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Majalah Patologi Klinik Indonesia dan Laboratorium Medik

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# INDONESIAN JOURNAL OF CLINICAL PATHOLOGY AND MEDICAL LABORATORY

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## CONTENTS

### RESEARCH

|   |         |
|---|---------|
| Leukocyte Interference on Hemoglobin Examination in Hematology Malignancy<br>(Pengaruh Jumlah Leukosit terhadap Kadar Hemoglobin pada Keganasan Hematologi)<br><b>Trinil Sulamit, Fery H. Soedewo, Arifoel Hajat</b> .....  | 203–207 |
| The Analysis of Calcium Level in Stored Packed Red Cells<br>(Analisa Kadar Kalsium Darah Simpan Packed Red Cells)<br><b>Suryani Jamal, Rachmawati Muhiddin, Mansyur Arif</b> .....  | 208–210 |
| Correlation between Matrix Metalloproteinase 1 Serum Levels and Model of End Stage Liver Disease Score in Patients with Hepatic Cirrhosis<br>(Kenasaban Kadar Matrix Metalloproteinase 1 Serum Terhadap Skor Model End Stage Liver Disease di Pasien Sirosis Hati)<br><b>Stephanus Yoanito, Siti Muchayat</b> ..... | 211–215 |
| Relationship between D-Dimer Level and Clinical Severity of Sepsis<br>(Hubungan antara Kadar D-dimer dan Tingkat Keparahan Klinis di Sepsis)<br><b>Yessy Puspitasari, Aryati, Arifoel Hajat, Bambang Pujo Semedi</b> .....  | 216–220 |
| Comparison of Factor VIII Activity in O and Non-O Blood Types<br>(Perbandingan Aktivitas Faktor VIII Antara Golongan Darah O dan Non-O)<br><b>Adil Dinata Simangunsong, Yetti Hernaningsih</b> .....  | 221–224 |
| Apo B/Apo A-I Ratio in Patients with Stenosis Coronary Heart Disease Greater or Less than 70%<br>(Rasio Apo B/Apo A-I di Pasien Penyakit Jantung Koroner dengan Stenosis Lebih Besar Atau Kecil 70%)<br><b>Dedi Ansyari, Tapisari Tambunan, Harris Hasan</b> .....  | 225–229 |
| Analysis of Dengue Specific Immune Response Based on Serotype, Type and Severity of Dengue Infection<br>(Analisis Respons Imun Spesifik Dengue terhadap Serotipe, Jenis dan Derajat Infeksi Virus Dengue)<br><b>Ade Rochaeni, Aryati Puspa Wardhani, Usman Hadi</b> .....   | 230–233 |
| Neutrophil/Lymphocyte Count Ratio on Dengue Hemorrhagic Fever<br>(Rasio Netrofil/Limfosit Pada Demam Berdarah Dengue)<br><b>Irmayanti, Asvin Nurulita, Nurhayana Sennang</b> .....  | 234–239 |
| Neutrophil-Lymphocyte Ratio and High Sensitivity C-Reactive Protein as Ischemic Stroke Outcome Predictor<br>(Rasio Neutrofil–Limfosit dan High Sensitivity C–Reactive Protein sebagai Peramal Hasil Strok Iskemik Akut)<br><b>Tissi Liskawini Putri, Ratna Akbari Ganie, Aldy S. Rambe</b> .....                    | 240–245 |
| Analysis of Rhesus and Kell Genotype in Patients with Transfusion Reaction<br>(Analisis Genotipe Rhesus dan Kell Pasien dengan Reaksi Transfusi)<br><b>Sukmawaty, Rachmawati Muhiddin, Mansyur Arif</b> .....   | 246–250 |

