.

This study did not find the D– phenotype in Lao blood donors (Table 4). This is different from studies in Northeast and Bangkok Thais, which found about 0.02 percent D– phenotype.[2,3,6,9] This may be related to the small sample size, as D– in Lao blood donors is usually found to be about 0.01 percent.[14] Other antigens in the Rh system, C, E, c, and e, have a similar distribution to that in Northeast Thais,[2] and slightly different from the Bangkok reports.[3] For example, the E antigen is found in 28.91 percent of Lao and 28.00 percent of Northeast Thais,[2] but approximately 38 percent of Bangkok blood donors were E+.[3,10] It is also interesting to note that the c antigen is found in fewer Asians, 31.67 to 51.6 percent,[2–4,8,10] but found much more often in Whites and Blacks, 80 percent and 96 percent, respectively.[1] With c and E antigens, based on the prevalence of negative and positive phenotypes found in Lao, alloimmunization of anti-E and anti-c may be commonly found in transfusion-dependent patients, but it would not be difficult to provide negative blood for transfusion. In terms of phenotype comparison, this study found that Rh phenotypes in Lao and Northeast Thais were similar.[2] As shown in Table 3, phenotype D+C+E–c–e+ was found in 60.43 percent of Lao and 61.00 percent in Northeast Thais, and D+C+E+c+e+ was found in 19.13 percent of Lao and 14.67 percent in Northeast Thais. In addition, some similarity and differences were observed in Bangkok Thais. Specifically, phenotype D+C+E–c–e+ was the one with the highest prevalence (60.43% in Lao, 55.6% in Bangkok Thais), whereas D+C–E+c+e– was found more often in Bangkok Thais than in Lao, 11.21 percent and 0.43 percent, respectively, and D+C–E+c+e+ was found at 3.90 percent in Bangkok Thais, but only 1.30 percent in Lao.[3,9,10]

In the Kell system, K was found at only 0.28 percent (Table 3). This prevalence is similar to those found in Thais and Taiwanese, but different from those in Whites and Blacks.[1,2,4] Because the K antigen is rarely found, anti-K would be expected to be an uncommon antibody among Lao.

This study's data on the Lewis blood group are interesting (Table 3). First, the prevalence of Le(a–b+) was lower than the finding in Thais and Taiwanese,[4,9,10] which was only 21.55 percent in Lao, but 40.65 percent in Thais[14] and 67.30 percent in Taiwanese.[4] Second, the phenotype Le(a+b–) at 28.45 percent in Lao was different from that in Taiwanese, which was only 10.3 percent ($p < 0.0001$).[4] Theoretically, the low prevalence of Le(a–b+) indicates the possibly lower prevalence of the *Se* gene than in Thais.[15] Lastly, Le(a–b–) at 50.43 percent in Lao was significantly higher than in Thais ($p < 0.0001$).[9] With the high prevalence of Le(a–b–), naturally occurring antibodies of Lewis would be commonly found in Lao.

Table 3 illustrates that this study found there were three main phenotypes in the Duffy system, Fy(a+b–), Fy(a–b+), and Fy(a+b+), and the prevalence was close to the finding in Northeast Thais,[2] but slightly different from that in Bangkok Thais.[3] In addition, phenotype Fy(a–b–) was not found in Lao as well as being missing in Thais,[2,3,9] confirming the lack of this phenotype in Asians.[15] In terms of individual antigens, Fy(a–) is very rare, but Fy(b–) is high in Lao, 0.22 percent and 80.82 percent, respectively. For the Kidd system, which is shown in Table 3, the prevalence of the Jk(a+b–) phenotype in Lao was 25.22 percent, which is close to those in Bangkok Thais,[3] Taiwanese,[4] and Whites.[1] However, phenotype Jk(a+b+) had a prevalence of only 39.44 percent in Lao, but was found in 50.30 percent of Thais,[3] 48.00 percent of Taiwanese,[4] and 49

percent of Whites.[1] The rare phenotype Jk(a–b–) was also found in 4.31 percent of Lao, but found in only 0.06 percent of Bangkok Thais,[3] not found in Taiwanese,[4] and found in less than 0.01 percent of Whites and Blacks.[1]

The rare phenotypes found in Lao donors are those lacking any of the following antigens: C, e, and Fy^a, which were found at 3.04 percent, 2.39 percent, and 0.22 percent respectively. It is possible that C, e, and Fy^a with their immunogenicity may induce alloimmunization in transfusion-dependent patients and contribute to difficulties providing blood from Lao donors.

The study of blood group antigen distribution in Lao blood donors of the National Blood Transfusion Centre, Vientiane, the Capital City of the Lao PDR, had some limitations. First, there were problems associated with the availability of samples. At the time of this study, the number of blood donors was quite small, so it took time to get enough blood samples. Second, some antisera were expensive and time-consuming to obtain from a foreign country. The expense associated with purchasing of the antisera led to a reduction in the number of some antigens typed, especially K and k.

Notwithstanding these limitations, our recommendation would be to carry out further studies in the near future to build on the benefits from this study. Additional studies about the distribution of blood group antigens in other provinces might be needed. Moreover, further studies about secretor status and prevalence of unexpected antibodies in blood donors and transfusion-dependent patients would be beneficial for transfusion therapy in the country.

In conclusion, this study is the first report of blood group antigen distribution among Lao blood donors. The information obtained will be useful for improving transfusion therapy in the country, especially for estimation of the availability of compatible blood for patients who have developed unexpected antibodies.

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