Effect of Storage Time of Packed Red Cells (PRC) on Hemoglobin Plasma, Plasma Glucose, pH, and Lactate Levels

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Abstract

Background: It has been shown that Red Blood Cells (RBC) undergo a number of changes during liquid storage which affect their viability and their ability to deliver oxygen to the tissues. These alterations can be classified in two major groups: biomechanical and biochemical. This study wanted to analyze the effect of storage time on plasma hemoglobin, plasma glucose, potential of hydrogen (pH), and lactate levels before and after storage on the 1st, 10th, 20th, and 30th days. **Method**: 50 Packed red cells bags were taken for this study. From the PRC, plasma was taken on 1st, 10th, 20th, 30th days for hemoglobin plasma, glucose plasma, pH, and lactate. Data obtained were analyzed using SPSS software for windows. **Result:** The results showed that the level of plasma hemoglobin (Hb) and lactate increased significantly from day 1 until day 30 ($P< 0.001$); on the other hand, the levels of plasma glucose and pH value showed a significant decrease from day 1 to day 30 ($P <0{,}001$). **Conclusion**: There is a significant difference in the length of storage of the Packed Red Cells on the results of the examination of Plasma Hemoglobin levels, Plasma Glucose levels, the pH value, and Lactate levels.

Key Words: Lactate, Packed Red Cells, pH, Plasma Glucose, Plasma Hemoglobin

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Introduction

Blood is fluid that transports oxygen and nutrients to the cells and carries away carbon dioxide and other waste products. Technically, blood is a transport liquid pumped by the heart (or an equivalent structure) to all parts of the body, after which it is returned to the heart to repeat the process. Blood is both a tissue and a fluid. It is a tissue because it is a collection of similar specialized cells that serve particular functions. Blood supllies oxygen to the tissues with hemoglobin. In addition, the blood also supplies nutrient, such as glucose and amino acids, and removes carbon dioxide, urea and lactic acid (Maulidan, E.B., Tambunan,B.A., & hajat, A. 2022).

Blood transfusion was an important part of modern health services. Blood transfusion was a processes sequence of transferring donor blood into the recipient's blood circulation as a treatment effort (WHO, 2009).

Before blood was transfused to a patient, it must be ensured that the blood was safe, that was, it must be free from blood-borne infectious diseases, so that after the blood was finished, a blood screening test was carried out which includes examination of HIV (Human Immunodeficiency Virus), HBsAg (Hepatitis B Surface Antigen), Anti HCV (Hepatitis C Virus), VDRL (Veneral Disease Research Laboratory), Malaria. Another examination that must be carried out before blood was transfused was a crossmatch examination. Blood that had passed the screening test and showed a negative result before there was a request for transfusion, the blood would be stored in a special refrigerator for blood storage until the expiration date of the blood (Naim, 2014).

According to Wahidiyat & Adnani (2017), blood products used for blood transfusion include whole blood (Whole Blood), concentrated red blood cells (Packed Red Cell / PRC), Washed erythrocytes / WE), platelets, fresh frozen plasma (FFP), cryoprecipitate. Each blood product was used based on the patient's medical condition, one of which used packed red cells. Packed red cells were derived from Whole blood which was precipitated during storage, or by high-speed centrifugation. Most (2/3) of the plasma was removed. One unit of PRC from 450 ml of whole blood. In the volume 200-250 ml with a hematocrit level of 70-80%, a plasma volume of 15-25 ml, and an anticoagulant volume of 10-15 ml. In general, the use of PRC (Packed red cells) for anemic patients who were not accompanied by a decrease in blood volume, for example, patients with hemolytic anemia, acute leukemia, chronic leukemia, malignancy, thalassemia, chronic kidney failure (Saraswati, 2015). Packed red cells consist mainly of erythrocytes that contain hemoglobin. Erythrocyte transfusions were given to replace or restore the capacity of the blood to carry oxygen, therefore good blood quality was very important (Fuadda et al., 2016; McC, 1998).

