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Original Article

Differences in Lactate Dehydrogenase (LDH) Levels in Packed Red Cells (PRC) with Various Storage Times

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Abstract

This research seeks to analyze the storage duration effect on PRC (Packed Red Cell) quality by assessing changes in LDH (Lactate Dehydrogenase) levels on storage days 1, 10, 20, and 30. This research is an observational analytical study with a time series design. The research was conducted at the Blood Transfusion Installation of Dr. Soetomo General Hospital Surabaya as the sampling site and the Clinical Laboratory of the Faculty of Health Sciences, Maarif Hasyim Latif University, Sidoarjo as the place for measuring research variables. The research sample consisted of fifty PRC bags from voluntary donors. LDH levels were examined with a Semi-Automatic Chemistry Analyzer Sinnowa BS-3000P Photometer (Sinnowa Medical Science & Technology Co., Ltd., China) and measured on days 1, 10, 20, and 30. The research data were analyzed using SPSS 25 statistical software. Data normality was analyzed using the Shapiro-Wilk test. Comparison of PRC quality was analyzed using repeated measure ANOVA analysis (same object analysis) if the data were normally distributed, or the Freedman test if the data distribution was not normal. The findings revealed that there was an increase in mean LDH levels with increasing PRC storage duration over 30 days. Repeated Measure ANOVA test and the Post Hoc Bonferroni test for LDH levels showed that there were statistically significant differences with a significance value of $p < 0.001$ at all observation times. In conclusion, there are significant differences in LDH levels during Packed Red Cell (PRC) storage from day 1, day 10, day 20, and day 30.

Keywords: Erythrocyte Hemolysis, Oxidative Stress, Stored Blood Quality, Blood Transfusion.

1. Introduction

Blood transfusion is one of the vital health treatments today, this health treatment is the activity of transferring donor blood to patients (Booth et al., 2021). Blood products produced include whole blood, packed red cells, thrombocyte concentrate, and blood plasma (Ministry of Health Regulation, 2015). Each type of blood product used refers to the patient's medical indications (Armaidi & Irawan, 2015). Packed Red Cells (PRC) is a type of blood component derived from Whole Blood (WB) that is settled during storage or with high-speed centrifugation, then most of the plasma is discarded. Packed Red Cells (PRC) has a volume of 200-250 mL with a hematocrit level of 70-80%, plasma volume of 15-25 mL, PRC bags use Citrate, Phosphate, Dextrose, Adenine-Formula 1 (CPDA-1) anticoagulant with a volume of anticoagulant precipitate fluid of 10-15 mL (Saraswati, 2015). PRC transfusion is used for patients with neonatal hyperbilirubinemia (Hosea et al., 2015), anemic patients not accompanied by decreased blood volume; thalassemia major (Sutrisnaningsih et al., 2017),

hemolytic anemia, acute leukemia, chronic leukemia, malignancy disease, thalassemia major, chronic kidney failure (Saraswati, 2015).

Packed Red Cells (PRC) is the most frequently transfused blood component. Data from the annual report of the Blood Transfusion Installation (ITD) of Dr. Soetomo General Hospital Surabaya in 2024 shows that PRC transfusion is the largest transfusion at Dr. Soetomo General Hospital, approximately 46,224 (49.9%) of 92,574 blood transfusion requests (ITD, 2021). The high PRC transfusion is related to the prevalence of anemia (Rahayu et al., 2023). Interventions related to anemia treatment are PRC transfusions, making the need for PRC transfusions high (Nasruddin, 2021). The prevalence of anemia in Indonesia is still quite high. Data from the 2018 Basic Health Research (Riskesdas) survey shows the national percentage of anemia in women of childbearing age is 48.9%, female toddlers 26.50%, adolescents (female and male) 22.7% and 16.6% respectively, and pregnant women 37.1% (Riskesdas, 2018).

