Potassium Levels and 2,3-Diphosphoglycerate (2,3-DPG) Levels in Packed red cells (PRC) at Different Storage Times

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Abstract

Background : During the storage period, erythrocytes would undergo biochemical changes known as storage lesions. Storage injury might lead erythrocytes to undergo hemolysis, impairing the PRC's quality, safety, and efficacy for recipients. The biochemical changes that occur were an increase in plasma potassium levels associated with cold storage temperatures causing the sodium/potassium exchange pump to became inactive and a decrease in pH-related 2,3-diphosphoglycerate (2,3-DPG) levels. This study sought to investigated the effect of storage duration on PRC quality by analyzing changes in potassium and 2,3-DPG levels on days 1, 10, 20, and 30 of storage. **Method** : This study used an observational analytical method with a time series design. There was a total of 50 PRC bags utilized as the samples. Potassium levels and 2,3-DPG were measured on day 1, day 10, day 20, and day 30. The study data were statistically analyzed using repeated measure ANOVA. **Conclusion** : According to the findings of a repeated measures analysis of variance performed on the levels of potassium and 2,3-DPG after 30 days of storage, there was a statistically significant difference ($p < 0.00$) in the levels of potassium and 2,3-DPG between day 1, day 10, day 20, and day 30.

Key Words: Packed red cells, Storage Lesion, Potassium, 2,3-DPG, Health Service

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Introduction

Packed red cells (PRC) was a blood component generated from whole blood (WB) precipitated during storage, or by high-speed centrifugation in which the majority of the plasma is eliminated. Packed red cells (PRC) had a volume of 200-250 mL with a hematocrit level of 70-80%, a plasma volume of 15-25 mL, a PRC bag using a Citrate, Phosphate, Dextrose, Adenine-Formula1 (CPDA-1) anticoagulant with an anticoagulant volume of 10-15 mL (Saraswati, 2015). Most blood transfusions involved packed red cellss (PRC).

PRC transfusion was applied for neonatal hyperbilirubinemia patients (Hosea, Ethics, & Lestari, 2015), anemic patients who did not accompanied by a decrease in blood volume; thalassemia major (Sutrisnaningsih, Suharjono, & Sudarmanto, 2016), hemolytic anemia, acute leukemia, chronic leukemia, malignancy, thalassemia major, as well as chronic kidney failure (Saraswati, 2015). The annual report data of the Blood Transfusion Department of Dr. Soetomo Hospital in 2020 the use of PRC blood components was 46.224 (49.9%) of the 92.574

requests for blood transfusion in Dr. Soetomo Surabaya Hospital (ITD, 2020). According to WHO and the results of the National Health survey in 2013 the prevalence of anemia in Indonesia was 29.7% male, 26.50% female, teenagers (female/male over the age of 15) 22.7% and 16.6%, respectively, and 37.1% of pregnant women (Indonesian Ministry of Health, 2013). Meanwhile, the prevalence of anemia had increased compared to the previous survey conducted in 2007, namely 27.7%, 9.4% and 6.9% respectively in children aged 1-4 years, 5-14 years and 15-24 years (Indonesian Ministry of Health, 2007). Based on these findings, Indonesia had a high prevalence of anemia. Due to the high prevalence of anemia, treatment needed to be directed properly and administered quickly. PRC blood transfusions were one of the interventions associated to the management of anemia; as a result, the demand for PRC blood transfusions had increased (Nursalam, 2003).

In order to achieved the best possible results from a PRC transfusion, it was recommended to use fresh blood, which was defined as blood that had been recently taken from a donor and must be used within 6 hours after collection. Using fresh blood had the benefit of preserving the majority of the erythrocyte and platelet functions (Bjerkvig et al., 2018). It was challenging to obtain fresh blood (fresh PRC) in Indonesia, thus transfusions could made used of stored blood instead (Indonesian Ministry of Health, 2013). Blood storage was necessary because the blood service unit cannot constantly offer fresh blood to patients at the request of the treating doctor. The use of stored blood had a number of benefits, one of which was that it was readily available at any moment; nevertheless, one of the drawbacks was that red blood cells go through a number of changes while they were being stored (Beliën & Forcé, 2012). These changes were known as storage lesions (Kim-Shapiro, Lee, & Gladwin, 2011).

