Vol. 23, No. 3, July 2017

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

INDONESIAN JOURNAL OF

Clinical Pathology and Medical Laboratory

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

IJCP & ML (Maj. Pat. Klin. Indonesia & Lab. Med.)	Vol. 23	No. 3	Page 203-309	Surabaya July 2017	ISSN 0854-4263

Published by Indonesian Association of Clinical Pathologists

Accredited No. 36a/E/KPT/2016, Tanggal 23 Mei 2016

p-ISSN 0854-4263
Vol. 23, No. 3, July 2017 e-ISSN 4277-4685

INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

Editorial Adress:
d/a Laboratorium Patologi Klinik RSUD Dr. Soetomo, Gedung Diagnostik Center Lt. IV
Jl. Mayjend. Prof. Dr Moestopo 6–8 Surabaya, Indonesia
Telp/Fax. (031) 5042113, 085-733220600 E-mail: majalah.ijcp@yahoo.com, jurnal.ijcp@gmail.com
Website: http://www.indonesianjournalofclinicalpathology.or.id

Accredited No. 36a/E/KPT/2016, Tanggal 23 Mei 2016

p-ISSN 0854-4263
Vol. 23, No. 3, July 2017 e-ISSN 4277-4685

INDONESIAN JOURNAL OF

CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

CONTENTS

Printed by Airlangga University Press. (OC 252/08.17/AUP-A1E). E-mail: aup.unair@gmail.com

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

2017 July; 23(3): 208–210
p-ISSN 0854-4263 | e-ISSN 4277-4685
Available at www.indonesianjournalofclinicalpathology.or.id

RESEARCH

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 7th, the 21st and the 35th days, and to compare the calcium level on the 7th, the 21st and 35th 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 7th and the 21st days, the 7th and the 35th days and also on the 21st and the 35th days. The reduction of calcium level occured 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 became 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 hypotesized to decrease the efficiency of stored blood and decrease their ability to act their required role after transfusion, but

Department of Clinical Pathology, Faculty of Medicine Hasanuddin University, Makassar, Indonesia. E-mail: suryani.jamal@gmail.com

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.[1]

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.[2,3]

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).[4,5]

Calcium ions (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 Ca^{2+} overload, which can make deformation of RBC and the structure of RBC becomes more rigid. In the state of ATP levels decreased, Na^{+} and Ca^{+} intracellular become accumulated and decreased levels of K^{+} and water intracellular leads to dehydration and stiffness of RBC.[6]

Based on research conducted by Sulemanji *et al.*[7] 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 hipocalcemia.[7]

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 7th day, 21st day and 35th 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 7th day, 21st day and 35th 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 7th day, 21st day and 35th 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 (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 7th day	6.70±0.93	<0.001
Calcium levels of 21st day	5.10±1.41	<0.001
Calcium levels of 35th day	3.89±0.90	<0.001

Table 2. Comparative test of calcium level in stored PRC on 7th day, 21th day and 35th day

Day	P value	Significant
7th day with 21th day	0.000	Different meaningful
21st day with 35th day	0.000	Different meaningful
7th day with 35th day	0.000	Different meaningful

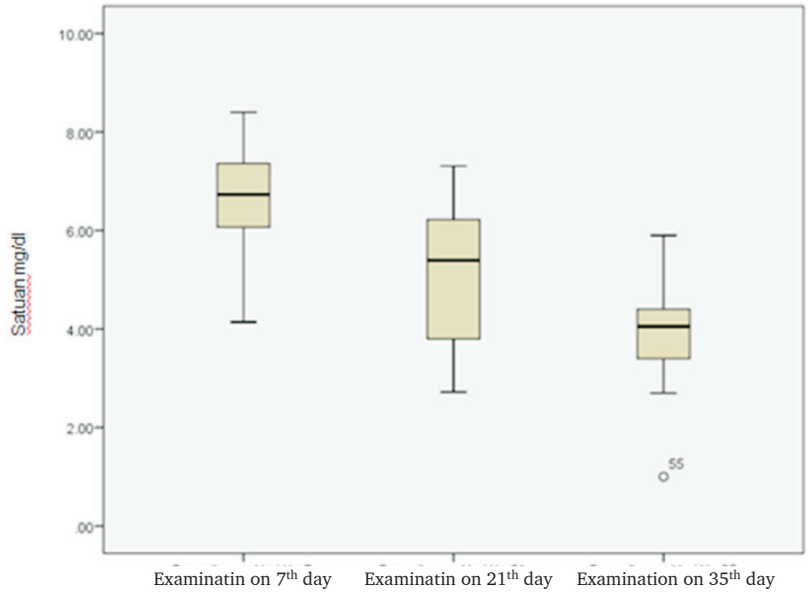

Figure 1. Calcium levels in stored PRC

through the energy that relies on calcium ATPase pump. Calmodulin, a cytoplasmic protein binding calcium, controls calcium pump to prevent intracellular Ca^{2+} overload, which can make deformation of RBC and structure of RBC becomes more rigid. In the state of decreased ATP levels, Na^{+} and Ca^{+} intracellular become accumulated and decreased levels of 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 7th day through 35th 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.

REFERENCES

1. Mustafa I, Al Marwani A, Nasr KM, Kano NA, Hadir T. Time Dependent Assesment of Morphological Changes: Leukodepleted Packed Red Blood Cells Stored in SAGM. BioMed Research International. Hindawi Publishing Corporation. 2016; Article ID 4529434: 7.
2. A Bogdanova, Makhro A, Wang J. Calcium in Red Blood Cells. International Journal of Molecular Sciences, 2013; 14(5): 9848-72.
3. Aubron C, Nichol A, Cooper DJ, Bellomo R. Age of red blood cells and transfusion in critically ill patients. Annals of Intensive Care: a Springer Open Journal. 2013; 3(2): 11.
4. Soepraptini J, K Widyayanti, Estoepangestie AT. Conversion of erythrocytes on blood smear Dog Before and After Storage by Using Citrate Phosphate Dextrose. Faculty of Veterinary Medicine, University of Airlangga. Scientific Journal of Veterinary Medicine, 2011; 4(1): 15-18.
5. Cluitmans JC, Chokkalingam V, Janssen AM. Alterations in Red Blood Cell deformability during Storage: A microfluidic Approach. BioMed Research International, 2014; 1-9.
6. Harmening DM, Moroff G. Modern Blood Banking and Transfusion Practices: Red Blood Cell and Platelet Preservation. Fifth Ed., Philadelphia, F.A. Davis Company, 2005; 1-21.
7. Sulemanji DS, Bloom JD. New insights into the effect of rapid transfusion of fresh frozen plasma on ionized calcium. Department of Anaesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston, 2012; 364-69.