Vol 25 No. 2 March 2019

p-ISSN 0854-4263
e-ISSN 4277-4685

INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

CONTENTS

RESEARCH

LITERATURE REVIEW

CASE REPORT

INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 155
2019 March; 25(2): 155-160
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

PROPORTION OF RHESUS BLOOD PHENOTYPES AT THE BLOOD DONOR UNIT IN BANDUNG CITY

Ivana Dewi, Nadjwa Zamalek Dalimoenthe, Anna Tjandrawati, Nida Suraya

Department of Clinical Pathology, Faculty of Medicine, Padjadjaran University/Dr. Hasan Sadikin Hospital Bandung, Indonesia. E-mail: ivanadewi.md@gmail.com

ABSTRACT

Blood type phenotype incompatibility including Rhesus between donors and patients may result in cross-matching problems, triggering alloimmunization, and causing Hemolytic Transfusion Reaction (HTR). Rhesus blood type incompatibility between mother and fetus may cause Hemolytic Disease of Fetus and Newborn (HDFN). Pretransfusion phenotypic blood groups matching also reduce cost efficiently which means antibody screening is no longer needed. The purpose of the study was to find the proportion of Rhesus blood type phenotypes in routine blood donors at the Blood Donor Unit, Indonesian Red Cross in Bandung city, Indonesia. The study was descriptive and observational with across-sectional design. The study was done at the Blood Donor Unit, Indonesian Red Cross in Bandung city from April 2016 to September 2017. The subjects were 142 routine blood donors. Blood sampling was done simultaneously with blood donation. Rhesus antigen examination of ethylene diamine tetraacetic acid (EDTA) blood was done by gel method. Subjects characteristics were males (68%) and females (32%), with the mean age of 39 years. Examination of Rhesus antigen foundantigen D(100%), antigen e(98.6%), antigen C(97.9%), antigen c(38.7%) and antigen E(31.7%). Results of Rhesus phenotypes were DCe/DCe(61.3%), DCe/DcE(29.6%), DCe/dce(7%), DcE/DcE(1.4%) and DcE/dce(0.7%). The distribution of Rhesus blood type was affected by factors such as genetics, race, ethnicity, marriage, demography, and migration. Rhesus blood phenotypes proportion in routine blood donors at Blood Donor Unit, Indonesian Red Cross in Bandung city sorted from the most were DCe/DCe, DCe/DcE, DCe/dce, DcE/DcE, and DcE/dce.

Key words: Blood donor, phenotype, rhesus

INTRODUCTION

Blood group phenotype is an antigen description which presents in the individual erythrocyte membrane. It is a gene product that can be detected by blood type examination.[1] Blood group system represent from a single gene or a cluster of two or more closely linked homologous genes.[2] Blood group genes can be detected reliably by genotyping, DNA-based techniques, these genes determine the expression of red cell antigen.[3]

The Rhesus (Rh), blood type system, is the primary blood group system besides ABO that essential for transfusion medicine. The Rh blood group is considered to have an essential role because in some cases the Rh incompatibility between donor and the patient showed Hemolytic Transfusion Reaction (HTR) and Rh incompatibility between the mother and fetus causing Hemolytic Disease of the Fetus and Newborn (HDFN).[2]

The cases of HDFN were associated with fetal erythroblastosis, severe icterus, and severe anemia. Study of HDFN in India found that the cause was 22% Rh incompatibility of 50 cases.[4] The HDFN cases were mostly due to Rhesus D antibody but also found other Rh antibodies such as anti-c and anti-E as in case reports in Thailand.[5] Since 1970 there has been a prevention of HDFN due to the incompatibility of Rh D by immunization, since then the incidence of HDFN has dropped dramatically. The case of HTR was reported in India, a pregnant female, multi-transfusion patient due to anti-E.[6] In Indonesia there is no evidence data of HDFN and HTR, it was suspected that the incidence rate is high but not recorded.

