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The Effect of Storage Period on the Platelet Levels on Whole Blood in the Blood Bank Refrigerator

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whole blood;

Abstract

The quality of blood treatment before and after transfusion must be maintained to prevent the potential for transfusion reactions. A blood storage process must meet the requirements that have been set. Platelets play a role in repairing the chain reaction of blood vessel damage and initiating bleeding cessation that results in blood clotting. Therefore, this study aimed to analyze the effect of the storage period on platelet levels in whole blood in a blood bank refrigerator using a quasi-experimental method (one-group pre-post test design). The population was 30 donors selected by simple random sampling. The data analysis used was the Friedman test. The results showed that there was a significant effect of storage period on platelet levels at 0, 10, 20 and 30 days with a p-value <001. The life span of platelets in vivo is about 7-10 days, whereas in vitro (without shaking) is only 3 days. The decrease in platelet levels is possible due to the short lifespan effect and changes in cell structure. This can cause the platelet cell morphology to change shape to become irreversible and can cause the loss of platelet viability. The blood storage period with the right duration is needed to maintain blood quality.

Contents

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1 Introduction

The first human blood transfusion was performed in 1667 in France using animal blood as a donor and resulted in transfusion reactions and severe complications. This causes a high mortality rate so some recipients die (Kaur et al., 2013; Rahman, 2018). Based on research by Muhiddin (2013), blood transfusion can trigger mild to severe transfusion reactions, where the incidence scale is <0.01% of cases. Transfusion reactions can occur in emergency surgery by giving whole blood (WB) in acute bleeding, hypovolemic shock, and major surgery with bleeding volume > 1500 ml (Laksono, 2013). The results of Fuadda et al. (2016), showed that the 95% average incidence of transfusion reactions in patients with WB transfusion was 0.71-1.89 with the lowest reaction 0 and the highest reaction 2 (Fuadda et al., 2016). WB transfusion reactions are more common in emergency surgery because the bloodstock in the Blood Donor Unit (UDD) is not always available (Kanamoto et al., 2012; Aziz et al., 2016). Based on data from the World Health Organization (2016) states that a country's blood needs are at least 2.5% of the total population. Meanwhile, the availability of blood in Indonesia in the last 6 years is still less than ideal compared to the total population, where the shortage of blood bags is 18.8%. This has an impact on the need for blood bags in each province, one of which is Central Java Province with a shortage of blood needs of 25,477 bags (Infodatin, 2016). One bag of whole blood can be stored for 35 days with the anticoagulant CPDA-1 (Citrate Phosphate Dextrose Adenine-1) at a temperature of 2-6 °C. Blood during storage will experience changes in blood components (Kiswari, 2014). One of the components of blood is blood platelets (platelets) which play an important role in hemostasis, damage to blood vessels, and initiating cessation of bleeding that results in blood clots (thrombus). Blood stored for 21 days experienced a decrease in leukocyte phagocytic power, decreased platelet activity and loss of clotting factors (Suciati, 2010).

This theory was strengthened by Na'im (2014), which showed that there was an effect of storage on platelet levels, with an average platelet level before storage of 172,000/µL and decreased after 21 days of storage to 81,000/µL (Naim, 2014). The normal concentration of platelets in the blood is 150,000-450,000/µL (Williams, 2021). The condition of lack of platelets in the blood (thrombocytopenia) is caused by the decomposition of circulating platelets below 100,000/µL. According to other studies, several factors cause a decrease in platelet levels, including age, gender, sex, anticoagulant, temperature and storage period (Anfossi et al., 2002; Kirkpatrick et al., 2019; Prodan et al., 2011).

The ability of metabolism in the body during puberty to adulthood will increase and metabolism will decrease as a person gets older. Guyton (1998), states that metabolism is closely related to the formation and decomposition of substances in the body. As a person gets older, his ability to form and decompose substances will also decrease (Guyton, 1989). Metabolic ability also affects bone growth and height at puberty. Bone growth in women will be relatively stable, while in men it will continue to increase. This causes the male bone proportion to appear larger, allowing for more metabolism and platelet production in megakaryocytes than in women (Linder, 1992).