During the storage period, blood components would experience changes, especially erythrocytes, would experience significant changes in shape over the length of time the blood was stored. During storage red blood cells were very sensitive to clotting. When the red blood cells freeze, the blood cell walls would break and the hemoglobin would come out. This situation could be fatal for recipients of blood transfusions (Tadjuddin, 2012). Donor blood stored using Citrate Phosphate (CPD) anticoagulant would last for 21-28 days while donor blood stored with Citrate Phosphate Dextrose Adenine one (CPDA 1) anticoagulant would last for 35 days after aftap (WHO, 2005). The quality of PRC during storage must be maintained even though there were changes in morphology, biochemistry, and metabolism called storage lesions. Oxidative damage was thought to be the most important factor in storage lesions caused by free radicals and reduces the quality of stored erythrocytes (Deyhim et al., 2014).

Blood storage was carried out considering that the blood service unit cannot always provide fresh blood to be given to patients according to the request of the treating doctor. To maintain the quality of blood, the blood storage process might meet the requirements set by the Ministry of Health, namely stored in the refrigerator at a temperature of 2^o-6^oC. When bloods are not stored at temperatures of 2^o- 6^oC, its ability to deliver oxygen was greatly reduced, leading in a decreased in ATP and 2,3 diphosphoglycerate (DPG) in erythrocytes, an increased in potassium and lactate dehydrogenase (LDH) plasma, and an increased in hemoglobin (Hb) levels (Kim-Shapiro et al., 2011). Another reason was to reduce the growth of bacteria that contaminate stored blood. Storage at temperatures above 6^oC caused the growth of bacteria very quickly so that blood transfusions could be fatal for patients who receive them. Likewise, 2^oC storage limit was also very important, because red blood cells were very sensitive to clotting. When red blood cells froze, the blood cell walls would break and hemoglobin would come out (hemolysis). This situation was also fatal for the recipient of the blood transfusion (Fuadda et al., 2016).

Proper storage of blood was one way to maintain the quality of erythrocytes. Hence, the purpose of blood storage was to maintain the viability and function of blood components for patients who require blood transfusions (Kor et al., 2009).

Several recent studies had shown that transfusion of PRC with longer shelf-life results in significantly worse clinical outcomes or improvements. This was due to the continued metabolism of blood cells during blood storage. Stored erythrocytes undergo complex changes in their membrane structure known as erythrocyte storage injury (Obike, 2016). According to Verma et al. (2015), although the pH value decreased but still in the normal range of 6,75 on day 21. This was caused by an increase in the concentration of lactic acid in the anaerobic glycolysis process. A study carried out by Wiwid (2019) found that the hemoglobin level of the PRC preparation continued to decrease during the shelf life. This occurred presumably due to PRC experiencing red blood cell damage / hemolysis. The shelf life caused changes in erythrocyte viscosity and increases stress cell induced hemolysis, causing death of erythrocyte cells. This erythrocyte damage has a significant impact on the decrease in hemoglobin levels. However, based on the results of Obike in 2016 there was a significant increase in Hb levels in PRC blood bags during the first and fifteenth day of storage with an average increase of 1,7 g/dL (Obike, 2016). During storage of PRC blood bags with anticoagulant CPDA-1, it was estimated that the levels of 2,3 DPG and ATP were suppressed to maintain oxygen diffusion and the biconcave structure of erythrocytes. The PRC blood bag went through a centrifuge process and the separation of blood components so that there was a change in plasma volume to get concentrated erythrocytes.

Plasma glucose decreased during storage, a significant decrease from the shelf life of 1-7 days but the decrease in levels was still above normal. This may be due to the need for glucose for erythrocyte metabolism during storage. Erythrocytes need energy for metabolism which came from glucose as the main source. The breakdown of glucose into lactate or pyruvate was commonly referred to as glycolysis. Glycolysis can also occurred outside the human body, as well as glucose in PRC, would be metabolized 10 times faster at 25^{o}C. So, that PRC stored at 25^{o}C would lose erythrocyte life 10 times faster (Saputra et al., 2019).