During the packed red cells (PRC) storage process, a storage lesion would occur. The elements of injury to blood storage included: morphological changes, biochemical changes and loss of function of the cation pump. This condition would affect the viability and function of RBC in transporting oxygen from the lungs to the tissues, causing the release of harmful substances such as; free haemoglobin as a source of Reactive Oxygen Species (ROS) thus the storage process will affected the quality and efficacy of PRC transfusion (Choudhury & Mathur, 2011). Storage lesions could be evidenced from a decrease in Adenosine Triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) in RBC, as well as an increased in potassium and an increased in free hemoglobin (Hb) levels in PRC (Saragih, 2019).

The most severe form of erythrocyte storage injury was hemolysis, which could occur both during the collection and storage of blood. Hemolysis was the rupture of erythrocytes by releasing hemoglobin (Hb) directly into the plasma or the loss of microvesicles containing lipids and hemoglobin from intact erythrocytes into the plasma. Hemolysis caused K+ cations to be released (Donati et al., 2014). It was estimated that 1 - 5% of erythrocytes would be damaged during donor collection and the viability of erythrocytes would continued to decreased by days as a result of decreased levels of Adenosine Triphosphate (ATP). Decreased ATP levels could caused erythrocytes to lose membrane lipids, the membrane becomes stiff and the shape of the erythrocytes changed from disc to spherical (without a central palor and small in size) (Orriss, Key, Hajjawi, & Arnett, 2013). The activity of the Na+/K+ATPase pump was strongly influenced by temperature. The pump becomes inactive at 4°C. All of these caused potassium leaked into plasma due to failure of the Na+/K+ATPase pump during blood storage, this process occured slowly and continuously (Asryani, Yaswir, & Rofinda, 2018). According to Asryani, Yaswir, & Rofinda (2018), a significant difference in PRC potassium levels based on storage time with a higher average PRC potassium level at > 14 days of storage was revealed. The average potassium level at 4-14 days of storage is 3.9 (0.8) mmol/L and >14 days of storage is 8.7 (4.9) mmol/L. There was a significant difference in the mean potassium level of PRC based on storage time was using the t test with the highest mean potassium level at storage > 14 days (p = 0.000).

Due to the absence of a nucleus and mitochondria in erythrocytes, hence oxidative metabolism of energy in erythrocytes was produced through the breakdown of glucose metabolic pathways of Embden-Meyerhof (glycolysis). In this pathway, ATP was produced by the anaerobic breakdown of glucose. The Hexose Monophosphate pathway produces NADPH to protected red blood cells from oxidative lesions and the Rapoport-Luebering pathway for the production of 2,3-

diphosphoglycerate (2,3-DPG) which played a role in the regulation of oxygen affinity. Glycolysis would caused a decrease in 2,3-DPG levels. 2,3-DPG acid was a product of glycolysis and was a three-carbon isomer of the intermediate glycolytic acid 1,3-biphosphoglycerate (1,3-BPG). Approximately 5 mmol/L of 2,3-DPG acid was found in human erythrocytes. High levels of 2,3-DPG in erythrocytes cause Hb's affinity for oxygen to decrease, whereas low levels of 2,3-DPG in erythrocytes causes Hb affinity for oxygen to increase. In blood transfusions, it is essential to control the level of 2,3-DPG since stored blood quickly loses 2,3-DPG, reducing its ability to transport oxygen (Scott, LeGrys, & Hood, 2016). According Sesmita (2017), it was revealed that there was a relationship between erythrocyte hemolysis index and 2,3-DPG levels of 0.49 during PRC storage with $p < 0.05$ which was statistically significant. Meanwhile, Mukherjee et al., 2010 found a negative correlation between levels of 2,3-DPG and lactic acid in WB blood bags with $p<0.005$. Those previous studies above had shown that during storage there were various changes that would affected the quality of blood and the efficacy of the PRC to be transfused.