|   |         |
|---|---------|
| Diagnostic Value of <i>Fastsure TB DNA Rapid Test</i> for Diagnosis of Pulmonary Tuberculosis<br>( <i>Nilai Diagnostik dari Uji Cepat Fastsure TB DNA untuk Diagnosis Tuberkulosis Paru</i> )<br><b>Diyan Wahyu Kurniasari, Jusak Nugraha, Aryati</b> .....   | 251–256 |
| Neutrophil-Lymphocyte Count Ratio in Bacterial Sepsis<br>( <i>Rasio Neutrofil-Limfosit Pada Sepsis Bakterial</i> )<br><b>Danny Luhulima, Marwito, Eva O</b> .....   | 257–262 |
| Comparison of Percentage Peripheral Blood Lymphoblast Proliferation and Apoptosis in Pediatric Acute Lymphoblastic Leukemia Before and After Chemotherapy Induction Phase<br>( <i>Perbandingan Persentase Proliferasi dan Apoptosis Limfoblas di Darah Tepi di Pasien Leukemia Limfoblastik Akut Anak Sebelum dan Sesudah Kemoterapi Tahap Induksi</i> )<br><b>Farida Nur'Aini, Endang Retnowati, Yetti Hernaningsih, Mia Ratwita A</b> ..... | 263–268 |
| Analysis of Erythrocyte Indices in Stored Packed Red Cells at The Blood Bank of Dr. Wahidin Sudirohusodo Hospital<br>( <i>Analisis Indeks Eritrosit Darah Simpan Packed Red Cells di Bank Darah RSUP Dr. Wahidin Sudirohusodo Makassar</i> )<br><b>Fitrie Octavia, Rachmawati Muhiddin, Mansyur Arif</b> .....  | 269–274 |
| Correlation of Urine N-Acetyl-Beta-D-Glucosaminidase Activity with Urine Albumin Creatinine Ratio in Type 2 Diabetes Mellitus<br>( <i>Kenasaban Aktivitas N-Asetil-Beta-D-Glukosaminidase Air Kemih dengan Air Kemih Albumin Kreatinin Rasio di Diabetes Melitus Tipe 2</i> )<br><b>Melly Ariyanti, Lillah, Ellyza Nasrul, Husni</b> .....  | 275–280 |
| Agreement of Simplified FencI-Stewart with Figge-Stewart Method in Diagnosing Metabolic Acidosis in Critically Ill Patients<br>( <i>Kesesuaian Metode FencI-Stewart yang Disederhanakan dengan Figge-Stewart dalam Mendiagnosis Asidosis Metabolik di Pasien Critically Ill</i> )<br><b>Reni Lenggogeni, Rismawati Yaswir, Efrida, Desywar</b> .....  | 281–286 |
| Comparison of Peripheral Blood Activated NK Cell Percentage Before and After Induction Phase Chemotherapy in Pediatric Acute Lymphoblastic Leukemia<br>( <i>Perbandingan Persentase Sel NK Teraktivasi Darah Tepi Sebelum dan Sesudah Kemoterapi Tahap Induksi di Pasien Leukemia Limfoblastik Akut Anak</i> )<br><b>Syntia TJ, Endang Retnowati, Yetti Hernaningsih, I Dewa Gede Ugrasena, Soeprapto Ma'at</b> .....                         | 287–293 |
| LITERATURE REVIEW   |         |
| Quality of Stored Red Blood Cells<br>( <i>Kualitas Sel Darah Merah Simpan</i> )<br><b>Anak Agung Wiradewi Lestari, Teguh Triyono, Usi Sukoroni</b> .....  | 294–302 |
| CASE REPORT   |         |
| A Thirty-One-Years-Old Female with SLE and Systemic Scleroderma<br>( <i>Perempuan Usia 31 Tahun dengan SLE dan Skleroderma Sistemik</i> )<br><b>Rahardjo, Rachmawati</b> .....  | 303–309 |

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Rismawati Yaswir, Nurhayana Sennang Andi Nanggung, Adi Koesoema Aman, Osman sianipar,  
Purwanto AP, Budi Mulyono, Jusak Nugraha, Rahajuningsih Dharma

