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RESEARCH

THE MORPHOLOGICAL FEATURES OF ERYTHROCYTES IN STORED PACKED RED CELLS

(Gambaran Morfologi Eritrosit di Packed Red Cells Simpan)

Dewi Sri Kartini, Rachmawati Muhiddin, Mansyur Arif

ABSTRAK

Morfologi eritrosit Packed Red Cells (PRC) akan mengalami perubahan selama penyimpanan di suhu 2°–8°C. Eritrosit dalam mempertahankan viabilitasnya membutuhkan adenosine triphosphate (ATP). Apabila kadar ATP intraseluler menurun, terjadi kerusakan lipid membran, penumpukan Natrium dan Kalsium intraseluler, penurunan kadar Kalium dan air intraseluler, dehidrasi sel, membran menjadi kaku dan bentuknya berubah dari cakram menjadi sel krenasi, sferosit dan bite cell. Penelitian ini bertujuan untuk melihat persentasi bentuk eritrosit crenated cell, sferosit dan bite cell PRC simpan pada hari ke-3 yang digunakan sebagai pembanding, hari ke-7, ke-14 dan ke-21 dari tanggal aktaf kantong darah. Penelitian observasional dengan pendekatan kajian kohort dilakukan pada bulan Agustus 2015 di BDRS RSUP Dr. Wahidin Sudirohusodo Makassar. Sampel sebanyak 30 selang kantong darah menjadi 120 hapusan darah slide. Dari 30 sampel kantong darah dengan golongan darah A 26,6%, B 13,3%, AB 16,6% dan O 43,3%, didapatkan peningkatan persentase jumlah crenated cell, sferosit dan bite cell setelah penyimpanan hari ke-3, ke-7, ke-14 dan ke-21. Penyimpanan hari ke-3 dijadikan pembanding. Data diolah dengan menggunakan uji Fiedman dan Wilcoxon dengan nilai kemaknaan p<0,001. Terdapat peningkatan persentase perubahan morfologi eritrosit (crenated cell, sferosit dan bite cell) seiring dengan lamanya penyimpanan darah PRC. Pemakaian darah PRC dianjurkan tidak boleh melebihi 21 hari penyimpanan.

Kata kunci: *Lama penyimpanan, crenated cell, sferosit dan bite cell*

ABSTRACT

The morphology of erythrocyte Packed Red Cells (PRC) is subject to changes during storage at 2º–8ºC. In maintaining their viability, erythrocytes require adenosine triphosphate (ATP). The decrease in intracellular ATP level results in damaged membrane lipid, accumulation of intracellular Sodium and Calcium, decreased intracellular potassium and water, cell dehydration, rigidity of membrane and changes in its shape from a disc into crenated red cells, spherocytes and bite cells. This study was aimed to observe the presentation of erythrocyte crenated cells, spherocytes and bite cells of stored PRC at day 3 (control), day 7, day 14 and day 21 from the blood collection date of the blood bag. An observational study with cohort study approach was conducted in August 2015 at the Blood Bank of Dr. Wahidin Sudirohusodo Hospital Makassar. The total sample was 30 blood bag that resulted in 120 slide blood smears. From 30 samples of blood bags comprising blood group A 26.6%, B 13.3%, AB 16.6% and O 43.3%, the increased percentages of crenated cells, spherocytes and bite cells were observed at day 3, day 7, day 14 and day 21. Day 3 of storage was taken as control. Data were processed by Fiedman test and Wilcoxon test with significance level at p<0.001. There was an increase in the percentage of erythrocyte morphology change (crenated cells, spherocytes and bite cells) along with the storage time of packed red blood cells. The use of packed red blood cells was recommended not more than 21 days of storage.

Key words: Storage time, crenated cells, spherocytes and bite cells

INTRODUCTION

Erythrocyte is a red blood cell with a diameter of 6-8 um and thickness of 1.5–2.5 um, spheric shape, flat edges and biconcave (resembling discs). Its cytoplasm is red with pale area in its center (acromia central), it has no cell nucleus. The erythrocyte membrane has a semipermeable protein and lipid layers. The outer layer of the membrane is rich with glycolipid and phospholipid choline and its inner layer is rich with

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amino phospholipids. The biochemical composition of erythrocyte membran consists of 52% protein, 40% lipid and 8% carbohydrates.[1,2]

In maintaining its viability erythrocyte needs adenosine triphosphate (ATP). The decrease in intracellular ATP will result in damaged membrane lipid, accumulation of intracellular Na^+ and Ca^+, decreased intracellular potassium and water level, cell dehydration, rigid membrane and altered shape from a disc into a spherocyte (spheric), crenated (serrated), and bite cell.[2,3]