The vital role of PRC transfusion in raising hemoglobin levels is inseparable from the fact that PRC continues to experience quality deterioration during storage. Erythrocytes undergo a number of changes during the storage period (Beliën & Forcé, 2012). These changes are known as storage lesions (Kim-Shapiro et al., 2011). Storage lesions will affect erythrocyte viability, erythrocyte function in transporting oxygen from the lungs to tissues, erythrocytes become susceptible to cell damage and hemolysis, thus affecting the quality of PRC and the efficacy of PRC transfusion for patients. Storage lesions can be proven by an increase in Lactate Dehydrogenase (LDH) in erythrocytes (Saragih et al., 2019).

The most serious outcome of erythrocyte storage lesions is the occurrence of hemolysis and cell damage during blood preservation. Hemolysis refers to the release of Hemoglobin (Hb) into the plasma when erythrocytes break down (Donati et al., 2014). The plasma shows higher levels of certain substances as a result of cell damage and hemolysis, including Lactate Dehydrogenase (LDH). LDH is found in erythrocytes in significant quantities, playing a role as a catalyst in the last step of erythrocyte glycolysis. When hemolysis happens, LDH is released into the bloodstream, where a greater amount of hemolysis results in an increased level of LDH in the plasma (Triyono et al., 2016). Saraswati (2015) research measured LDH levels in 10 PRC blood bags with a storage period of 28 days. The research findings indicated that there were notable variances ($p < 0.05$) in LDH levels observed on the 5th, 15th, and 28th days. The longer the PRC is stored, the higher the LDH level (Saraswati, 2015). The results of Saraswati (2015) research are supported by other studies showing significant increases in hemolysis and LDH levels ($p < 0.05$) during five weeks of PRC storage (Sawant et al., 2007).

PRC anticoagulant-preservative materials are phosphate and adenine which allow for a longer storage period. Citrate-phosphate-dextrose (CPD) became the standard anticoagulant at the end of the 1950s. Blood bags containing CPD anticoagulant-preservative can last up to 21 days. This anticoagulant-preservative was then developed in 1978 with the addition of adenine formula 1 (CPDA-1) so that the shelf life of blood with this anticoagulant-preservative increased to 35 days (Maulidan et al., 2022). Several studies that have been conducted prove that during the PRC storage period of more than 3 days, various changes occur that will affect blood quality and the efficacy of PRC transfusion for patients, and supported by observations made by researchers showing the average use of stored PRC at the Blood Transfusion Installation of Dr. Soetomo General Hospital Surabaya is below 20 days, so in this study, the examination time for the variables studied was chosen on days 1, 10, 20, and 30 of PRC storage.

Based on the changes explained above and the fact that many PRCs are transfused at Dr. Soetomo General Hospital Surabaya, this research is very important to be carried out regarding the impact of storage on the quality of PRC blood products at the Blood Transfusion Installation of Dr. Soetomo General Hospital. The researcher wants to study how many days

after PRC production is still suitable for transfusion by looking at changes that occur in LDH levels according to storage time.

Based on the research background, the purpose of this study is to analyze the effect of storage duration on the quality of Packed Red Cell (PRC) by observing changes in LDH levels in stored PRC on days 1, 10, 20, and 30. The results of this research are expected to contribute knowledge about the effect of storage on the quality of PRC that is still safe to use, thereby increasing the safety of recipients who undergo stored PRC transfusion.

2. Literature Review

2.1. Blood

Blood plays a crucial role in the body as it carries oxygen to cells, provides nutrients to tissues, removes waste products, and houses immune system components to fight off diseases (Maharani & Noviar, 2018). Red blood cells play a crucial role in the respiratory system of the body by carrying oxygen to every cell and tissue and removing carbon dioxide to the lungs. Oxygen is essential for life, but excess oxygen will have harmful effects and can cause tissue damage, therefore every living creature has a system that strictly regulates oxygen transport to tissues (Hamasaki & Yamamoto, 2000).