Storage of PRC in the presence of phosphate and adenine allowed for a longer storage period. Citrate-phosphate-dextrose (CPD) became the standard anticoagulant in the late 1950s. Blood stored with CPD anticoagulant could last up to 21 days. This anticoagulant was later developed in 1978 with the addition of adenine (CPDA-1) so that the shelf life of blood with this anticoagulant increased to 35 days (Maulidan, Tambunan, & Hajat, 2022). The average used of PRC storage at the Blood Transfusion Department of Dr. Soetomo Hospital was less than 20 days, thus the examination times for the 1st, 10th, 20th, and 30th days were selected for this study based on the maximum storage time and average use.

Based on the many references and the fact that demand for PRC transfusions at the Blood Transfusion Department of Dr. Soetomo Hospital was rising, this study was essential for determining the impact of storage on the quality of PRC blood products at the Blood Transfusion Department of Dr. Soetomo Hospital. Looking at the changes that occured in the value of potassium and 2,3-diphosphoglycerate (2,3-DPG) according to storage time would allow us to figure out how many days after producing PRC it was still possible to be transfused.

In general, this study sought to examine the impact of storage duration on the quality of packed red cellss (PRCs) by analyzing changes in the levels of potassium and 2,3-diphosphoglycerate (2,3-DPG) in PRCs stored on 1st, 10th, 20th, and 30th days.

Materials and Methods

This study was an observational analytical study type with a time series design. The population in this study were packed red cells (PRC) donor blood products in the Blood Transfusion Department of Dr. Soetomo Surabaya Hospital from July to August 2022. Ethical approved was released by the Health Research Ethics Commission, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. All recruited donors had received information related to this study and had given a letter of approval for the action related to the purpose of taking blood for study purposes. The sample used was packed red cells (PRC) blood obtained from the production process and stored at the refrigerator blood bank temperature of the Blood Transfusion Department of Dr Soetomo Hospital with a temperature of 1 - 6°C which met the inclusion and exclusion criteria. The inclusion criteria in this study were packed red cells (PRC) blood components obtained during the study period at the Blood Transfusion Department of Dr. Soetomo Surabaya Hospital which was in accordance with Indonesian Health Ministry regulation number 91 of 2015 on the 1st day of storage, 10th day, 20th day, and 30th day. Exclusion criteria in this study were damaged packed red cells (PRC) components (such as any signs of hemolysis, lipemic and icteric). The sampling technique used in this study used a consecutive sampling technique. The sample size used in this study used 50 samples. In this study, the independent variable was the storage time of packed red cells (PRC), 1st day, 10th day, 20th day, and 30th day. Likewise, the dependent variables were potassium levels and 2,3-diphosphoglycerate levels.

The tools used in this study were PRC donor bags, blood bank refrigerator, test tube racks, gloves, plain tubes, clamp scissors, heat sealers, surgical scissors, blood transport boxes, ice packs, tube racks, centrifuges, Semi Automatic Chemistry Analyzer Sinnowa BS-3000P (Sinnowa Medical Science & Technology Co., Ltd., China) and the

human 2,3-DPG ELISA kit (CUSABIO Technology., LLC., United States). The materials used in this study were blood and plasma samples from Packed red cellss (PRC). The study was conducted at the Blood Transfusion Department of Dr. Soetomo Surabaya Hopital as the sampling site and the Hematology Laboratory and Chemistry Laboratory, Faculty of Health Sciences, Maarif Hasyim Latif University, Sidoarjo.