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## RESEARCH

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# THE ANALYSIS OF CALCIUM LEVEL IN STORED PACKED RED CELLS

*(Analisa Kadar Kalsium Darah Simpan Packed Red Cells)*

**Suryani Jamal, Rachmawati Muhiddin, Mansyur Arif**

### ABSTRAK

Penelitian ini bertujuan mengetahui; pengaruh penyimpanan terhadap kadar kalsium pada darah simpan Packed Red Cells (PRC); menentukan kadar kalsium pada hari ke-7, hari ke-21 dan hari ke-35 dan membandingkan kadar kalsium hari ke-7 dan hari ke-21, hari ke-7 dan hari ke-35, hari ke-21 dan hari ke-35. Penelitian ini dilaksanakan di Bank Darah dan Laboratorium Patologi Klinik RSUP Dr. Wahidin Sudirohusodo. Metode yang digunakan dalam penelitian ini adalah observasional dengan pendekatan potong lintang. Pengambilan sampel dilakukan di semua darah simpan Packed Red Cells (PRC) yang disalurkan di Bank Darah RSUP Dr. Wahidin Sudirohusodo. Data dianalisis dengan uji statistik Repeated Anova Test. Hasil penelitian menunjukkan bahwa terdapat perbedaan bermakna kadar kalsium darah simpan PRC akibat pengaruh penyimpanan pada hari ke-7 dan hari ke-21, pada hari ke-7 dan hari ke-35 serta hari ke-21 dan hari ke-35. Penurunan kadar kalsium terjadi karena eritrosit sudah mulai terjadi lisis.

**Kata kunci:** Kadar kalsium, packed red cells, lama penyimpanan

### ABSTRACT

This study purpose was to know; the effects of storage to calcium level in Packed Red Cells (PRC); to determine the calcium level on the 7<sup>th</sup>, the 21<sup>st</sup> and the 35<sup>th</sup> days, and to compare the calcium level on the 7<sup>th</sup>, the 21<sup>st</sup> and 35<sup>th</sup> days. This study was conducted at the Blood Bank and Clinical Pathology Laboratory of the Dr. Wahidin Sudirohusodo Hospital. The method used in this study was an observational method with cross-sectional approach. The samples were taken from all Packed Red Cells (PRC) distributed in the Blood Bank of the Dr. Wahidin Sudirohusodo Hospital. The data were analyzed with Repeated Anova test. The result of study showed that there were significant differences of calcium levels in blood stored Packed Red Cells (PRC) because of the effect of storage on the 7<sup>th</sup> and the 21<sup>st</sup> days, the 7<sup>th</sup> and the 35<sup>th</sup> days and also on the 21<sup>st</sup> and the 35<sup>th</sup> days. The reduction of calcium level occurred because of lyses in erythrocytes.

**Key words:** Calcium level, packed red cells, periods of storage

## INTRODUCTION

Storage of red blood cells in a preservative medium is associated with harmful metabolic, biochemical and molecular changes to erythrocytes: these changes are collectively referred to as "storage lesions". Blood products such as RBC stored with additive solutions in different temperatures contribute significantly storage lesions. The most probable sites of damage will be cytoskeletal proteins in RBC membrane. These membrane changes will lead RBC to become fragile and increased osmotic fragility and changes in electrolyte imbalance. Great efforts have been done

to provide a suitable and a safe supply of blood with more benefits than side effects. Currently, the storage procedures of blood bags in blood banks require some conditions to ensure the maximum storage time for a healthy and safe blood supply. Nowadays, blood bags can be stored up to 42 days at 2–6°C, as long as the mean hemolysis does not exceed 0.8% and more than 75% red blood cells survive in the first 24 hours after transfusion. However, pathological consequences can affect the stored blood: they are termed as storage lesions. Storage lesions are hypothesized to decrease the efficiency of stored blood and decrease their ability to act their required role after transfusion, but

these hypotheses have no clear evidences yet. Some reversible changes result from stored blood such as decreased ATP dan 2,3 DPG. However, some other damage is irreversible and includes increased osmotic fragility, small echinocytic rigid red blood cells with reduced function, microvesiculation and hemolysis.<sup>1</sup>