2.2. Red Blood Cell (RBC) Metabolism

Red Blood Cells (RBC) do not have a nucleus, ribosomes, mitochondria, and other organelles that are important for performing specific functions of cell survival, thus red blood cells are unable to synthesize nucleic acids, proteins, and lipids through denovo pathways. The shape of Red Blood Cells (RBC) is a flexible biconcave disc. The *Embden-Meyerhof* pathway (glycolysis) is the only metabolic pathway to produce energy (J. Adams, 2010). The Embden-*Meyerhof pathway* (glycolysis) also produces Nicotinamide Adenosine Dinucleotide Hydrogen (NADH) which is needed by the methemoglobin reductase enzyme to reduce methemoglobin (oxidized hemoglobin) to reduced hemoglobin. 2,3-DPG produced in the *Luebering-Rapoport Shunt* or side path in this pathway forms a 1:1 complex with hemoglobin, and as mentioned above, is important in regulating hemoglobin's affinity for oxygen. The Hexose Monophosphate Pathway (*pentose phosphate*) about 5% of glycolysis occurs through this oxidative pathway, with the conversion of glucose-6-phosphate to 6-phospho-gluconate and then to ribose-5-phosphate producing Nicotinamide Adenine Dinucleotide Phosphate (NADPH). NADPH is associated with glutathione which maintains intact Sulfhydryl Groups (SH) in cells, including the sulfhydryl groups of hemoglobin and erythrocyte membranes. NADPH is used by another methemoglobin reductase to maintain hemoglobin iron in the functionally active Fe^{2+} state (Holffbrand et al., 1981).

2.3. Packed Red Cell (PRC)

Packed Red Cell (PRC) is the most requested blood component in hospital blood banks. Packed Red Cell (PRC) is a blood unit obtained by cold centrifugation method with high speed from Whole Blood (WB) then most of the plasma is discarded (Ministry of Health Regulation, 2015). The volume of one PRC unit is 200-250 mL. PRC blood unit transfusion is used for patients with neonatal hyperbilirubinemia (Hosea et al., 2015), anemic patients not accompanied by decreased blood volume; thalassemia major (Sutrisnaningsih et al., 2017), hemolytic anemia, acute leukemia, chronic leukemia, malignancy disease, thalassemia major, chronic kidney failure (Saraswati, 2015). Patients receive PRC transfusion in order to enhance the delivery of oxygen to their tissues. This type of transfusion is administered to patients exhibiting symptoms or requiring a rapid elevation in Hb levels. PRC transfusion remains

advisable in cases of acute severe bleeding, symptomatic severe anemia, as well as in certain specific conditions like hemoglobinopathy and kernicterus. Transfusions are not usually suggested when hemoglobin levels are more than 10 g/dL. Conditions that make patients unsuitable for PRC transfusions include stable acute and chronic anemia. These conditions encompass autoimmune anemia, megaloblastic anemia, iron deficiency, and anemia in patients with kidney failure, all of which can be managed with alternative treatments instead of transfusions (Seeber & Shander, 2012). One unit of PRC has a volume of 150-250 mL. The expiration limit for PRC is 30 days (Ministry of Health Regulation, 2015).

2.4. Storage Conditions and Duration

Blood units have the ability to be preserved for an extended period by incorporating an anticoagulant solution (Hamasaki & Yamamoto, 2000). Blood is kept in a fridge at a temperature between 1-6°C in a blood bank for 21-42 days, depending on the anticoagulant solution used to keep it viable (Lockwood et al., 2008; McCullough, 2011). Blood Bank Refrigerator is a refrigerator with good air circulation and equipped with a temperature controller specially designed to store blood units. Household refrigerators are not suitable for blood storage, as there is no alarm system in place to notify blood service employees if the temperature exceeds the permitted limits. It is necessary to check and document the temperature of the Blood Bank Refrigerator regularly, specifically every 4 hours. Prior to being given to patients, blood units are heated to 10°C. It is imperative to ensure that blood components are stored correctly while being transported from the blood transfusion service center to the hospital. The type of container that meets blood transportation standards has been available in developed countries, but this type of container is often not available in developing or underdeveloped countries and blood products may not be stored properly during transportation (Almac & Ince, 2007).