Potassium was measured using the Sinnowa BS-3000P Semi Automatic Chemistry Analyzer and 2,3-DPG was measured by a CUSABIO human 2,3-DPG ELISA kit. The data obtained were entered into tables and processed using the Statistical Package for Social Science (SPSS) 25 software. The collected data were cleaned, coding, tabulated, and entered into the computer. Data were calculated by mean, standard deviation and confidence interval (CI) 95% of the mean. The normality test of the data was analyzed using the Shapiro-Wilk test. The comparison of PRC quality on the 1st, 10th, 20th, and 30th days was analyzed using repeated measure ANOVA analysis (analysis with one object) when the data was normally distributed, or Freedman's test when the data distribution was not normal with p value of < 0.05 considered statistically significant.

Results and Discussion

Characteristics of Samples

The sample size in this study was 50 PRC bags from 50 donors. This amount had met the minimum sample size specified. The characteristics of the sample in the form of donor blood group were shown in table 1 as follows.

Table 1: Characteristics of Samples

Blood types	PRC (bag)	Amount (%)
A Rh +	9	18%
B Rh +	15	30%
O Rh +	21	42%
AB Rh +	5	10%
Total	50	100%

Data Normality Test

The results of the Shapiro Wilk normality test, which examined the levels of potassium and the 2,3-DPG during the observation period showed that the data on potassium levels and 2,3-DPG were normally distributed at all observation times (p > 0.05), thus the analysis of differences between observations was carried out using the repeated measure ANOVA test.

Potassium Difference Test

The results of the potassium difference test on the observation of day 1, day 10, day 20, and day 30 were analyzed using the repeated measure ANOVA test which was presented in table 2 and figure 1 bellow.

Table 2: The difference in potassium level between observations

Observation time	N	Potassium Mean ± SD	p value
D1	50	3.93 ± 0.40	0.00
D10	50	11.86 ± 0.67	
D20	50	18.78 ± 0.54	
D30	50	24.36 ± 0.78	

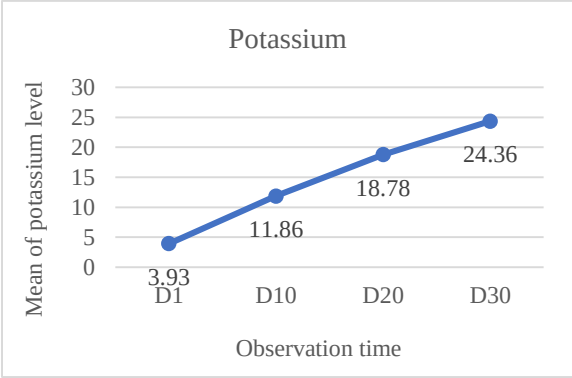

Figure 1: The mean of potassium levels between observations

Based on the above data, the results of the repeated measure ANOVA test showed that there was a significant difference (p < 0.05) in potassium levels between observations with p value = 0.00.

Difference Test Results of 2,3-DPG

The results of the 2,3-DPG difference test on the observation of 1st, 10th, 20th, and 30th days were analyzed using the repeated measure ANOVA test which was presented in table 4 and figure 2 below.

Table 3: The difference of 2,3-DPG between observation times

Observation time	N	2,3-DPG Mean ± SD	p value
D1	50	4.35 ± 0.23	0.00
D10	50	2.75 ± 0.27	
D20	50	1.42 ± 0.19	
D30	50	0.48 ± 0.12	

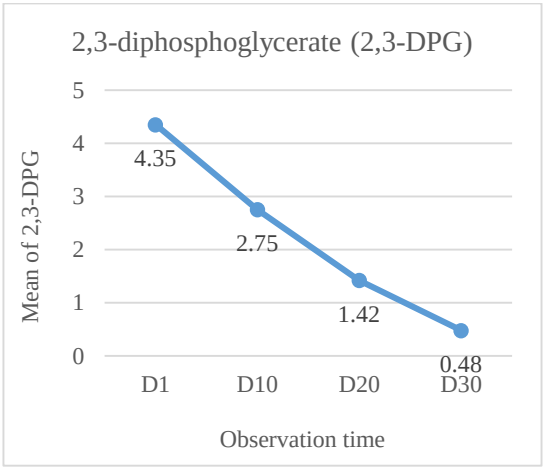

Figure 2: The mean of 2,3-DPG between observations

The results of the Repeated Measure ANOVA test above showed that there was a significant difference ($p < 0.05$) in the 2,3-DPG level between the observation time with p value = 0,00.

