Jk(a-b-) phenotype screening by the urea lysis test in Thai blood donors

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Background. The Jk(a-b-) phenotype is rare in most populations and often detected after transfusion or pregnancy. After immunisation, anti-Jk3 forms and it can be difficult to find compatible Jk(a-b-) donors. Using anti-Jk^a and anti-Jk^b in a conventional tube method is unsuitable for identifying Jk(a-b-) in mass screening of blood donors. Jk(a-b-) phenotypes are associated with the absence of urea transporters on erythrocytes, making red blood cells (RBC) resistant to lysis by 2M urea, while Jk(a+b-), Jk(a-b+) and Jk(a+b+) phenotypes are susceptible to lysis.

Materials and methods. We screened for Jk(a-b-) phenotypes in blood donors by the urea lysis test using a 96-well microplate. The Jk(a-b-) phenotypes were confirmed by the indirect antiglobulin test (IAT).

Results. Altogether, 20,163 blood samples from Thai blood donors were tested and only RBC from five samples were resistant to lysis by 2M urea, while 20,158 samples were completely lysed within 5 min. In an IAT, both anti-Jk^a and anti-Jk^b failed to agglutinate RBC from all five samples. Using a micro-titre plate, the direct urea lysis test, costs • 0.01, about 480 times less than IAT. Moreover, the test time for each plate (94 samples) is about 18 times less than that for IAT.

Conclusion. Jk(a-b-) phenotype screening by the direct urea lysis test on samples in a micro-titre plate is simple, cost-effective and practical for mass screening of blood donors.

Key words: Jk(a-b-) phenotype, Thai blood donors, urea lysis test.

The Kidd blood group system (ISBT 009) was discovered in 1951. A specific antibody against an unknown red cell antigen was found in a patient's serum (Mrs. Kidd) during pregnancy[1]. In general, Kidd antibodies are a significant cause of delayed haemolytic transfusion reaction and haemolytic disease of the newborn; the severity of the disease varies but tends to be mild. Importantly, the antibodies are often difficult to detect, making them hazardous in transfusion medicine[2-7]. Three antigens have been found in this blood group system: Jk^a, Jk^b and Jk3. However, only three phenotypes, Jk(a+b-), Jk(a-b+), and Jk(a+b+), are common among different populations. The Jk(a-b-) phenotype is rare in most populations and is usually detected after a transfusion or during a pregnancy. After immunization, anti-Jk3 can be found in patients with the Jk(a-b-) recessive phenotype, causing acute and delayed haemolytic transfusion reactions, and creating difficulties in finding compatible blood donors. The Jk (a-b-) phenotype can be routinely identified by the absence of Jk^a and Jk^b antigens when testing red blood cells (RBC) with specific antiserum using the indirect antiglobulin test (IAT)[8]. The IAT by the conventional tube method is expensive, time-consuming, labour intensive and unsuitable for mass screening in blood donors. The Kidd glycoprotein is a urea transporter and the Jk(a-b-) phenotype is associated with the absence of this transporter. Therefore, RBC of subjects with the Jk(a-b-) phenotype were resistant to lysis by

2M urea in distilled water, as reported by Heaton *et al*. in 1982 [9], whereas RBC from subjects with the other phenotypes were not; hence, the urea lysis test could be used as a screening test for Jk(a-b-) phenotype as well as for the detection of Jk heterozygotes in paternity testing[10,11]. Various studies suggest that the Jk(a-b-) phenotype is rare among Thai people[12,13]. In this study we screened for Jk(a-b-) phenotypes in Thai blood donors using the urea lysis test.

Materials and methods

Subjects

Blood samples taken between May 2008 and March 2009 from 20,163 Thai blood donors at the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand were included in this study.

Methods

Samples were screened for Jk(a-b-) phenotypes by the direct urea lysis test as previously described with some modifications using a 96-well microtitre plate[10]. Jk^a and Jk^b antigens in the Jk(a-b-) phenotype, identified by the urea lysis test, were confirmed by IAT using anti-Jk^a and anti-Jk^b via the conventional tube method.

Direct urea lysis test

In order to determine the characteristics of the direct urea lysis test, red cells of known Jk(a+b-), Jk(a-b+), Jk(a+b+) and Jk(a-b-) phenotypes were suspended in phosphate-buffered saline (PBS) pH 7.2, in various concentrations (1%, 2%, 3%, 4% and 5%). Twenty-five microlitres of RBC suspension were placed into each well of the microtitre plate. The urea solution, 2M urea in distilled water, was prepared and kept at 4 °C until use. One hundred and fifty microlitres of 2 M urea were added to each well. The plate was mixed on a plate rotator, incubated at room temperature for 5, 10, 15, 20, 25, 30, 35, 45 and 50 mins, and then centrifuged at 1,800 rpm for 2 min (Eppendorf Centrifuge 5810R, Eppendorf, Germany). The plate was read for haemolysis by naked eye. Negative Jk(a-b-) and positive Jk(a+b+) controls for haemolysis were included in the plate.