2.5. Changes in Red Blood Cells During Storage

Transfusion medical science continues to develop, increasing the demand for blood transfusions, this causes millions of units of stored Packed Red Cell (PRC) blood to be transfused to patients worldwide. The basic theoretical assumption is that an increase in intravascular erythrocyte mass will increase oxygen supply to tissues, but based on the results of several studies that have been conducted, it shows that this theoretical assumption is wrong and there is a positive correlation between storage duration and erythrocyte viability and function. Research results show that PRC undergoes a number of changes during the storage period that affect the survival and ability to provide oxygen to tissues, but field facts prove that PRC is the blood unit most often transfused (Almac & Ince, 2007).

Red Blood Cell (RBC) storage lesion is a series of biochemical, metabolic, and structural changes that emerge when RBCs are stored outside the body. These changes determine the effectiveness and safety of transfusions using stored blood. Many things need to be considered when giving transfusions with stored blood. Some things that need to be considered are the effectiveness of tissue oxygenation and clinical side effects on giving old stored donor blood compared to fresh donor blood before being given to recipients (Glynn et al., 2016).

Storage has a negative effect on the ability of erythrocytes to distribute oxygen and many events that support erythrocyte transfusion can harm recipients. Several events show an increased risk of severe complications and death in critically ill patients, especially patients undergoing cardiac surgery. Recent research indicates erythrocyte damage in low-temperature storage as a factor responsible in blood transfusion if associated with increased length of hospitalization, decreased tissue oxygenation, pro-inflammatory and immunomodulatory effects, increased risk of infection, multiple organ failure, and increased

morbidity and mortality (Adias et al., 2012). During storage, red blood cells experience various alterations that impact their longevity and their capacity to transport oxygen to body tissues. These modifications can be categorized into two main groups: biomechanical transformations and biochemical transformations (Almac & Ince, 2007).

2.6. Lactate Dehydrogenase (LDH) Levels

Lactate Dehydrogenase (LDH) is an intracellular enzyme that functions in catalyzing pyruvate to lactate and NADH to NAD+ in glycolysis metabolism (Amorini et al., 2007; Murray et al., 2003). The process of glycolysis is one of the stages to synthesize ATP which is used as an energy source for red blood cells. The energy produced will be used to maintain the shape of red blood cells and the viability of red blood cells (Kholmukhamedov & Jobe, 2019). At the end of the glycolysis process, it will produce 2 molecules of ATP, 2 molecules of ADP, and 2 molecules of CO2. This dissolved CO2 compound will cause conditions inside the blood bag to become acidic, resulting in a decrease in pH (Lodish, 2008).

In general, LDH levels will increase during the storage period of donor red blood cells. The increase in LDH levels during the storage period indicates the occurrence of glycolysis metabolism. The glycolysis process is also seen from the decrease in glucose levels along with the length of storage time of donor red blood cells because glucose undergoes oxidation to produce ATP (Amorini et al., 2007; Murray et al., 2003). LDH levels will increase along with the length of storage time, this is positively related to the decrease in glucose levels. The increase in LDH levels can also be caused by the increasingly low pH conditions in the blood bag. Decreased pH, glucose, and increased LDH can cause damage to the permeability of the erythrocyte cell membrane (Marpaung et al., 2015).

3. Methods

3.1. Research Type and Design

This research is an observational analytical study with a time series design, which is research conducted without intervening on the variables to be studied, carried out at a certain time period, to see changes that occur from the beginning to the specified time in sequence.

3.2. Research Population and Sample

The population in this study is donor blood products Packed Red Cell (PRC) at the Blood Transfusion Installation of Dr. Soetomo General Hospital Surabaya from July to August 2024. The sample in this study is Packed Red Cell (PRC) containing Citrate Phosphate Dextrose Adenine CPDA-1 anticoagulant-preservative material from voluntary blood donors who have gone through the production process at the Blood Component Laboratory and have passed screening tests related to transfusion-transmitted diseases (TTD) at the Blood Transfusion Installation of Dr. Soetomo General Hospital Surabaya, then stored in a Blood Bank Refrigerator with a temperature of 1-6˚C. Voluntary blood donors who meet the blood donation requirements have provided a consent letter regarding the purpose of blood collection for research purposes.