Rh blood group differences between donors and patients can also becaused by incompatibility problems in cross-matching. In blood bank of Hasan Sadikin General Hospital, Bandung was found cross-matching incompatibility sometimes although ABO and Rhesus blood group were the same. This finding can be caused by the compatibility of the Rhesus blood group to the donor, and the patient is only based on D antigen alone. In 2016 there were 3-7 incompatible cases of cross-matching tests even though ABO and Rh D blood type of donors and

patients were the same.[7] The impact of incompatible cross-match testing is the increased cost of pretransfusion examination because cross-match testing had to be reperformed with other donor blood flasks.

Another problem in Rh blood group that is still possible that alloimmunization occurred and formation of alloantibody in patients, although pretransfusion examination had been done before. Routine pretransfusion examinations include the same groups of ABO and Rh D blood type and passing a cross-matching test.

Rh phenotypes determination has an important role in preventing incompatibility of cross-matching test, an occurrence of alloimmunization and incidence of transfusion reaction. Data of Rh phenotypes are required in blood banks for various purposes.[8] Rh phenotypes types differ by population, until now there are several known Rh phenotypes which are DCe/dce, DCe/DCe, DcE/dce, DcE/DcE, dce/dce, Dce/dceas in South African population and DCe/dce, DCe/Dce, DCe/DCe, DcE/dce, DcE/DcE, DCe/DcE, DCe/dcE, Dce/dce, dce/dce in Caucasian populations.[9]

The optimal selection of blood donors is essential to achieve safe blood transfusions. Donor phenotypes can influenced donor selection from donor characteristics, ideally, the blood group phenotypes between donors and patients should be the same.[10]

The American Association of Blood Banks (AABB) recommends shorter cross-matching test when antibody screening is added to the pretransfusion examination. Studies have shown that compatible ABO with negative antibody screening makes the patient have no history of unexpected antibodies.[11]

According to Setia *et al*. study in India, pretransfusionscreening and typing of blood types are needed. It is mentioned that the presence of alloantibodies was found in 89 cases in 17,896 subjects.[12] The Chaudhary study in India showed that the evaluation of screening and antibody typing provided 91.6% safety with unexpected antibody 0.75% (15 of 2026 cases).[13]

Unexpected erythrocyte alloantibodies can occur in transfusions by healthy donors. The presence of these antibodies can lead to severe transfusion reactions. There are several cases reports in patients with alloantibodies due to blood transfusion.[14]

Distinct distribution of blood group antigen is clinically important for transfusion medicine. Data of donors blood type antigen is important to adjust between donor and patient antigens. It is hoped that this can reduce the occurrence of alloimmunization, especially in multi-transfusion patients.[15] According to Lau *et al*. study in China, transfusion was done by simply matching the phenotypes between patient and donor without antibody screening, this proved to be more cost-effective.[16] Similar to Pachaury *et al*., and Gundrajakuppam *et al*. study in India, examination of blood type phenotypes as a pretransfusion examination was also suggested, with the phenotypic blood group data banks.The cost of pretransfusion would be much more efficient, particularly in developing countries.[17,18] The HTR case report in Malaysia from Asnawi *et al*. study suggested that screening and identification of antibodies was necessary for patients at the high-risk occurrence of alloantibody and a system for providing phenotypic data to prevent alloimmunization is required.[6]

Pretransfusion examination in Bandung city, for now, is done by ABO and Rhesus D blood type antigen examination. Examination of Rh blood group antigen consists of five antigens which are D, C, c, E, and e. Examination of antigen C, c, E, and e is not routinely performed at the Blood Donor Unit (BDU) Indonesian Red Cross (IRC) in Bandung city.

