CONTENTS

RESEARCH

Differences of Plasma Interleukin-6 and Tumor Necrosis Factor-A Levels in Healthy People, Rifampicin Resistant and Sensitive Pulmonary Tuberculosis Patients
Wahyu Setiani Wibowo, Jusak Nugraha, Soedarsono .............................................................. 129 - 134

Association between Specific Enolase Serum Levels and Outcome Acute Ischemic Stroke One Month After Onset
Yuri Haiga, Darwin Amir, Yuliarni Syafrita ................................................................................ 135 - 139

Analysis of Hemoglobin Levels And Leukocyte Count in Neonates with Hyperbilirubinemia
Dewi Suharti, Sulina Yanti Wibawa, Muthmainnah ............................................................... 140 - 144

Diagnostic Value of Ca-125 in Patients with Epithelial Ovarian Cancer at the Dr. Soetomo General Hospital Surabaya in 2016
Kintan P. R. Kania, Betty A. Tambunan, Willy Sandhika .......................................................... 145 - 149

Analysis of Vitamin D in Patients with Type 2 Diabetes Mellitus
Arfandhy Sanda, Uleng Bahrun, Ruland DN. Pakasi, Andi Makbul Aman ....................................... 150 - 154

Proportion of Rhesus Blood Phenotypes at the Blood Donor Unit in Bandung City
Ivana Dewi, Nadjwa Zamalek Dalimoenthe, Anna Tjandrawati, Nida Suraya ............................... 155 - 160

Correlation of Total Lymphocyte Count with CD4 Count in HIV/TB Coinfected Patients
Herniaty Rampo, Uleng Bahrun, Mansyur Arif ......................................................................... 161 - 164

Using Six Sigma to Evaluate Analytical Performance of Hematology Analyzer
Robiul Fuadi ............................................................................................................................... 165 - 169

Correlation of AA Index with Degree of Liver Fibrosis in Chronic Hepatitis B Patients
Rika Andriany, Ibrahim Abdul Samad, Mansyur Arif ................................................................. 170 - 173

Difference in HbA1c Level between Boronate Affinity and Ion Exchange-High Performance Liquid Chromatography Method in Diabetic Patient
Tuti Asryani, Elyza Nasrul, Rikarni, Tutty Prihandani ............................................................... 174 - 179

Diagnostic Value of Neutrophil Lymphocyte Ratio to Differentiate Ischemic and Hemorrhagic Stroke
Martina Rentaulli Shombing, Liong Boy Kurniawan, Darwati Muhadi ......................................... 180 - 183

D-Dimer and Fibrinogen in Patients Underwent Surgery in Malignant and Benign Ovarian Tumor
Ismail Aswin, Herman Hariman, Fauzie Sahil ............................................................................... 184 - 190
Relationship between Specific Gravity of Cupric Sulfate and Saturation of Blood Droplets During Donor’s Hemoglobin Screening

**Resna Hermawati, Solichul Hadi** .......................................................... 191 - 193

Vancomycin-Resistant *Staphylococcus aureus* at the Dr. Wahidin Sudirohusodo Hospital Makassar

**Fatmawaty Ahmad, Nurhayana Sennang, Benny Rusli** ........................................ 194 - 198

The Levels of Interleucin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-ALFA) in Preeclampsia Patient and Normal Pregnancy

**Mawardi, Ratna Akbari Ganie, Sarma N. Lumbanraja** .................................................. 199 - 201

Analysis of Platelet Volume Mean, Platelet Distribution Width, and Platelet Count in Hemorrhagic and Non-Hemorrhagic Stroke

**Gita Medita Sunusi, Darwati Muhadi, Mansyur Arif** .................................................. 202 - 206

High Fluorescent Lymphocyte Count Examination in Dengue Hemorrhagic Patients with Sysmex Xn-1000 Hematology Analyzer

**Budiono Raharjo, Solichul Hadi** .......................................................... 207 - 210

Prevalence and Characteristics of Multidrug-Resistant *Acinetobacter baumannii* Cases at the Dr. Wahidin Sudirohusodo General Hospital in Makassar

**Dewi Kartika Tungadi, Nurhayana Sennang, Benny Rusli** ........................................ 211 - 217

The Correlation of Anemia and Hepcidin Serum Levels in Regular Hemodialysis Patients with Chronic Hepatitis C

**Wingsar Indrawanto, Adi Koesoema Aman, Alwi Thamrin** ........................................ 218 - 223

The Comparison between HbA1c and Glycated Albumin Level Patient with Type II Diabetes Mellitus with or without CKD

**M. Rusli, Zulfikar, Santi Syafril** .......................................................... 224 - 227

Differentiation of γδ Lymphocyte Cells Expressing Interleukin-17 on Healthy Persons and Adult Acute Myeloid Leukemia Patients

**Elvan Dwi Widyadi, Yetti Hernaningsih, Endang Retnowati, Ugroseno, Ryzky Widi Atmaja** ........................................ 228 - 232