Samples Characteristics

The study sample consisted of 50 PRC bags with Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant obtained from voluntary of blood donation activities. The highest proportion of blood types was O Rh+ with 21 bags (42%), folowed by B Rh+ with 15 bags (30%), blood type A Rh+ and AB Rh+ with 9 bags (18%) and 5 bags (10%), respectively. Similar to the studies by Sutjianto, Nurulita, & Mangarengi (2014) and Asryani et al. (2018), samples were taken from the PRC bag hose in order to analyzed the potassium levels. Likewise, as carried out by Sesmita (2017) which utilize a PRC bag hose to examined the 2,3-DPG.

Examination of potassium parameters and 2,3-DPG level as storage lesion markers was carried out on PRC with CPDA-1 anticoagulant, which was the same as carried by Sutjianto et al. (2014) who examined 16 PRC bags with the CPDA-1 anticoagulant. Meanwhile, Sesmita (2017) used 14 bags of PRC with anticoagulant CPDA-1 obtained from healthy donors, where the number of PRC examined was less than Asryani et al. (2018) who examined 22 CPDA-1 PRC bags.

The difference of potassium in time series observations

The repeated measures ANOVA data presented in Table 2 reveal a statistically significant difference in potassium levels between Day 1, Day 10, Day 20, and Day 30 ($p = 0.00$). Figure 1 presents a line graph that illustrates the mean increase in potassium levels that occurs during the storage of PRC. The mean potassium level on the first day was 3.93 mmol/L, compared to 11.86 mmol/L on the 10th day, 18.78 mmol/L on the 20th day, and 24.36 mmol/L on the 30th day. This indicated that hemolysis occurred during storage causing an increase in potassium levels in the plasma.

The results of this study were in accordance with the study of Sutjianto et al. (2014) which showed a significant difference ($p = 0.00$) in the mean potassium levels of PRC during the storage period on the 10th day, on the 20th day compared to storage on the first day. Furthermore, Uvizl, Klementa, Adamus, & Neiser (2011) reported an increase in potassium levels with storage time. Likewise, Asryani et al. (2018) highlighted a significant increase ($p=0.00$) of potassium PRC at 4-14 days of storage and >14 days of storage.

Erythrocytes in stored blood would experience storage lesions that damage erythrocyte function, causing a decrease in the effectiveness and safety of transfusion for the recipient (Glynn, Klein, & Ness, 2016). Hence, Adenosine Triphosphate (ATP) was required to maintain the viability of erythrocytes, phosphorylate glucose and maintain the sodium/potassium exchange pump. Refrigeration during storage would cause ATP

levels to decrease thereby stimulating an ATP-dependent sodium/potassium exchange pump (Na-K ATPase) to balance intracellular and extracellular levels gradually by releasing potassium out of cells and sodium into erythrocytes (Sutjianto et al., 2014).

The balance of intracellular and extracellular potassium levels related to the activity of the Na-K ATPase pump. The erythrocyte membrane had very little permeability to potassium, so its transfer was associated with an energy-dependent transport mechanism (glycolysis). Storage temperature of 1-6°C would inhibit glycolysis so that ATP levels decrease, this results in the Na-K ATPase pump unable to maintain the cation gradient. Intracellular potassium would come out of erythrocyte cells and there would be an increase in plasma potassium levels (Scott et al., 2016). Hemolysis occurred during storage causing an increase in potassium levels in the plasma (Sutjianto et al., 2014).

The increase in potassium levels in the stored PRC plasma allowed the occurrence of hyperkalemia in the recipient. The prevalence of hyperkalemia increased with the amount of blood and the storage time of the transfused PRC. Hyperkalemia caused problems in the blood vessels of the heart that could be life-threatening, especially in patients with kidney failure, cardiovascular disorders and massive transfusions (Uvizl et al., 2011).