PRC continued to experienced quality degradation during storage. This condition was due to the blood cells undergolysis, which has a direct effect on cell morphology, hemoglobin levels, blood pH, and blood ion levels (Sepvianti & Kusumaningrum, 2019).

The United States recommended a 1% limit for the hemolysis index at the end of PRC storage although it had not established an acceptable free Hb level (Cluitmans et al., 2012; Sowemimo-Coker, 2002). Storage of PRC in the presence of phosphate and adenine allowed for a longer storage period. These advanced encourage the use of additive solutions that would not only extend the shelf life but also maintained the quality of the PRC concentrate during storage, namely solutions containing saline adenine glucose mannitol (SAGM) added to erythrocytes, increasing the shelf life of PRC when stored at 2°C to 6°C. Addition of salt and mannitol decreases the rate of hemolysis, and glucose provides an energy substrate pathway and adenine maintains ATP levels (Fuadda et al., 2016).

Based on the phenomenon described above, the researchers wanted to compared the condition of PRC before and after the storage period. We wanted to know about the condition of the PRC whether it was still feasible to immediately transfuse by looking at the changes that occurred in the value of plasma hemoglobin (Hb), plasma glucose, potential of hydrogen (pH), and lactate. Hence, this work aimed to analyzed the effect of storage time on plasma hemoglobin, plasma glucose, potential of hydrogen (pH), and lactate levels before and after storage on the 1st, 10th, 20th, and 30th days.

Material and Method

Research Type and Design

This type of research was an analytical observational study with a time series design, to determine the effect of PRC blood storage time on increasing levels of plasma hemoglobin, plasma glucose, pH, and lactate. The sample was collected using a consecutive sampling technique from July-August 2022. The inclusion criteria were PRC components (stored on day 1, day 10, day 20, and day 30) obtained during the study period at Dr. Soetomo Blood Bank Unit.

Sample Size

Estimated sample size used for testing the hypothesis of one population, using the sample formula according to Lemeshow (1997) as follows (Lemeshow et al, 1997):

$$n = \frac{\sigma^2 \, (Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu 1 - \mu 2)^2}$$

n : number of samples required

$Z_{1-\alpha/2}$: Relative deviation that described the degree of confidence in drawing conclusions (α = 5%), then $Z_{1-\alpha/2}$= 1.96

$Z_{1-\beta}$: The relative deviation that described the level of statistical test power in determined the significance of 90% ($Z_{1-\beta} = 1{,}28$)

1 : Estimated mean in the standard population

2 : Estimated mean in the study population

σ : Standard Deviation

Based on previous research by Chaundhary et al 2014 by using the above formula calculation, the minimum of samples (n) was 30 samples. The sample size used in this study used 50 samples.

Research Location and Time

This investigation was carried-out in July 2022 - August 2022 at Dr. Soetomo Hospital Surabaya Blood Bank unit as a place for sample collection and processing. As well as at the Clinical Chemistry Laboratory, Faculty of Health Sciences, Maarif Hasyim Latif Sidoarjo University as a place for sample examination. Ethical approved was released by the Health Research Ethics Commision, Dr Soetomo General Academic Hospital, Surabaya, Indonesia.

Research Variable

a. Independent variable: Packed Red Cell (PRC) storage time; i.e. 1st, 10th, 20th, 30th day
b. Dependent variables: Levels of plasma hemoglobin, plasma glucose, pH value, and lactate

Processing and Data Analysis

In this investigation, we used a comparative statistical test of more than two pairs of sample groups, namely the Repeated-measures ANOVA test to saw the differences in the average storage time (X)/factor and examination level (Y)/dependent.

Repeated-measures were used in this study because the same subject participates in an experiment with two or more treatments. In addition, when using Repeated-measures analysis, the effect of the experiment is more clearly visible. Where we could determine whether there was an interaction (dependence) between the variables if the standardized residual value of all variables had a significant value of more than 0,05 or was normally distributed. Meanwhile, if the standardized residual value of one variable or all variables had a significant value of less than 0,05 or was not normally distributed, then the Friedman test was used. Friedman test was used to test the differences of 3 or more samples that were interconnected.