Another factor that causes a decrease in platelet levels is the inaccurate comparison of anticoagulants and blood. According to Artha (2017), the ratio of anticoagulants is too little, causing platelets to coagulate. Conversely, if the volume of anticoagulant is too much, the platelets will disintegrate and enlarge so that the platelet count will be low. Based on research conducted by Wirawan (2011) showed that platelet levels in whole blood with the anticoagulant EDTA (Ethylene Diamine Tetra Acetic Acid) can show pseudo thrombocytopenia (false thrombocytopenia) during the examination. This decrease in platelet levels was due to EDTA having a too long storage period which could trigger agglutination and clumping of platelets in vitro (Gevi et al., 2012; Wong et al., 2013; Ocké et al., 1995).

Temperature and storage period can affect the results of platelet examination (Worek et al., 1999; Xu et al., 2011). Platelets in fresh blood that have not experienced the storage period still have complete clotting factors including unstable clotting factors and stable clotting factors (Sutapa et al., 2021). Another cause of a decrease in platelet levels due to temperature which causes changes in cell structure, according to research conducted

by Ariani et al. (2021), shows that a decrease in pH (<6.8) causes the morphology of platelet cells to change shape to become irreversible and can reduce platelet viability (Ariani et al., 2021).

Decreased platelet formation causes inhibition of bone marrow function which will prolong the coagulation time and increase the risk of blood vessel bleeding. Meanwhile, an uncontrolled increase in the number of platelets (thrombocytosis) occurs due to the acute phase response to inflammation or infection (Sloane, 2004). Based on this background, the researchers were interested in examining the effect of storage periods of 0, 10, 20 and 30 days on platelet levels in whole blood in a blood bank refrigerator. The results of this study are expected to determine the effect of the storage period on platelet levels in whole blood. It is also hoped that the most optimum storage period can be obtained in the blood bank refrigerator to maintain the components in the blood to reduce the presence of transfusion reactions in the recipient (AL-Otaibi, 2021).

2 Materials and Methods

This research is a quasi-experimental research design with one group pre and post-test design. The population in this study were all donors in the Semarang Regency area. The sample in this study was voluntary donors who met the inclusion criteria and successfully passed the blood donor selection and blood collection was carried out (aftap). Sampling was carried out in the UDD PMI unit car located at the Wiratama KAS No. Dormitory. 66 RT.5/9 Watugong. The sampling technique used is simple random sampling with a total sample of 30 people. The research sample was a whole blood donor sample that was given anticoagulant CPDA-1 and put into an EDTA tube. Whole blood samples were stored in the Blood Bank Refrigerator at a temperature of 2-6ºC and checked for platelet levels for 0, 10, 20 and 30 days at the Regional Health Laboratory of Semarang Regency. The research data were analyzed using the Shapiro-Wilk normality test. The data shows that it is not normally distributed, so statistical analysis uses Friedman's difference test. The data can be declared statistically significant if the p-value <0.05. The research data collection scheme is shown in Figure 1.

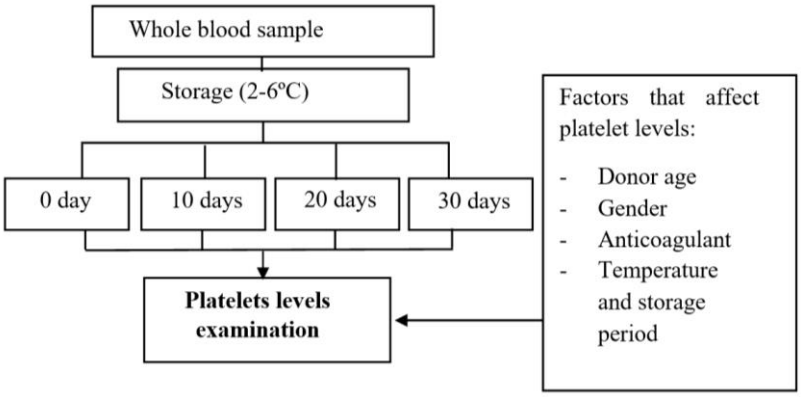

Figure 1. Schematic of research data collection

3 Results and Discussions

Examination of whole blood platelet levels conducted at the Regional Health Laboratory of Semarang Regency showed that the average platelet count decreased significantly. Based on the results of the non-parametric test, namely the Friedman test, there was a significant difference between platelet levels at 0, 10, 20 and 30 days of storage in a blood bank refrigerator. Table 1-5 shows the distribution of respondents' characteristics, the frequency of platelet storage time, the frequency of platelet decline, and the percentage decrease in platelet value as well as a comparative analysis of platelet levels. The donor characteristics are shown in Table 1.