Examination of Rh phenotypes in blood donors in some countries, for example in Karim study in Pakistan, showed that the most phenotype was Dce/Dce (44%). Chitra study in India showed that the most phenotype was Dce/Dce (35%), rare phenotypes were dce/dce (7%), DcE/DcE (2%), and dCE/dce (2%). Gundrajukuppam study in India showed that the most phenotype was Dce/Dce (43.4%). Musa *et al*. stated the most phenotype was DCe/DCe, the rare phenotypes were DcE/DcE and DcE/DCE in Malaysia. In Indonesia, Rh phenotype study on blood donors has been published before.[19,20,18,21]

The importance of the Rh phenotypes data in blood bank is to find the rare blood type to provide blood donors that have negative antigen for the patient (blood transfusion recipients), especially multi-transfusion patients. The purpose of donor data bank is to minimize the occurrence of alloimmunization and ensuring the safety of blood transfusions for patients besides pretransfusion examination cost may also be lower because screening antibody screening is eliminated.[18,16]

If a patient with a majority Rh phenotype such as Dce/Dce is transfused with donor blood with a rare phenotype such as DcE/DcE then there is a high probability of a transfusion reaction. Transfusion reactions often occur in the Hasan Sadikin General Hospital (HSGH), from January 2016 to April 2017 0.24-0, 60% of cases were recorded.[22] The target of

HSGH quality indicator for transfusion reaction is <0.01%. Blood Donor Unit (BDU) of IRC in Bandung was reported distributed most of the blood donor flasks to HSGH.

Blood Donor Unit of IRC in Bandung city is a reference unit of West Java Province, it is the third largest BDU in Indonesia after DKI Jakarta and East Java. In 2016, the number of blood donors in the Blood Donor Unit of Bandung was as many as 127,764 people, and the number of blood components produced was 159,894 components.[23]

A routine blood donor is a blood donor who has donated blood at least twice a year and has been performed for at least two consecutive years.[24] Research subjects are routine blood donors because routine blood donors have the most significant contribution to the number of blood flasks transfused to patients. The study was conducted at BDU IRC in Bandung city because all the blood donors activities for Bandung area centered in BDU IRC.

The purpose of this study was to find the proportion of Rh phenotypes in routine blood donors in BDU IRC in Bandung city. Based on this study it was expected to find the proportion of routine blood donors phenotypes to obtain the blood donors Rh phenotypes data and hopefully in the future can be used for safe transfusion by adjusting the donors and patients Rh phenotypes. When safe transfusion is achieved then the incidence of transfusion reactions and alloimmunization can be minimized.

METHODS

The study was descriptive and observational with across-sectional design. The subjects of the study were routine blood donors who donated blood to BDU IRC in Bandung city. Inclusion criteria were reguler blood donors who made a blood donation (after declared being healthy by physician). Exclusion criteria were not 5% erythrocyte suspension from the examination of Rh antigen, and the result does not come out or invalid results.

The research material used was ethylenediaminetetraacetic acid (EDTA) blood for Rh phenotypes examination, using 5% erythrocyte suspension. Investigation performed was the examination of Rh phenotypes based on antigen C, c, E, and e, using the principle of antibody-antigen examination: hemagglutination by gel method.

RESULTS AND DISCUSSION

During the sample collection period, the subjects were 142 donors. Characteristics of the study subjects covering the gender are listed in Table 1. In this study, the number of male blood donors was more frequent than females, similarly found in Sarkar *et al*. and Gundrajukuppam *et al*. study.[8,18]

Table 1. The characteristics of subjects

Characteristic	Total (n=142)	
	n	%
Gender		
Male	97	68
Female	45	32

In this study the age of the subjects was 18-63 years, with a mean age 39 years, the normality test data showed normal distribution. The results of the Rh antigen examination in 142 subjects are shown in Figure 1.

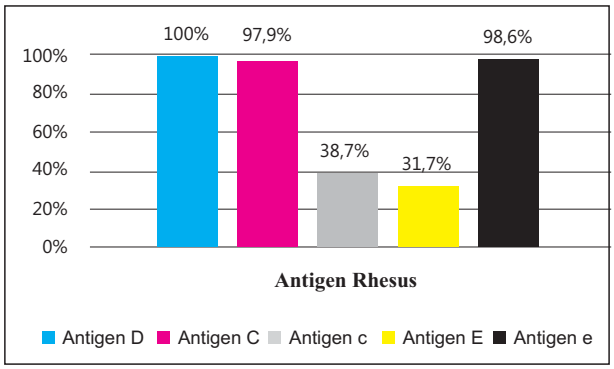

Figure 1. Rhesus antigen examination

Based on the results of examination of Rhesus antigen, and then performed an interpretation of Rhesus blood type phenotypes and obtained five Rhesus blood type phenotypes, which can be seen in Table 2.