Results and Discussion

Quality assurance of inspection results

Quality assurance inspection in this study was carried out before taking measurements on the sample. Quality assurance was carried out by checking the expired date, a lot of number of each kit and storage of reagents according to the instructions on the kit. Quality assurance was also carried out by controlling. Control examinations were carried out 20 times each day.

Table 1: Values Quality Control

Observation	SD Value	CV Value
Hemoglobin	0,09 mg/dL	3,82 %
Glukosa	0,89 mg/dL	0,89 %
Laktat	0,49 mg/dL	4,77 %
pH	0,07	0,98%

Quality Consolidation

The results of the Quality control inspection would be shown in graphical form. If the quality control value were beyond the Standard Deviation (SD) then the problem could be from the tool, reagent or control reagent. If no one of these problems was discovered, calibration can be carried out.

Research Sample Characteristics

The sample size used in this study was 50 samples. This amount had met the minimum sample size set in this study. Sampling was carried out in August-September 2022 at the Blood Bank unit of Dr. Soetomo Hospital Surabaya.

Table 2: Average values ± standard deviation Plasma hemoglobin, Plasma Glucose, pH and lactate on day 1, day 10, day 20 and day 30

Variable	n	PRC Storage Time			
		Day 1	Day 10	Day 20	Day 30
Plasma Hemoglobin ± SD (mg/dL)	50	1.534±0.45	1.983±0.080	2.7487±0.129	3.911±0.208
Plasma Glucose ± SD (mg/dL)	50	391.39±49.876	316.65±64.99	281.98±69,027	248.72±64,496
pH ± SD	50	7.754±0.131	7.386±0.162	7.066±0.172	6.776±0.1333
Lactate ± SD (mg/dL)	50	50,449±5,240	95,872±11,730	121.292±8.891	146.332±9.149

This effect was seen by comparing plasma hemoglobin, plasma glucose, pH and lactate levels at several PRC storage times, started from the first day of examination until the 30th day. The Statistical test used was a comparison test of more than two groups of paired samples, namely the repeated measured ANOVA, where the storage time (X)/factor and plasma levels of hemoglobin, plasma glucose, pH, and lactate (Y)/factor as dependent list.

Data Normality Test Results

Table 3: Plasma Hemoglobin, Plasma Glucose, pH, and Lactate Data Normality Test Results

Examination (day)	Saphiro-Wilk (P)			
	Plasma Hb	Plasma Glucose	pH	Lactate
D 1	0.004	0.136	0.013	0.002
D 10	0.001	0.087	0.026	0.177
D 20	0.000	0.222	0.019	0.000
D 30	0.001	0.747	0.001	0.033

Table 3 showed the results of the normality test used the sig. value Saphiro Wilk. The sig. value compared with the alpha value (0,05). Based on the table, it can be seen that the value of sig. plasma hemoglobin levels from day 1 to day 30 have a significance value of less than 0,05; which means that the data was not normally distributed. Thus, it would be continued with the Friedman test.

Based on the table, it can be seen that the value of sig. plasma glucose levels from day 1 to day 30, the significance value was more than 0,05, which means the data was normally distributed. Hence, it would be continued with repeated measures ANOVA test.

The pH value from day 1 to day 30 had a significance value of less than 0,05 , which means that the data was not normally distributed. Therefore, it would be continued with the Friedman test.

Lactate levels only on day 1, day 20 and day 30 the significance value was less than 0,05. In day 10, the significance value was more than 0,05, which means the data was not normally distributed. Thus, it would be continued with the Friedman test.

Plasma Hemoglobin Difference Test Results

Comparative analysis of the results of examination of plasma glucose levels on day 1, day 10, day 20, day 30 on PRC storage used the Friedman test.