Table 1
Distribution of donor characteristics

Variable	n	Min	Max	Mean
Age (years)	30	32	55	46
Hemoglobin (g/dL)	30	10,1	14,33	12,41
Gender :				
Male	22	73,33	%	
Female	8	26,67	%	

Source: Primary Data

Table 1. shows the distribution of donor characteristics the average age of the respondents is 46 years, (minimum 32 years and maximum 55 years) the average respondent's haemoglobin is 12.41 g/dL (minimum 10.1 g/dL and maximum 14.33 g/dL) and the sex of the respondents were 22 (73.33%) male and 8 (26.67%) female, respectively.

Table 2
Frequency distribution of platelet storage period ($1x10^3$µL)

Variable	n	Min	Max	Mean
0 (day)	30	139	416	261,50
10 (days)	30	126	386	225,33
20 (days)	30	100	364	187,67
30 (days)	30	84,0	347	159,50

Source: Primary Data

Table 2. The frequency distribution of the platelet storage period shows that the platelet value based on the 0-day storage period obtained a minimum value of 139,000 µL, a maximum of 416,000 µL and an average of 261,500 µL. The storage period of 10 days obtained a minimum value of 126,000 µL, a maximum of 386,000 µL and an average of 225,330 µL. A storage period of 20 days obtained a minimum value of 100,000 µL, a maximum of 364,000 µL and an average of 187,670 µL. A storage period of 30 days obtained a minimum value of 84,000 µL, a maximum of 347,000 µL and an average of 159,500 µL.

Table 3
Frequency distribution of decreased platelet values ($1x10^3$µL)

Variable	n	Min	Max	Mean
0 - 10 (days)	30	13	81	36,17
0 - 20 (days)	30	24	212	73,83
0 - 30 (days)	30	29	288	102,00

Source: Primary Data

Based on Table 3. The frequency distribution of the decrease in platelet value shows that the decrease in platelet value based on a storage period of 0-10 days obtained a minimum value of 13,000 µL, a maximum of 81,000 µL and an average of 36,170 µL. Storage period 0-20 days obtained a minimum value of 24,000 µL, a maximum of 212,000 µL and an average of 73,830 µL. Storage period 0-30 days obtained a minimum value of 29,000 µL, a maximum of 288,000 L and an average of 102,000 µL.

Table 4
Distribution of the percentage decrease in platelet value

Variable	n	Min (%)	Max (%)	Mean (%)
0 - 10 (days)	30	5,46	28,15	14,05
0 - 20 (days)	30	11,38	50,96	28,51
0 - 30 (days)	30	20,86	69,23	38,55

Source: Primary Data

Based on Table 4. The distribution of the percentage decrease in platelet value shows that the percentage decrease in platelet value based on a storage period of 0-10 days is obtained at a minimum of 5.46%, a maximum of 28.15% and an average of 14.05%. Storage period 0-20 days obtained a minimum of 11.38%, a maximum of 50.96% and an average of 34.35%. Storage period 0-30 days obtained a minimum of 20.86%, a maximum of 69.23% and an average of 38.55%.

Table 5
Results of comparative analysis of platelet levels

Platelet Levels	n	Average ±SD	*P value*
0 (day)	30	261,50±55,05	<0,001
10 (days)	30	225,33±56,21	
20 (days)	30	166,64±56,59	
30 (days)	30	159,50±48,88	

Source: Primary Data

Table 5. The results of the comparative analysis of platelet levels showed that platelet levels with a storage period of 0 days had a mean value of 261.50 and SD 55.05 with a p-value of <0.001; platelet levels with a storage period of 10 days with a mean value of 225.33 and SD 56.21 with a p-value of <0.001; platelet levels with a storage period of 20 days with a mean value of 166.64 and SD 56.59 with a p-value <0.001; platelet levels with a storage period of 30 days, the mean value was 159.50, and SD was 48.88 with p <0.001.