In 142 subjects antigen D was 100% (n = 142). Other studies, where the majority of blood donors had antigen D were Musa *et al*. (97.5%), Garg *et al*. (93.8%), Gundrajukuppam *et al*. (94.1%), Karim *et al*. (97%), and Thakral *et al*. (93.4%).[21,25,18,19,26]

Antigen e was the second commonly found in blood donors antigen which was 98.6% (n = 140), then the next antigen often found was 97.9% (n = 139) C antigen, e antigen, and C antigen were frequent antigens found to be similar to those studies of Garg *et al*., Gundrajukuppam *et al*., Karim *et al*., Thakral *et al*.[25,18,19]

Antigen c and E were found to vary between this study with other studies such as Garg *et al*., Gundrajukuppam *et al*., Karim *et al*., Thakral *et al*., Sarkar *et al*.[25,18,19,26,8] Comparison of Rh blood group antigens in this study with other studies are shown in Table 3.

Table 2. Rhesus antigen examination and phenotypes interpretation

No	Antigen					Phenotypes Interpretation	Total (n=142)	
	D	C	c	E	e		n	%
1	+	+	-	-	+	DCe/Dce (R1R1)	87	61.3
2	+	+	+	+	+	DCe/DcE(R1R2)	42	29.6
3	+	+	+	-	+	DCe/dce(R1r)	10	7.0
4	+	-	+	+	-	DcE/DcE(R2R2)	2	1.4
5	+	-	+	+	+	DcE/dce(R2r)	1	0.7
Total							**142**	**100**

Table 3. Comparison of Rhesus antigen

Studies	Population	Rhesus Antigen					Total sample
		D(%)	C(%)	c(%)	E(%)	e(%)	
This study	Indonesia	100	97.9	38.7	31.7	98.6	142
Garg *et al.*[25]	India	93.8	91.8	55.2	21.1	98.7	2.769
Gundrajukuppam *et al.*[18]	India	94.1	88	54.9	18.8	98.4	1.000
Karim *et al.*[19]	Pakistan	97	87	57	19	99	100
Thakral *et al.*[26]	India	93.4	84.8	52.8	17.9	98.3	1.240

Table 4. Rhesus blood type phenotypes comparison

The proportion of Rhesus phenotypes	This study		Musa *et al.*[21]		Garg *et al.*[25]		Karim *et al.*[19]		Thakral *et al*[26]	
	n	%	n	%	N	%	N	%	n	%
Population	**Indonesia**		**Malaysia**		**India**		**Pakistan**		**India**	
DCe/DCe (R1R1)	87	61.3	330	56	1235	44.6	41	41	543	43.8
DCe/DcE (R1R2)	42	29.6	113	19	389	14	10	10	102	8.3
DcE/DcE (R2R2)	2	1.4	28	5	23	0.8	1	1	18	1.5
DCe/dce (R1r)	10	7.0	76	13	902	32.6	34	34	372	30
DcE/dce (R2r)	1	0.7	19	3	163	5.9	8	8	111	8.9
Dce/dce (R0r)	-	-	-	-	55	2	3	3	12	1
Dce/dce (rr)	-	-	12	2	2	0.1	1	1	72	5.8
dCe/dCe (r′ r′)	-	-	-	-	-	-	1	1	7	0.5
dCe/dcE (r′ r′ ′)	-	-	-	-	-	-	1	1	3	0.2
DcE/DCE (R1Rz)	-	-	14	2	-	-	-	-	-	-
DcE/DCE (R2Rz)	-	-	2	0	-	-	-	-	-	-
Total	142		594		2,769		100		1,240	

In the results of this study obtained five Rh blood group phenotypes. The comparison between the Rh-blood phenotypes between this study and other studies is shown in Table 4.