Jk (a-b-) phenotype confirmation by the IAT

One drop of human anti-Jk^a and one drop of human anti-Jk^b (DiaMed AG, Switzerland), were added into the corresponding test tubes and then one drop of 3-5% RBC suspension in 0.9% normal saline was added into each tube. The contents of the test tubes were mixed and incubated at 37 °C for 30 min. The cells and serum mixtures were washed three times with normal saline and two drops of antiglobulin serum (National Blood Centre, Thai Red Cross Society, Bangkok, Thailand) were added to each tube. After centrifugation, the reactions were read macroscopically and the agglutination reactions were graded as 4+, 3+, 2+, 1+ and w+, respectively. After reading a negative reaction under the microscope, IgG-coated RBC were added to check the validity of the antiglobulin test.

Results

When different concentrations (from 1 to 5 %) of RBC suspension in PBS were tested with 2M urea in the microtitre plate, it was found that similar results were obtained for non-haemolytic reactions for Jk(a-b-) phenotypes and complete haemolysis for common Jk phenotypes. With regards to these findings, in this study, first 25 µL of PBS were added, followed by preparation of the 1-5% RBC suspensions using the stick method. The incubation time of the RBC and 2M urea was also studied. It was found that positive and negative reactions were clear-cut after 5 min of incubation, but Jk(a-b-) RBC showed haemolysis when the incubation period exceeded 40 min, as shown in Table I.

Table I - Comparison of results of the direct urea lysis test with different incubation periods.

Phenotype	Incubation time (mins)									
	5	10	15	20	25	30	35	40	45	50
Jk(a+b-)	H	H	H	H	H	H	H	H	H	H
Jk(a-b+)	H	H	H	H	H	H	H	H	H	H
Jk(a+b+)	H	H	H	H	H	H	H	H	H	H
Jk(a-b-)	0	0	0	0	0	0	0	0	H	H

H= Complete haemolysis, 0= No haemolysis

Altogether, 20,163 blood samples were screened for the Jk(a-b-) phenotype using the direct urea lysis test. Interestingly, RBC from five samples (0.025%) were resistant to lysis by 2M urea in distilled water in

a 96-well microtitre plate, while the other 20,158 samples (99.97%) were completely lysed within 5 min. Both anti-Jk^a and anti-Jk^b failed to agglutinate RBC from all five samples by IAT. Hence, these five samples were identified as having the Jk(a-b-) phenotype but adsorption/elution tests with anti-Jk^a and anti-Jk^b and/or family studies are necessary to differentiate Jk(a-b-) phenotype of recessive type for transfusion to patients with anti-Jk3.

Discussion

The prevalence of thalassaemia and haemoglobinopathies is high in Thailand and other Southeast Asian countries and many of the patients with these conditions require repeated blood transfusions in order to maintain their normal activity[14]. Although all patients receive group-and type-specific red cells that are compatible, an increase in the incidence of alloantibodies and autocontrol positive responses occurs, possibly due to alloimmunisation[15]. The most common alloantibodies found in multitransfused patients are anti-E, anti-Mi (Mur), anti-c and anti-Jk^a [16,17]. Thus, the rapid availability of fully phenotyped RBC antigens is of great advantage. Until now, only limited blood group antigen typing in blood donors has been carried out because complete phenotyping is time-consuming, cumbersome and expensive with the conventional tube technique. Although previous studies determined the prevalence of phenotypes of blood group systems in 500 Thai blood donors, using the gel test in order to save time and reduce laboratory procedures, the rare Jk(a-b-) phenotype was not found[12,18]. When a patient with a Jk(a-b-) phenotype with anti-Jk3 requires blood transfusion for surgery or emergency situations, it is a great concern for the blood bank because few donors can be found throughout the country.

A recent report showed that Jk(a-b-) RBC were resistant to lysis by 2M urea, a common diluent in automated blood analysers[9]. A previous study compared direct and indirect urea-dependent lysis methods for screening for Jk(a-b-) RBC. The indirect method was recommended because the results do not change with extended incubation in the urea solution[10]. In this present study, the direct urea lysis test was used to screen for the Jk(a-b-) phenotype and the incubation time was extended from 5 min up to 40 min with no interference, confirming previous findings that Jk(a-b-) RBC did not haemolyse for at least 15 min in either 2M urea or methylurea, whereas common Jk phenotype RBC were completely haemolysed in 2 min[11,19]. Additionally, the concentration of RBC used for this test can vary from 1 to 5% suspended in PBS, which is convenient for routine testing. However, one limitation of using the urea lysis test alone is that common Jk phenotypes, Jk(a+b-), Jk(a-b+), Jk(a+b+) and Jk(a-b-), cannot be differentiated. The cost of the direct urea lysis test is only • 0.01 per test, about 480 times less than that for an IAT and the test time for each plate (94 tests plus two controls) is approximately 20 min (about 18 times less than IAT).

Even though the prevalence of Jk(a-b-) phenotype was found to be low in the Thai population, as in previous studies, additional screening of blood donors using the urea lysis test is necessary in order to identify more donors suitable for Jk(a-b-) patients. This test can also be used to screen patients' relatives as potential donors, especially when volunteer donors are not available. In addition, further study of this rare phenotype in Thai people, in collaboration with other reference laboratories, is suggested.

In conclusion, screening for Jk(a-b-) phenotypes by the direct urea lysis test using microtitre plates is simple, cost-effective and convenient for mass screening in blood donors, especially in emergency situations.

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