Calcium is an universal molecule involved in the cell cycle, metabolism and structural integrity, motility, and volume. The calcium levels in the intracellular of erythrocytes in the human circulation, are not only for controlling biophysical membrane composition, the volume but also physiologic parameters such as metabolic activity and cell clearance. The low basal permeability of erythrocyte membranes to calcium and the power calcium pump maintain free calcium levels intracellular between 30 and 60 nM while average the blood plasma calcium 1.8 nM. Thus, activation of taking calcium had a strong influence on many processes in cells that make calcium as a regulator in erythrocytes.<sup>2,3</sup>

Packed Red Cells (PRC) obtained from the centrifugation of whole blood by eliminating the plasma. The solution most commonly used as an anticoagulant is CPDA-1 (Citrate Phosphate Dextrose Adenine-1), that purposed to maintain the viability of erythrocytes, optimizing pH during storage and can store blood up to 35 days at temperatures 1°-6°C. During storage the erythrocytes undergo similar changes occurring in the body (in vivo).<sup>4,5</sup>

Calcium ions ( $\text{Ca}^{2+}$ ) actively pumped from intracellular to extracellular, through the energy that relies on calcium ATPase pump. Calmodulin, a cytoplasmic protein binding calcium, controls calcium pump to prevent intracellular  $\text{Ca}^{2+}$  overload, which can make deformation of RBC and the structure of RBC becomes more rigid. In the state of ATP levels decreased,  $\text{Na}^+$  and  $\text{Ca}^+$  intracellular become accumulated and decreased levels of  $\text{K}^+$  and water intracellular leads to dehydration and stiffness of RBC.<sup>6</sup>

Based on research conducted by Sulemanji *et al.*<sup>7</sup> in 2011 in Boston, there was a decrease in calcium levels up to 50% of baseline values in vitro experiments on fresh frozen plasma and whole blood. This study found that citrate binds with calcium, causing hypocalcemia.<sup>7</sup>

Based on the theory of calcium in erythrocytes and from previous studies that support this theory, it was found that calcium levels getting low in stored PRC. It indicated that the lysis of erythrocytes had already occurred.

## METHODS

This study was an observational study with cross-sectional study. Research was conducted at the Blood Bank Unit Dr. Wahidin Sudirohusodo Hospital Makassar starting in July–August 2015. Feasibility of ethics derived from Health Research Ethics Committee (KEPK) Faculty of Medicine, Hasanuddin University.

The samples were stored PRC taken from blood bag tubing which was divided into 3 parts and then stored in the blood bank refrigerator (2°–8°C) and levels of calcium was examined by using ABX Pentra 400.

Test results were analyzed using SPSS version 22. The statistic method used was a numerical comparative test pairing more than two measurements. (Repeated Anova Test) to know the difference in calcium levels between the storage time 7<sup>th</sup> day, 21<sup>st</sup> day and 35<sup>th</sup> day and are presented in tables and figures. The test results were statistically significant if the p-value <0.05.

## RESULTS AND DISCUSSION

The research was conducted at the Blood Bank Dr. Wahidin Sudirohusodo Hospital Makassar in July–August 2015 with results of the calcium levels in 26 samples each measured on 7<sup>th</sup> day, 21<sup>st</sup> day and 35<sup>th</sup> day, then obtained the following results:

Based on normality test to determine the distribution of the data, obtained normally distributed data, therefore to distinguish the level of calcium 7<sup>th</sup> day, 21<sup>st</sup> day and 35<sup>th</sup> day carried Repeated Anova Test was carried on as in table and graph below.