Table 4: Differences in mean plasma hemoglobin over time

	n	Median (Min-Maks)	Value of P
Day 1 Plasma Hemoglobin Level	50	1,580 (1,08 - 2,60)	< 0.001
Day 10 Plasma Hemoglobin Level	50	1,960 (1,24 - 4,90)	
Day 20 Plasma Hemoglobin Level	50	2,245 (1,16 – 7,30)	
Day 30 Plasma Hemoglobin Level	50	3,170 (1,32 – 9,20)	

Friedman test results showed that there was a significant difference in plasma hemoglobin at the four times of observation with a significance value of <0.05. Hence, it can be concluded that there was an effect of the storage time of packed red cells on hemoglobin levels.

Table 5: Significance Value of Wilcoxon test Results on Plasma Hemoglobin Levels

Observation Time	n	P Value
Hb1-Hb10	50	< 0,001
Hb1-Hb20	50	< 0,001
Hb1-Hb30	50	< 0,001
Hb10-Hb20	50	< 0,001
Hb10-Hb30	50	< 0,001
Hb20-Hb30	50	< 0,001

Plasma Glucose Difference Test Results

The results of the plasma glucose difference test on the 1st day, 10th day, 20th day, and 30th day were analyzed using repeated measures ANOVA test analysis.

Table 6: The Differences in plasma glucose

Observation Time	n	Mean ± Standard deviation	P Value
D1	50	391,394 ± 49,876	<0.001
D10	50	316,654 ± 64,991	
D20	50	281,988 ± 69,027	
D30	50	248,725 ± 64,496	

The results of the repeated measure ANOVA test showed a significant difference in plasma glucose at the four times of observation with the greenhouse-gewasser sig. < 0,05; which means that there was a significant difference in the average decrease over time, so it can be concluded that there was an effect of the shelf life of packed red cells on plasma glucose levels.

Table 7: The mean value of decreasing plasma glucose levels over time

Observation Time	n	Average-difference	Value of P
D1-D10	50	74.740	<0.001
D1-D20	50	109.406	<0.001
D1-D30	50	142.669	<0.001
D10-D20	50	34.665	<0.001
D10-D30	50	67.929	<0.001
D20-D30	50	33.264	<0.001

Test Results for Different pH value

Comparative analysis of the results of examination of pH values on day 1, day 10, day 20, and day 30 on PRC storage used the Friedman test.

Table 8: The Differences in pH values over time

	n	Median (Min-Maks)	P Value
pH Value Day 1	50	7,750 (7,50-8,00)	
pH Value Day 10	50	7,400 (7,00-7,70)	< 0.001
pH Value Day 20	50	7,100 (6,80-7,40)	
pH Value Day 30	50	6,800 (6,50-7,00)	

Friedman test results showed that there was a significant difference in the pH value at the four times of observation with a significance value of < 0,05. It can be concluded that there was an effect of the storage time of packed red cells on the pH value.

Table 9: Significance Value of Wilcoxon test Results on pH Values

Observation Time	n	P Value
pH1-pH10	50	< 0,001
pH1-pH20	50	< 0,001
pH1-pH30	50	< 0,001
pH10-pH20	50	< 0,001
pH10-pH30	50	< 0,001
pH20-pH30	50	< 0,001

Test Results for Different Lactate Levels

Comparative analysis of the results of examination of lactate levels on day 1, day 10, day 20, and day 30 on PRC storage used the Friedman test.

Table 10: The Differences in lactate levels over time

	n	Median (Min-Maks)	P Value
Day 1 Lactate Level	50	50,405 (36,21-62,91)	
Day 10 Lactate Level	50	96,820 (76,19-120,16)	<0.001
Day 20 Lactate Level	50	123,305 (96,42-130,17)	
Day 30 Lactate Level	50	143,423 (126,91-161,71)	

Friedman test results showed that there was a significant difference in plasma hemoglobin at the four times of observation with a significance value of < 0,05, means that there was an effect of packed red cell storage time on lactate levels.