The distribution of blood types including Rhesus are influenced by various factors such as genetics, race, ethnicity, marriage, demography, and migration.[27,28]

In the study of Karim *et al*., with the Pakistani population, more phenotypic variants were found than this study where as the number of subjects was fewer.[19] This is probably because Pakistan is a multi-racial and multi-ethnic country, where all of these factors can lead to more phenotypic variants.[29]

The Rhesus blood type phenotypes variation in this study played a role in the frequency of alloimmunization and alloantibodies if the donor and patient phenotypes were different. In thalassemia patients, the prevalence of alloimmunizationwas about 4-50%, the more homogeneous phenotype in a population the lower the risk of alloimmunization. In Dhawan *et al*. study mentioned that the alloimmunization prevalence was 5.64%, in which 52.17% was caused by Rh antibodies (Anti E 17%, Anti D 13%, and Anti C 13%).[30] These Rh antibodies could result in incompatibilities in cross-matching, an occurrence of HDFN and HTR. Agnihotri study in India, the

prevalence of alloantibodies was 0.8% and the Rh antibody found was 41.6%.[31,18] Limitations of the study was the number of subjects, if the amount was larger, there was a possibility that more variations of Rhesus blood type phenotypes could be found.

CONCLUSION AND SUGGESTION

The proportion of Rhesus blood type phenotypes in routine blood donor in BDU IRC Bandung, sorted from the most were DCe/DCe, DCe/DcE, DCe/dce, DcE/DcE, and DcE/dce. It is needed to do another research with a more significant number of samples so the possibility of complete phenotypes variation may be found.

Rhesus phenotypes data bank for donors and patients is needed to be created so that in the future it can be used to improve the efficiency of the blood bank and reduce the risk of alloimmunization and transfusion reaction.