**LITERATURE REVIEW**

Hormone Examination in Menopause

**Ferdy Royland Marpaung, Trieva Verawaty Butarbutar, Sidarti Soehita** ........................................ 233 - 239

**CASE REPORT**

Chronic Myelogeneous Leukemia Transformation into Acute Lymphoblastic Leukemia

**Endah Indriastuti, Arifoel Hajat** .......................................................... 240 - 245

Rapid Progression of Clavicular Solitary Plasmacytoma to Multiple Myeloma

**Hantoro Gunawan, Paulus Budiono Notopuro** .......................................................... 246 - 249
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 found antigen 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. Blood group system represent from a single gene or a cluster of two or more closely linked homologous genes. Blood group genes can be detected reliably by genotyping, DNA-based techniques, these genes determine the expression of red cell antigen.

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).

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. 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. 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. 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 because 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
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. Rh phenotypes 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 in Caucasian populations.

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.

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.

According to Setia et al. study in India, pretransfusion screening and typing of blood types are needed. It is mentioned that the presence of alloantibodies was found in 89 cases in 17,896 subjects. 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).

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.

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. 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. Similar to Pachauri 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. 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.

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%). Gundrajakuppam 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.

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.

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. 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.

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. 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 regular 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.

Table 1. The characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n=142)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>n</td>
</tr>
<tr>
<td>Male</td>
<td>97</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
</tr>
</tbody>
</table>

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](image)

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%), Gundrajukuppam et al. (94.1%), Karim et al. (97%), and Thakral et al. (93.4%).

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., Sarkar et al.

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. Comparison of Rh blood group antigens in this study with other studies are shown in Table 3.
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.  

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. This is probably because Pakistan is a multi-racial and multi-ethnic country, where all of these factors can lead to more phenotypic variants.  

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%). These Rh antibodies could result in incompatibilities in cross-matching, an occurrence of HDFN and HTR. Agnihotri study in India, the

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### Table 2. Rhesus antigen examination and phenotypes interpretation

<table>
<thead>
<tr>
<th>No</th>
<th>D</th>
<th>C</th>
<th>c</th>
<th>E</th>
<th>e</th>
<th>Phenotypes Interpretation</th>
<th>Total (n=142)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>DcE/Dce (R1R1)</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>DcE/DcE (R1R2)</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>DcE/dce (R1r)</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>DcE/DcE (R2R2)</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>DcE/dce (R2r)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Comparison of Rhesus antigen

<table>
<thead>
<tr>
<th>Studies</th>
<th>Population</th>
<th>Rhesus Antigen</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D(%)</td>
<td>C(%)</td>
</tr>
<tr>
<td>This study</td>
<td>Indonesia</td>
<td>100</td>
<td>97.9</td>
</tr>
<tr>
<td>Garg et al.</td>
<td>India</td>
<td>93.8</td>
<td>91.8</td>
</tr>
<tr>
<td>Gundrajukuppam et al.</td>
<td>India</td>
<td>94.1</td>
<td>88.0</td>
</tr>
<tr>
<td>Karim et al.</td>
<td>Pakistan</td>
<td>97</td>
<td>87</td>
</tr>
<tr>
<td>Thakral et al.</td>
<td>India</td>
<td>93.4</td>
<td>84.8</td>
</tr>
</tbody>
</table>

### Table 4. Rhesus blood type phenotypes comparison

<table>
<thead>
<tr>
<th>The proportion of Rhesus phenotypes</th>
<th>This study</th>
<th>Musa et al.</th>
<th>Garg et al.</th>
<th>Karim et al.</th>
<th>Thakral et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Indonesia</td>
<td>Malaysia</td>
<td>India</td>
<td>Pakistan</td>
<td>India</td>
</tr>
<tr>
<td>DcE/DcE (R1R1)</td>
<td>87</td>
<td>61.3</td>
<td>330</td>
<td>56</td>
<td>1235</td>
</tr>
<tr>
<td>DcE/DcE (R1R2)</td>
<td>42</td>
<td>29.6</td>
<td>113</td>
<td>19</td>
<td>389</td>
</tr>
<tr>
<td>DcE/dce (R2R2)</td>
<td>2</td>
<td>1.4</td>
<td>28</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>DcE/dce (R1r)</td>
<td>10</td>
<td>7.0</td>
<td>76</td>
<td>13</td>
<td>902</td>
</tr>
<tr>
<td>DcE/dce (R2r)</td>
<td>1</td>
<td>0.7</td>
<td>19</td>
<td>3</td>
<td>163</td>
</tr>
<tr>
<td>Dce/dce (R0r)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Dce/dce (rr)</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>dce/dCe (r’ r )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>dce/dCe (r’ r’ )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>DcE/DcE (R1Rz)</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>DcE/DcE (R2Rz)</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>594</td>
<td>2,769</td>
<td>100</td>
<td>1,240</td>
</tr>
</tbody>
</table>
prevalence of alloantibodies was 0.8% and the Rh antibody found was 41.6%.

**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.

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