Table 11: Significance Value of Wilcoxon test Results on Laktat Levels

Observation Time	n	P Value
Lak1-Lak10	50	< 0,001
Lak1-Lak20	50	< 0,001
Lak1-Lak30	50	< 0,001
Lak10-Lak20	50	< 0,001
Lak10-Lak30	50	< 0,001
Lak20-Lak30	50	< 0,001

Discussion

The sample of this study used 50 bags of Packed Red Cell (PRC) blood components with the highest proportion of blood types. Normality test was performed on all parameters examined in this study. The results of the normality test showed that only plasma glucose had a normal distribution at all four examination times (p > 0,05); so that the differences between times were carried out by repeated measure ANOVA test analysis. Other parameters that were not normally distributed were analyzed by Friedman's test.

Plasma Hemoglobin Difference Test Results

The results of the different normality test of hemoglobin plasma showed that the distribution of the data was not normally distributed (p<0,05) so that the differences in plasma hemoglobin levels were analyzed used the Friedman test. Friedman test results showed a p value < 0,05 which means that there were significant differences on day 1, day 10, day 20, and day 30. On the results of the Friedman test, the average plasma hemoglobin level on day 1 was 1,534 with a standard deviation of 0,318; on day 10 was 1,982 with a standard deviation of 0,565; on day 20 was 2,748 with a standard deviation of 0,911; and day 30 was 3,911 with a standard deviation 1,468.

This means that started from the 10th day of storage, the destruction of erythrocytes had occurred. This stated that the effect of storing packed red cells would made many erythrocytes died immediately after the blood was transfused due to a decrease in ATP (Adenosine Tri Phosphate) levels. Blood that had been stored for 30 days, 20% of the erythrocyte content in it would die after was being transfused. Oxygen was tightly bound to hemoglobin and too little was delivered to issues. Because many erythrocytes were lysed, it was possible that the stored blood would experience an increase in hemoglobin levels (Sumaningsih et al., 2018).

According to Karon et al., and Spinelli et al., the United States proved that an increase in plasma free hemoglobin levels occurred during PRC storage. This increase was thought to be a contributing factor to poor outcomes in PRC transfusion recipients although the underlying mechanism was not fully understood (Karon et al., 2012; Spinelli et al., 2014).

There was a significant increase in Hb levels in PRC blood bags during the first and fifteenth days of storage with an average increase of 1.7 g/dL. During storage of PRC blood bags with anticoagulant CPDA-1, it was estimated that the levels of 2,3 DPG and ATP were suppressed to maintain oxygen diffusion and the biconcave structure of erythrocytes. In this case, PRC blood bags went through a centrifuge process and separated blood components so that there was a change in plasma volume to get concentrated erythrocytes (Achor, 2016)

It was found that the hemoglobin level of the PRC preparation continued to decrease during the shelf life. This occurred presumably due to PRC experiencing red blood cell damage / hemolysis. The shelf life causes changes in erythrocyte viscosity and increases stress cell induced hemolysis, causing death of erythrocyte cells (wiwid et al, 2019)

Based on the results of research conducted by Rosyidah et al. (2020), it was revealed that the average value of hemoglobin level examination decreased on the first day to the 10th day but on the 14th day it increased. Likewise, Naim (2014) showed that there was no effect of the duration of blood storage on hemoglobin levels.

Based on the Friedman test table, the value of p < 0,05 was 0,000, so the null hypothesis (Ho) was rejected and H1 was accepted, which means there was an effect of donor blood storage on the results of the examination of plasma hemoglobin levels. This showed during the storage process showed the destruction of erythrocytes. The longer the blood was stored, the erythrocytes swell due to the loss of viability of erythrocyte cells caused by the rigidity of the membrane and the unavoidable loss of erythrocyte cell membrane lipids during storage, the stiffness of the erythrocyte cells holds the trapped plasma, and this causes the hemoglobin level to increase in storage. The results of this study were almost the same as Saini et al. (2015) which showed a significant increase from day 1 to day 28 of storage (Saini et al, 2015).