Table 1. Operational Definition of Research

	Definition	Measurement			
		Method	Tool	Result	Scale
Packed Red Cell (PRC)	Blood component obtained from whole blood by centrifugation; plasma mostly removed. Used PRC is from eligible voluntary donors from Dr. Soetomo General Hospital Surabaya				
Storage Time	Day 1: 24 hours post-production; Day 10/20/30: PRC stored for 10/20/30 days respectively.				
Lactate Dehydrogenase (LDH)	Intracellular enzyme abundant in erythrocytes; elevated levels indicate hemolysis.	Kinetics - IFCC	Semi-Automatic Clinical Chemistry Analyzer Sinnowa BS-3000P Photometer	U/L	Interval

3.3. Research Equipment and Materials

The equipment used in this research is PRC donor bags with Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant solution, Blood Bank Refrigerator, test tube rack, gloves, plain tubes, clamp scissors, heat sealer, surgical scissors, blood transportation box, ice pack, tube rack, centrifuge, and Semi-Automatic Chemistry Analyzer Sinnowa BS-3000P (Sinnowa Medical Science & Technology Co., Ltd., China). The materials used in this research are blood samples and plasma from Packed Red Cell (PRC).

3.4. Research Location and Time

The research was conducted at the Blood Transfusion Installation of Dr. Soetomo General Hospital Surabaya as the sampling site and the Clinical Laboratory of the Faculty of Health Sciences, Maarif Hasyim Latif University, Sidoarjo as the place for measuring research variables, for approximately 62 days from July to August 2024.

3.5. Research Procedure

3.5.1. Research Sample Preparation

Packed Red Cell (PRC) that has been produced at the Blood Component Laboratory of the Blood Transfusion Installation of Dr. Soetomo General Hospital Surabaya is stored for 24 hours in a Blood Bank Refrigerator with a temperature of 1-6°C (day 1), samples are obtained from one part of the PRC bag tube then put in plain tubes and centrifuged at a speed of 3500 rpm for 10 minutes. Plasma is taken and placed in plain tubes. PRC plasma samples are used for examining Lactate Dehydrogenase (LDH) levels using the spectrophotometer method. Three other parts of the PRC bag tube are stored in a Blood Bank Refrigerator with a temperature of 1-6°C for examination of Lactate Dehydrogenase (LDH) levels in storage on days 10, 20, and 30.

3.5.2. Examination of Lactate Dehydrogenase (LDH) Levels

a. Preparation of Working Reagent and Sample Setup

The examination of Lactate Dehydrogenase (LDH) levels begins with the preparation of the working reagent by mixing reagent R1 and reagent R2 in a 4:1 ratio. Sample preparation involves four tubes, each labeled as Blank, Standard, Control, and Sample. Into the Blank tube, 500 µL of working reagent is added. The Standard tube receives 500 µL of working reagent and 5 µL of standard reagent. The Control tube is filled with 500 µL of working reagent and 5

µL of control reagent. Lastly, the Sample tube is prepared by adding 500 µL of working reagent and 5 µL of plasma. After preparation, the samples are ready for analysis according to the instrument's protocol.

Table 2. Composition of LDH Reagent Mixture with Sample

Pipetting	Blank	Standard	Control	Sample
Working Reagent	500 µL	500 µL	500 µL	500 µL
Standard	-	5 µL	-	-
Control	-	-	5 µL	-
Plasma	-	-	-	3 µL

b. Performing Standards and Quality Control

To begin, turn on the device by pressing the switch located at the back. Once the green screen appears, tap the LCD screen to access the main menu. Before performing any tests, the device must be washed. Prepare a tube containing aquadest (distilled water), insert it into the device's suction hose, and press the "Wash" button. This washing process must be repeated three times to ensure cleanliness.

Next, access the testing program by pressing "Test" on the menu display and selecting the "LDH" parameter. To perform standard calibration, first insert a tube containing aquadest into the suction hose and press the green button, allowing the device to draw the liquid. Once the device completes the suction, a reading will appear on the screen. If the result falls within the expected range, press the "Continue" button to proceed.