The difference of 2,3-DPG in time series observations

The Repeated Measures ANOVA test for 2,3-DPG levels after 30 days of storage revealed a statistically significant difference ($p = 0.00$) between the levels on 1st, 10th, 20th, and 30th days (see Table 4). The line diagram in Figure 2 showed the decrease in the mean level of 2,3-DPG with the storage time of PRC for 30 days. The results of measuring the average level of 2,3-DPG on day 1 was 4.35 mol/mL to 2.75 mol/mL on day 10, on day 20 it was 1.42 mol/mL to 0.48 mol/mL on day 30.

This study was consistent with Sesmita (2017), who found that the average level of 2,3-DPG in 14 PRC bags decreased significantly ($p < 0.05$) after being stored for 28 days. Study by Mukherjee et al., (2010) showed the average decrease in levels of 2,3-DPG in 25 PRC bags was 11.5±6.2 mol/gHb (100%) on the 1st day of storage, 4.1±1.6 mol/gHb on day 7, and 0.4 ± 0.2 mol/gHb on day 14 which were statistically significant ($p = 0.001$). Further, study conducted by Donati et al. (2014) found a decrease in 2,3-DPG levels during leukoreduction PRC storage on day 1 (1.19 mol/mL), day 7 (0.62 mol/mL), day 14 (0.19 mol/mL), day 21 (0.15 mol/mL), day 28 (0.11 mol/mL), day 35 (0.07 mol/mL), and day 42 (0.03µmol/mL) which was statistically significant ($p < 0.05$).

The decrease in 2,3-DPG levels during PRC storage was dependent on ATP and intracellular pH. Adenosine triphosphate was the energy produced during the metabolic process of breaking down glucose (Sesmita, 2017). The decrease in 2,3-DPG was passive, associated with changes in the specificity of the enzyme diphosphoglycerate mutase/diphosphoglycerate phosphatase with a decrease in pH (Lestari, Triyono, & Sukorini, 2018). Besides, storage temperature could affect pH, glucose and lactate production (Armenia & Tambunan, 2020). The normal value for erythrocyte intracellular 2,3-DPG was about 4.5 mmol/L. After 24 hours of refrigeration, the 2,3-DPG level decreases, and the total supply of 2,3-DPG was depleted after two weeks of refrigeration. 2,3-DPG was an allosteric modulator of oxygen and hemoglobin binding. 2,3-DPG had the ability to exclusively attach to hemoglobin, which in turn lowers its affinity for oxygen. Low levels of 2.3 caused hemoglobin's affinity for oxygen partial pressure to rise, reducing erythrocytes' ability to deliver oxygen to tissues (Evans et al., 2021; Srinivasan, Morkane, Martin, & Welsby, 2017; Yudin & Verhovsek, 2019). Because most PRC transfusions were administered to anemic patients, the level of 2,3-DPG was a critical point to keep in mind when evaluating the effectiveness of a PRC transfusion for the recipient (Lestari et al., 2018).

Conclusions

This study highlight that there are differences in potassium levels during the storage period of Packed red cells (PRC) from day 1, day 10, day 20 and day 30. Likewise, there are also differences in 2,3-DPG levels during the storage period of Packed red cells (PRC) from day 1, day 10, day 20 and day 30.

We suggest that further study needs to be done on PRC units with leukoreduction to determine the

effect of leukocytes on increasing erythrocyte potassium levels and decreasing 2,3-DPG levels during PRC storage. In addition, it is necessary to measure additional parameters as indicators of storage lesions on PRC storage. These parameters include intracellular pH of erythrocytes, plasma hemoglobin, ATP levels, glucose levels, antioxidants in erythrocytes such as Glutathione Sulfhydril (GSH) and Superoxid Dismutase (SOD), as well as leukocyte parameters in PRC that are believed to influence the increase in potassium erythrocyte levels and decrease in 2,3-DPG levels during the PRC storage period.

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