Glucose Plasma Difference Test Results

The results of the normality difference test for hemoglobin plasma showed that the data was normally distributed (p > 0,05), so that the differences in plasma hemoglobin levels were analyzed used the repeated measures ANOVA test. The results of the repeated measures ANOVA test showed a p value <0,05, which means that there were significant differences on day 1, day 10, day 20, and day 30. On the results of the repeated measure ANOVA test, the mean plasma glucose level on day 1 was 391,394 with a standard deviation of 49,876; on day 10 was 316,654 with a standard deviation of 64,991; day

20 was 281,988 with a standard deviation of 69,027; and day 30 was 248,725 with a standard deviation of 64,496.

In this study, there was a significant decrease in plasma glucose due to the need for glucose for erythrocyte metabolism during storage. Erythrocytes needed energy for metabolism which came from glucose as the main source. Hence during storage, glucose was taken up from the plasma during storage. Erythrocytes did not have nuclei and mitochondria so that oxidative metabolic energy was generated through the breakdown of glucose. The breakdown of glucose into lactate or pyruvate was commonly referred to as glycolysis. Glycolysis can also occur outside the human body. Glucose would be stable within 24 hours in plasma or serum stored at 20°C, whereas at room temperature, blood samples for glucose testing in the absence of glycolysis inhibitors would be metabolized at about 7 mg/dL/day. Hence, glucose in packed red cells will be metabolized 10 times faster at 25°C; so Packed Red Cells stored at 25°C will lose their erythrocyte life 10 times faster (Donadee et al., 2011).

Test Results for Differences in pH Value

The results of the normality test of pH values showed that the distribution of the data was not normally distributed ($p<0,05$); so that the differences in pH values were analyzed using the Friedman test. Friedman test results showed a p value < 0,05 which means that there were significant differences on day 1, day 10, day 20, and day 30. On the results of the Friedman test, the average pH value on the 1st day was 7,754 with a standard deviation of 0.131; on the 10th day of 7,386 with a standard deviation of 0.162; on the 20th day of 7,066 with a standard deviation of 0,721; and the 30th day of 6,776 with a standard deviation 0,133.

Based on the results of the examination above, it was found that the pH value had decreased. This result was in accordance with the statement of Isti et al, 2018 that the cell metabolic process continued as storage time increases; ATP and glucose levels in the blood bag would decrease because they were used for cell metabolism whose energy came from the glycolysis process. Lactic acid as a result of glycolysis would continue to accumulate caused the environmental conditions to become acidic (pH decreases). (Isti et al, 2018).

Test Results for Different Lactate Levels

The results of the normality test of lactate levels showed that the distribution of the data was not normally distributed ($p<0,05$); so that the differences in lactate levels were analyzed used the Friedman test. Friedman test results showed a p value <0,05 which means that there were significant differences on day 1, day 10, day 20, and day 30. On the results of the Friedman test, the average lactate level on day 1 was 50,449 with a standard deviation of 5,241; on day 10 was 95,873 with a standard deviation of 11,730; on day 20 was 121,29 with a standard deviation of 8,892; and on day 30 was 146, 33 with a standard deviation of 9,149.

According to Isti et al. (2018), when lactate levels have increased, lactic acid as a result of glycolysis would continue to accumulate causing the environmental conditions became acidic (pH decreases) (Isti et al, 2018)

Factors that affected the blood in the blood bag were acidity or pH, storage time, and temperature. Erythrocytes would be destroyed if there was no rejuvenation with the blood storage process, which was the process of slowing down the destruction so that the absence of rejuvenation can be overcome. One way was to store blood at a low temperature of 2°C-6°C. This was also one method that could be used to slow down the metabolism. As according to Saidjao (2019), the maximum temperature for storing blood was 6°C, while over 6°C bacterial growth occurs. Meanwhile, temperatures less than 2°C could damage cell membranes because blood clots occurred which cause hemolysis (Saidjao, 2019).

Conclusions

Based on the results of the storage investigations in Blood Transfusion Unit of Dr. Soetomo Hospital Surabaya, it can be concluded that there is a difference level on the results of the examination of Plasma Hemoglobin levels, Plasma Glucose levels, pH and Lactate levels on Packed red Cells. Further research is needed with shorter storage interval variations so that it can predict the occurrence of hemolysis and changes in levels of biochemical parameters un PRC accurately.

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