The device will prompt for a blank test. Press "Yes," then insert the prepared Blank into the suction hose and press the green button. Wait until the Blank is fully drawn in. The result will appear, after which you should press "Continue" to begin the standard testing process. Confirm by selecting "Yes" on the "Test STD" display. Insert the prepared Standard tube into the suction hose and press the green button.

After the standard is processed, the factor result will be shown. To proceed to the Quality Control step, press "Continue" and confirm "Yes" when prompted. Insert the Control tube into the suction hose and press the green button. The Control result will be displayed on the screen. You may select "Rerun" to repeat the quality control process or "Continue" to move on. Quality control testing should be replicated 20 times for accuracy.

c. Performing Sample Examination

Once quality control is complete, press "Continue" on the control result display. Begin the sample test by entering the sample ID and necessary information on the screen, then press "Continue." Insert the prepared sample tube into the suction hose and press the green button. Wait for the device to finish processing. Once complete, the sample result will be displayed on the screen, and the result should be recorded in the examination result table.

To examine the next sample using the same parameter, press "Continue." If there are no further samples, perform the washing procedure again as described earlier (three times with aquadest), and finally, turn off the device by pressing the switch on the back (Nugraha & Badrawi, 2018).

The results obtained from the examination are presented in the form of tables, diagrams, and written descriptions to provide a clear, structured, and comprehensive overview of the data. This presentation method facilitates better understanding, comparison, and interpretation of the findings.

3.6. Data Analysis Method

The research data obtained during the observation period are entered into tables and processed using the Statistical Package for Social Science (SPSS) 25 computer program. The collected data undergo cleaning, coding, tabulation, and entry into the computer. The data calculates the mean, standard deviation, and 95% confidence interval (CI) of the mean. Data normality test is analyzed using the Shapiro-Wilk test. Comparison of PRC quality on days 1, 10, 20, and 30 is analyzed using repeated measure ANOVA analysis (same object analysis) because the data are normally distributed, or the Freedman test if the data distribution is not normal with a p-value < 0.05 considered statistically significant.

4. Results and Discussion

4.1. Research Results

The research sample consisted of 50 PRC (Packed Red Cell) blood units from 50 voluntary blood donors who had received information regarding this research and had provided consent forms for blood collection for research purposes.

Table 3. Research Sample Characteristics

Characteristics	Mean ± SD	n	Percentage (%)
Gender			
Male		10	20%
Female		40	80%
Hemoglobin Level	14.7 ± 0.9 g/dL		
Blood Type			
A Rh+		10	20%
B Rh+		21	42%
O Rh+		16	32%
AB Rh+		3	6%
Total		**50**	**100%**

Table 3 shows that female voluntary donors (80%) were more numerous than male donors (20%) with a mean Hb level of 14.7 g/dL. The most common ABO blood type was type B at 42%. All research samples had Rh-positive (D antigen) blood type. Figure 1 shows that the mean Lactate Dehydrogenase (LDH) levels in PRC increased with storage duration over 30 days.