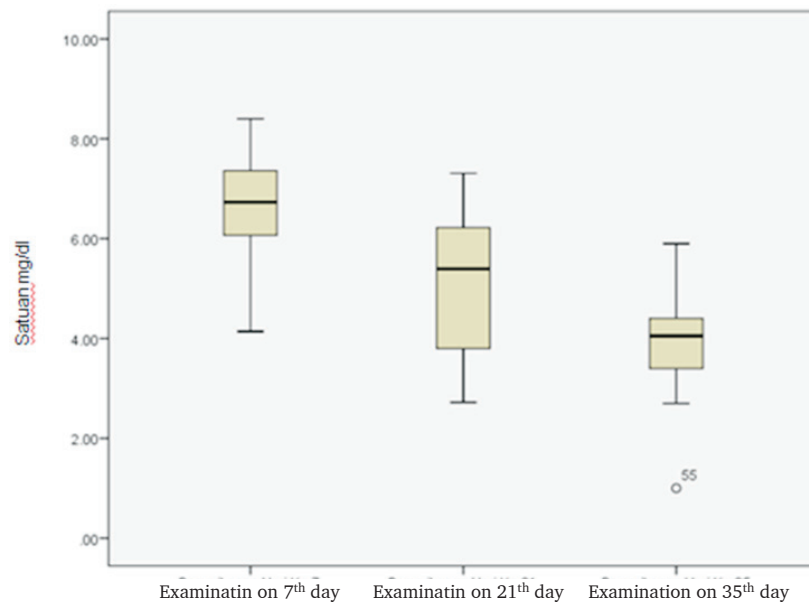
During the storage period, calcium levels tended to decrease. This was because the calcium ion ( $\text{Ca}^{2+}$ ) actively pumped from intracellular to extracellular,

**Table 1.** Mean levels of calcium based on storage duration

|  | Mean ± Standard deviation | P value |
|--|---------------------------|---------|
| Calcium levels of 7 <sup>th</sup> day  | 6.70±0.93                 | <0.001  |
| Calcium levels of 21 <sup>st</sup> day | 5.10±1.41                 | <0.001  |
| Calcium levels of 35 <sup>th</sup> day | 3.89±0.90                 | <0.001  |

**Table 2.** Comparative test of calcium level in stored PRC on 7<sup>th</sup> day, 21<sup>th</sup> day and 35<sup>th</sup> day

| Day  | P value | Significant          |
|--|---------|----------------------|
| 7 <sup>th</sup> day with 21 <sup>th</sup> day  | 0.000   | Different meaningful |
| 21 <sup>st</sup> day with 35 <sup>th</sup> day | 0.000   | Different meaningful |
| 7 <sup>th</sup> day with 35 <sup>th</sup> day  | 0.000   | Different meaningful |



**Figure 1.** Calcium levels in stored PRC

through the energy that relies on calcium ATPase pump. Calmodulin, a cytoplasmic protein binding calcium, controls calcium pump to prevent intracellular  $\text{Ca}^{2+}$  overload, which can make deformation of RBC and structure of RBC becomes more rigid. In the state of decreased ATP levels,  $\text{Na}^+$  and  $\text{Ca}^+$  intracellular become accumulated and decreased levels of  $\text{K}^+$  and water intracellular leads to dehydration and stiffness of RBC. Low calcium levels in the plasma caused by the accumulation of intracellular calcium ions caused erythrocyte membrane rigidity. The rigid erythrocyte membrane deformability of red cells will cause disturbance. At the beginning of erythrocytes transformed into spherocytes experiencing further rigid breakable or lysis.

The changes show a significant difference between the levels of calcium from 7<sup>th</sup> day through 35<sup>th</sup> day showed that the younger PRC showed better quality, especially in maintaining of the function of erythrocyte membrane deformability so that the function of erythrocytes in the management of patients become more leverage.

## CONCLUSION AND SUGGESTION

Decreased levels of calcium was in line with the length of storage period and in line with the

decline in the quality of erythrocytes in PRC. Based on this study that recommend using storage PRC with a shortened period of storage.

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