Based on the results of the study, it was shown that those aged >46 years experienced a decrease in platelet levels in a donor's body. This is strengthened by the theory of Guyton (1998), which states that metabolism is closely related to the formation and decomposition of substances in the body whereas as a person ages, his ability to form and decompose substances will decrease as well. However, with increasing age, the decline in platelet levels will occur more quickly in male donors, while in female donors the decline tends to be slower. Bone growth and height at puberty in girls will be relatively stable, while in boys will continue to increase. The greater proportion of male bone allows the metabolism and production of platelets in megakaryocytes more than in women (Linder, 1992).

Based on the results of the examination, the average platelet level at 0 days of storage is in the normal category with a range of 150,000 - 450,000 µL of platelets in the blood (Williams, 2021). Normal platelet levels are because at 0 days of storage, the blood has not yet experienced a storage period, so it still has complete clotting factors including unstable clotting factors and stable clotting factors. However, blood that has undergone a storage period tends to have decreased platelet levels. Permenkes No. 91 states that the life span of platelets in vivo is only about 7-10 days, whereas if in vitro (without any shaking) the life span will be shorter, namely only 3 days. The results of this study proved to show a decrease in platelet levels that varied, the highest frequency of decrease occurred at 0-30 days of storage with an average of 102,000 µL (38.55%).

The cause of the decrease in platelet levels, according to Armenia & Tambunan (2020), is possible due to the influence of the short lifespan of platelets so that platelet cells will lyse during the storage process (platelet storage lesions) (Permenkes, 2016) (Armenia & Tambunan, 2020). This is referred to as a storage lesion caused by prolonged contact of plasma with red blood cells in the exchange of contents between plasma and red blood cells causing dilution (dilution) resulting in a change in the concentration of the analyte. (Bhargava et al., 2016). Hemolysis of red blood cells may persist during storage. The degree of hemolysis in

the blood bag depends on the donor's physical condition, anticoagulant, and storage period. It can also occur as a result of the mishandling of blood during delivery and storage. Low temperatures during delivery and storage reduce the risk of bacterial growth although cold-tolerant bacteria can survive and grow slowly during storage (Tzounakas et al., 2017).

Platelets also have adhesion properties that make it easier for platelets to stick to the surface of foreign objects in the delayed sample so that the yield is low. Cooling platelets (< 21 days) inhibits the release of granules such as thromboglobulin (Josefsson et al., 2007). This theory is evidenced by the results of the comparative analysis of data which showed that there was an effect of the average platelet level on storage for 10, 20 and 30 days, resulting in a significant decrease ($p < 0.001$). This opinion is evidenced by the results of the research on the percentage decrease in platelet value on days 0 to 20 days as much as 73,830 L (28.51%). This happens because spontaneously cooled platelets can form aggregates, causing platelets to clump together and swell and then break into fragments smaller than platelets so they are not counted as platelets. In addition, it causes platelet cells to become enlarged and damaged (Josefsson et al., 2007; Kaufman, 2006).
During storage, red blood cells undergo progressive structural and functional changes that can reduce their function and viability of red blood cells. This is corroborated by a study by Mane et al. (2015), which stated that there was a significant decrease in serum albumin levels in whole blood stored in blood banks ($n=20$, $p<0.05$). The release of oxygen from red blood cells decreases during storage. Certain chemicals such as histamine, lipids, and cytokines released by leukocytes during storage directly affect the physical function and metabolism of red blood cells. The length of storage of whole blood affects changes in serum albumin levels, this is also similar to a study conducted by Bhargava et al in 2016 in India ($n=30$, $p<0.05$) (Bhargava et al, 2016; Mane et al., 2015).

4 Conclusion

Based on the results of data analysis of research conducted on platelet levels in whole blood for 0, 10, 20 and 30 days in a blood bank refrigerator, it was concluded that there was an effect of blood bank refrigerator storage period on platelet levels in whole blood with p-value < 0.001. The results showed that the optimum storage period in a blood bank refrigerator was 10 days. This is shown in the frequency of the decrease of 36,170 µL in the lowest platelet level from 0 to 10 days, with 14.05% smallest decrease percentage. Based on the research result we can conclude that whole blood components must be distributed or transfused before 10 days of the storage period in the blood bank refrigerator.

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