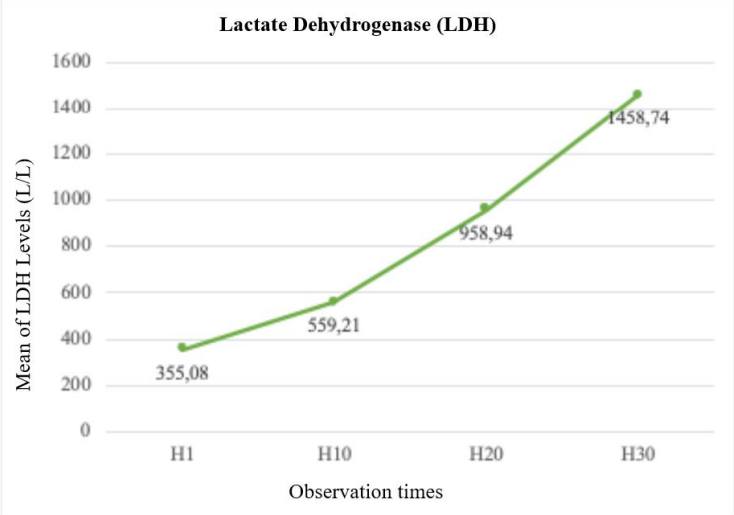

Figure 1. Graph of Mean LDH Levels Across Observation Times

The Shapiro-Wilk normality test results for LDH measurements across observation times showed a normal distribution at all observation times with significance values of $p > 0.05$. The Repeated Measure ANOVA test results for PRC LDH levels in Table 4 show statistically significant differences at all observation times: day 1, day 10, day 20, and day 30, with a significance value of $p < 0.001$.

Table 4. Results of Mean Difference Test for PRC LDH Levels Across Observation Times

Observation Time	n	LDH (U/L)	P Value
		Mean ± SD	
Day 1	50	355.08 ± 9.31	< 0.001
Day 10	50	559.21 ± 14.17	
Day 20	50	958.94 ± 15.08	
Day 30	50	1458.74 ± 15.31	

The Bonferroni Post Hoc test needed to be conducted to determine which observation times were different. The results of the Bonferroni Post Hoc analysis for PRC LDH levels across observation times in Table 5 show that there were significant differences in PRC LDH levels at all observation times.

Table 5. Results of Bonferroni Post Hoc Test for PRC LDH Levels Across Observation Times

Observation Time	Day 10	Day 20	Day 30
Day 1	P < 0.001	P < 0.001	P < 0.001
Day 10		P < 0.001	P < 0.001
Day 20			P < 0.001

4.2. Discussion

The research sample consisted of 50 PRC bags with Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant obtained from voluntary blood donation activities. Voluntary donors who met blood donation requirements were given consent forms regarding the purpose of blood collection for research purposes. The PRC samples in this study had passed screening tests for Transfusion-Transmitted Infections (TTI) conducted by the Transfusion-Transmitted Infections Laboratory at the Blood Transfusion Installation of Dr. Soetomo Hospital, Surabaya. Table 3 indicates that the majority of subjects were female (80%), while male subjects accounted for 20%. The average hemoglobin (Hb) level among all subjects was 14.7 g/dL. Blood type B was the most prevalent ABO group, observed in 42% of the subjects. Additionally, all participants had Rh-positive (D antigen) blood type.

The research samples were obtained from one part of the PRC bag tubing containing CPDA-1 anticoagulant-preservative, similar to the research by Triyono et al. (2013), which analyzed Lactate Dehydrogenase (LDH) levels from PRC and Whole Blood (WB) CPDA-1 bag tubing. The number of samples analyzed was 10 PRC CPDA-1 bags and 11 WB bags with a storage time of 28 days (Triyono et al., 2016). This research differs from the research conducted by Triyono et al. (2016). LDH levels were analyzed from 50 PRC CPDA-1 bags with a storage duration of 30 days.

The Repeated Measure ANOVA test results for PRC LDH levels in Table 4.2 show statistically significant differences at all observation times: day 1, day 10, day 20, and day 30, with a significance value of $p < 0.001$. The line diagram in Figure 4.1 shows that the mean PRC LDH levels increased with PRC storage duration. The results of the Bonferroni Post Hoc

analysis for PRC LDH levels across observation times in Table 5.4 show that there were significant differences ($p < 0.001$) in PRC LDH levels at all observation times.

The results of this research are similar to the research conducted by Triyono et al. (2016), which showed that the increase in PRC LDH levels on day 1 and day 3 had no statistically significant difference ($p = 0.508$), while the increase in LDH levels on day 7, day 14, and day 28 had statistically significant differences ($p = 0.005$) (Triyono et al., 2016). Research by Marjani et al. (2007) showed a statistically significant increase in PRC LDH levels ($p < 0.001$) on day 5 of storage (Marjani et al., 2007). This increase is supported by research from Aslan et al. (1997), which found that the activity of glutathione peroxidase enzyme and superoxide dismutase enzyme decreased significantly ($p < 0.05$) after storage on day 9 and day 13 (Aslan et al., 1997). The activity of these enzymes decreased, weakening the erythrocyte antioxidant system, thereby facilitating hemolysis (Triyono et al., 2016). This finding is supported by research by Arun et al. (1999), which stated that there are significant changes in the integrity of the erythrocyte membrane with increasing storage duration (Arun et al., 1999).