REFERENCES

1. Leger RM. Blood group terminology and other blood groups. In: Modern blood banking & transfusion practices. 6th Ed., Philadelphia, FA Davis Company, 2012; 175-91.
2. Daniels G. Human blood groups. 3rd Ed., West Sussex, Wiley-Blackwell, John Wiley and Sons Ltd, 2013; 182-236.
3. Flegel WA, Gottschall JL, Denomme GA. Implementing mass-scale red cell genotyping at the blood center. Transfusion, 2015; 55(11): 2610-15.
4. Kumar A, Patel MK, Chavda B, Ranjan A, Ahmad F. Hemolytic disease of the newborn: A study of 50 cases. IJSS, 2013; 1(3): 1-5.
5. Menuam T, Juengpichanvanich N, Charoenkwan P, Sakulwattana M, Cheepsattayakorn R, Fongsatitkul L, *et al*. Hemolytic disease of the fetus and newborn due to antibodies to minor red blood cell groups: Two case reports. Chiang Mai Med J, 2012; 51(4): 119-23.
6. Asnawi AWA, Sathar J, Mohamed R, Deraman R, Kumaran S, Hamid SSA, *et al*. Fatal delayed hemolytic transfusion reaction and hyperhemolysis syndrome in pregnant women with sickle cell anemia. Indian J Hematol Blood Transfus, 2015; 32(1): 251-3.
7. Pencatatan Darah Donor Inkompatibel. Bank Darah Rumah Sakit Hasan Sadikin. Bandung. 2016.
8. Sarkar BRS, Philip CJ, Mallhi SCR, Yadav DCP. The proportion of Rh phenotypes in voluntary blood donors. Med J Armed Forces India, 2013; 69(4): 330-4.
9. Blaney KD, Howard PR. Basic and applied concepts of blood banking and transfusion practices. 3rd Ed., Missouri, Elsevier, 2013; 1-18, 107-125.
10. Chasse M, Mc Intyre L, English SW, Tinmouth A, Knoll G, Wolfe D, *et al*. Effect of blood donors characteristics on transfusion outcomes: A systematic review and meta-analysis. Transfus Med Rev, 2016; 30: 69-80.
11. Downes KA, Shulman IA. Pretransfusion testing. Roback JD, Grossman BJ, Harris T, Hillyer CD, editor. In: AABB technical manual. 18th Ed., Bethesda, AABB Press, 2014; 367-82.
12. Setia R, Sachdeva P, Arora S, Handoo A, Kapoor M. Making type and screen policy an essential component of pretransfusion testing: Need of the hour in India. Glob J Transfus Med, 2017; 2(1): 34-7.
13. Chaudhary R, Agarwal N. Safety of type and screen method compared to conventional antiglobulin cross-match procedures for compatibility testing in Indian setting. Asian J Transfus Sci, 2011; 5(2): 157-9.
14. Patidar GK. Antibody screening of healthy blood donors: It is time to make it mandatory. J Blood Disorders Transf, 2015; 6(1): 245.
15. Lamba DS, Kaur R, Basu S. Clinically significant minor blood group antigens amongst North Indian donor population. Adv Hematol, 2013; 2013.
16. Lau FY, Wong R, Chan NPH, Chui CH, Ng E, Ng MHL, *et al*. Provision of phenotype-matched blood units: No need for pretransfusion antibody screening. Hematol, 2001; 86(7): 742-8.
17. Pachaury R, Arya DR, Mahawar NL, Bharti A, Das PK. The frequency of Rh phenotypes in voluntary blood donors. JMSCR, 2017; 5(7): 25083-8.
18. Gundrajukuppam DK, Vijaya SBK, Rajendran A, Sarella JD. Prevalence of principal Rh blood group antigens in blood donors at the blood bank of a tertiary care hospital in Southern India. J Clin Diagn Res, 2016; 10(5): EC07-10.
19. Karim F, Moiz B, Muhammad FJ, Ausat F, Khurshid M. Rhesus and Kell phenotyping of voluntary blood donors: Foundation of a donor data bank. J Coll Physicians Surg Pak, 2015; 25(10): 757-60.
20. Chitra M, Jagannathan SY, Arumugam P, Ravishankar J. Prevalence of Rh antigens among voluntary blood donors in Chennai, Tamil Nadu, India. Int J Res Med Sci, 2016; 4(12): 5360-3.
21. Musa RH, Ahmed SA, Hashim H, Ayob Y, Asidin NH, Choo PY, *et al*. Red cell phenotyping of blood from donors at the national blood center of Malaysia. Asian J Transfus Sci, 2012; 6(1): 3-9.
22. Laporan indikator mutu. Bank Darah Rumah Sakit Hasan Sadikin. Bandung.
23. Laporan kegiatan unit donor darah tahun 2016. Palang Merah Indonesia kota Bandung.
24. WHO. Blood donor selection. Guidelines on assessing donor suitability for blood donation. Geneva, Switzerland, WHO Press, 2012; 16-48.
25. Garg N, Singh DK, Tomar R, Singh B. Phenotype prevalence of blood group systems (ABO, Rh, Kell) involuntary, healthy donors-experience of a tertiary care hospital in Delhi, North India. J Blood Disord Transfus, 2015; 6(4): 1-4.
26. Thakral B, Saluja K, Sharma RR, Marwaha N. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in North Indian blood donors. Transfus Apher Sci, 2010; 43(1): 17-22.
27. Dewan G. Comparative frequency and allelic

distribution of ABO and Rh (D) blood groups of major tribal communities of Southern Bangladesh with general population and their determinants. EJMHG, 2015; 16(2): 141-7.
28. Tesfaye K, Petros Y, Andargie M. Frequency distribution of ABO and Rh (D) blood group alleles in Silte Zone, Ethiopia. EJMHG, 2015; 16(1): 71-6.
29. Shehzad A. The issue of ethnicity in Pakistan: Historical background. J Pakistan Vision, 2011; 12(2): 124-30.
30. Dhawan H, Kumawat V, Marwaha N, Sharma R, Sachdev S, Bansai D. Alloimmunization and autoimmunization in transfusion-dependent thalassemia major patients: Study on 319 patients. Asian J Transfus Sci, 2014; 8(2): 84-8.
31. Agnihotri A. Antibody screening with the Asian cell panel: Can we still do away with cross-match Asian J Transfus Sci, 2014; 8(2): 26-8.