Packed Red Cells (PRC) is prepared from whole blood by centrifugation and removing the plasma. The most commonly used solvent as anticoagulant is CPDA-1 (Citrate Phosphate Dextrose Adenin-1) to preserve the erythrocyte viability, optimize the pH during storage, and is capable of storing blood up to 35 days at temperature of 1–6°C. The citrate compound contained in the blood bags is useful in preventing coagulation by binding calcium in donor plasma. Phosphate functions as buffer to maintain blood pH and to prevent the decrease of 2.3 Diphosphoglycerate (DPG). Dextrose is also added to blood bags to meet the energy need of the cells by serving as a substrate to produce ATP. Adenine functions to regenerate adenosine triphosphate (ATP).[2–4]

According to its storage time, the transfused blood is classified as; fresh blood aged six hours after collection; new blood aged more than six hours to six days after the collection of blood from donors; stored blood aged more than six days since collection from donors at optimum temperature.[4,5]

During the storage time of PRC in the Blood Bank, erythrocytes are subject to metabolic changes due to different situation in vivo, namely the decreased concentration of Adenosine 5'-triphosphate (ATP) and 2.3 Diphosphoglycerate (DPG), decreased blood pH, increased kalium and lactate concentration, erythrocyte cell shape change, the loss of erythrocyte viability and hemolysis.[5,6]

According to Haradin and colleagues in 1969, during storage the ATP level decreased and this related to changes in erythrocyte, including the changes in cell shape from a disc to more spheric shape.

According to the study of Esper and colleagues in 2012, there is a correlation between blood storage time and abnormal morphological presentation of erythrocyte in stored bllod that will be transfused.[7]

Soepraptini and colleagues in 2001 stated that the storage of dog blood using CPD for 21 days resulted in erythrocyte cell change into crenation cells due to erythrocyte cell dehidration.[8]

METHODS

This study was an observational study with cohort study approach. Packed Red Cells in Blood Bank of Wahidin Sudirohusodo Hospital was sampled from blood bag with the same bag number, which were then divided into four parts and stored in a refrigerator at 2–8°C.

Blood smears were prepared at day 3 of storage (control) and then at day 7, day 14 and day 21. Blood smears were prepared from 5 μL of blood sample and after drying were fixated with methanol and stained with May Grundwald-Giemsa (MGG). After that, the blood smears were read using a microscope at 100x10 magnification. Morphology of the erythrocytes in the form of crenated cells, spherocytes and bite cells were counted in 1000 erythrocytes and multiplied by 100% to obtain the percentage. There was no normal value limit for crenated cells, spherocytes and bite cells.

Data were then analyzed using SPSS software and Friedman test because data were not normally distributed, and then continued with Post Hoc Test – Wilcoxon. Calculation results were presented in the form of tables and graphs.

RESULTS AND DISCUSSION

According to the results of study conducted during August 2015, there were 30 samples of stored PRC with blood smear number of 120 slides. The blood smears at day 3 were used as a control. Characteristics of stored PRC blood based on glood group can be seen in Table 1.

Erythrocyte morphology appears to have changes. Smears of stored PRC at day 3, day 7, day 14 and day 21 can be seen in Figure 1.

The smears of stored PRC were observed under microscope to assess the number of changes in the morphology of erythrocyte with forms of crenated cells, spherocytes and bite cells. The calculation results were then processed using Friedman test as shown in Table 2 with graphical representation in Figure 2.

Table 1. Distribution of study samples

Blood group	n(30)	(%)
B	4	13.3%
O	13	43,3%

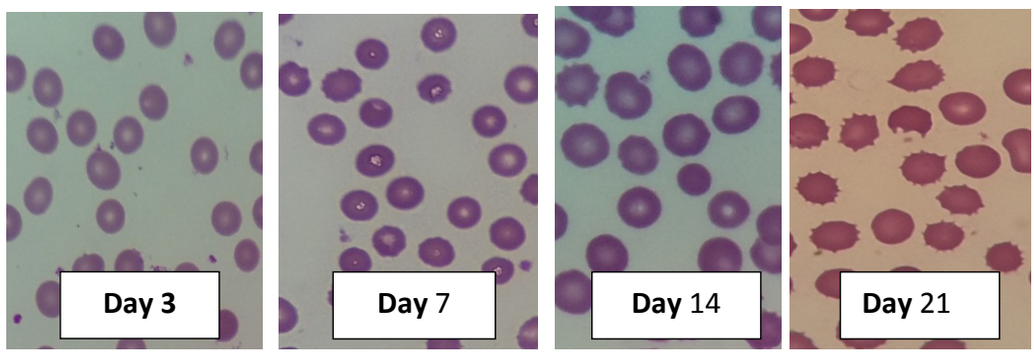

Figure 1. Stored PRC blood smears.