Prolonged storage causes increased exposure of erythrocytes to oxidative damage, which is reinforced by the rate of hemolysis that increases with increasing storage duration (Gkoumassi et al., 2012). The higher the hemolysis, the higher the LDH levels (Triyono et al., 2016). This finding is supported by research by Sawant et al. (2007), which showed a significant increase in hemolysis and LDH levels ($p < 0.05$) during storage (Sawant et al., 2007). PRC bags contain less anticoagulant, higher friction between erythrocytes, and more concentrated leukocytes, making PRC erythrocytes more susceptible to hemolysis (Triyono et al., 2016).

The results of this study indicate that there are significant differences in LDH levels at all observation times. The increase in PRC LDH levels appears to progress with increasing storage duration. The increase in LDH levels is an indication of hemolysis in the PRC unit (Saraswati, 2015). The clinical implications of hemolysis in stored blood units for patients receiving transfusions are very serious and cause redox injury to tissues, endothelium, or kidney proximal tubules, while procoagulant and proinflammatory surfaces emerge due to microvesicle infusion affecting microcirculation, leading to systemic hemodynamics (F. Adams et al., 2015). PRC units with a storage period of more than 20 days are not recommended to be given to patients. Longer PRC storage causes an increase in LDH levels, which may result in more PRC transfusions being required. This process leads to greater transfusion reactions due to alloimmunization. Alloimmunization is an immune reaction in the form of antibody formation that occurs when blood group antigens not possessed by a person enter their blood circulation. The antibodies formed are called alloantibodies, and the antigens that enter are called alloantigens. Alloimmunization occurs due to alloantigens present in erythrocytes, leukocytes, or platelets from donor blood components. Alloantibodies produced from alloimmunization are clinically significant if the alloantibodies can cause hemolysis (erythrocyte alloantibodies), febrile non-hemolytic transfusion reactions (leukocyte alloantibodies), or refractory platelet transfusions (platelet alloantibodies) (Zwaginga & van Ham, 2017).

5. Conclusion

This research analyzed Lactate Dehydrogenase (LDH) levels in Packed Red Cell (PRC) bags stored with CPDA-1 anticoagulant for 30 days. The sample consisted of 50 PRC bags obtained from voluntary blood donors who had passed infectious disease screening tests. The results showed that LDH levels increased significantly ($p < 0.001$) with increasing storage time, which was confirmed through Repeated Measure ANOVA and Bonferroni Post Hoc tests. The increase in LDH levels indicates the occurrence of erythrocyte hemolysis due to oxidative stress and degradation of antioxidant enzyme function. There are significant differences in Lactate Dehydrogenase (LDH) levels during the storage period of Packed Red Cells (PRC) from day 1, day 10, day 20, and day 30.

These findings are consistent with various previous studies showing that hemolysis increases significantly during blood storage, especially after days 9 to 30. This indicates that long-term storage contributes to erythrocyte membrane damage and the release of LDH enzyme into the plasma. Clinically, increased LDH levels and hemolysis in stored PRC units can pose risks to recipient patients, including tissue injury, microcirculation disorders, and the risk of alloimmunization that can reduce transfusion effectiveness and cause serious transfusion reactions.

The limitation of this research is that the measurement of parameters that could affect the parameters analyzed in this study was not performed. These parameters include the hemolysis index and glucose levels. Varying hemolysis indices and glucose levels may influence the increase in LDH levels. This research is a basic study that can be continued with the addition of hemolysis index and PRC glucose level analysis to determine their relationship with the increase in Lactate Dehydrogenase (LDH) levels.

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