Table 2. Mean and standard deviation with Friedman test

Day	Mean ± Standard deviation			P
3	1.06±0.46	0.08±0.10	0.05±0.05	<0.001
14	7.93±.66	0.52±0.36	0.18±0.12	

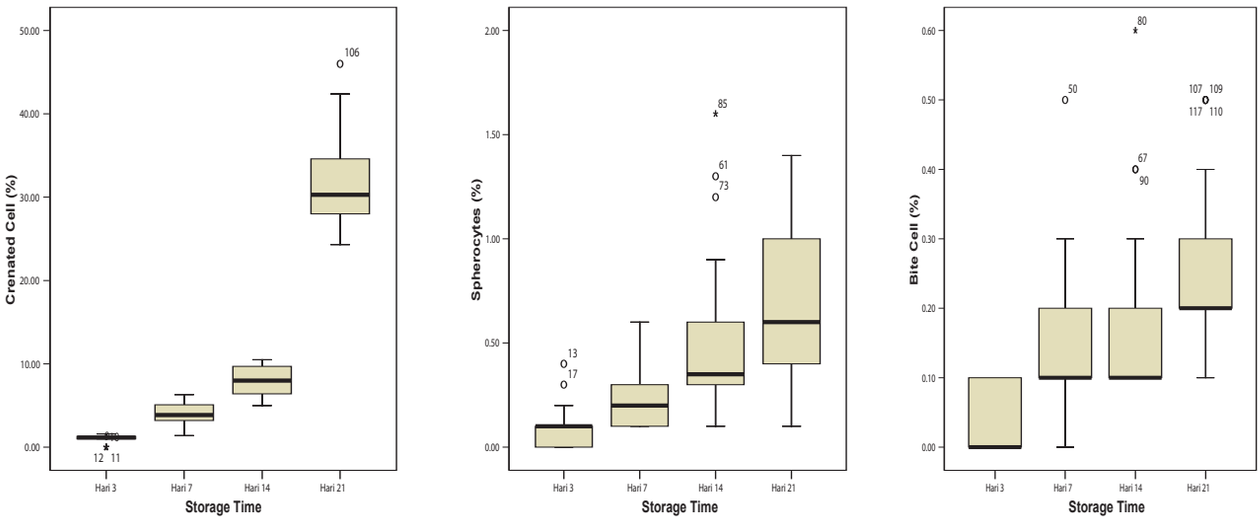

Figure 2. Comparison of percentage and storage time.

The changes in erythrocyte morphology appeared in blood smear according to observation day 3 and day 7, day 7 and day 14 and then day 14 and day 21 using post Hoc-Wilcoxon test can be seen in Table 3, Table 4 and Table 5.

Data were analyzed using Friedman test because the data were not normally distributed. Evaluation was continued by Post Hoc–Wilcoxon test to examine the difference between the four different storage time.

Table 3. Crenated cells with Post Hoc-Wilcoxon test

Crenated cells	P
Day 7 and Day 14	0.000 (<0.05)

Table 4. Spherocytes with Post Hoc Test–Wilcoxon

Spherocytes	P
Day 7 and Day 14	0.000 (<0.05)

Table 5. Bite cells with Post Hoc Test-Wilcoxon

Bite cells	P
Day 7 and Day 14	0.026 (<0.05)

This study evaluated the morphological features of erythrocytes in blood smears from 30 samples of stored PRC blood bag that were divided into 4 parts, resulting in 120 blood smears. The blood smears were intended to evaluate the percentage of morphological features of erythrocte in the form crenated cells, spherocytes and bite cells at day 3 (control), day 7, day 14 and day 21.

The study findings indicated a relationship between storage time and the increase in erythrocyte morphology percentage (crenated cells, spherocytes and bite cells). According to statistical evaluation, a significant result was found in all evaluated morphological forms of erythrocytes, including crenated cells, spherocytes and bite cells using Friedman test ($P<0.001$) and post hoc test–Wilcoxon ($p<0.05$) except for spherocytes at day 14 and day 21 showed no significant difference ($p=0.061$).

This study findings support the previous studies suggesting that erythrocyte morphology is subject to changes with the storage time. The longer the storage time, the higher the erythrocyte morphology change.

Erythrocyte morphological change in stored PRC into crenated cells, spherocytes and bite cells is caused by metabolic changes occuring due to different conditions from in vivo condition, namely the change in ATP and DPG susceptibility, decreased blood pH, increased kalium and lactate concentration, change in erythrocyte cells shapes, loss of erythrocyte viability and hemolysis. This is in accordance to the theory that erythrocytes need ATP in maintaining their viability. The decrease in ATP level will result in damaged membrane lipid, accumulated intracellular natrium and calcium, decreased intracellular potessium and water level, cell dehydration, rigid and easily ruptured membrane. Cell dehydration was shown by increased crenated cell shape, accumulation of intracellular calcium as indicated by the increased spherocyte shape and rigid and easily ruptured cells as indicated by the increased bite cell shape.[3,5,6]

CONCLUSION AND SUGGESTION

There was an increase in morphological change percentage of erythrocytes (crenated cells, spherocytes and bite cells) with the storage time of PRC blood. The use of PRC blood was recommended not more than 21 days of storage.

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