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The Effect Of Storage Time And Type Of Anticoagulant In Platelet Rich Plasma (PRP) On Platelet Counts

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Abstract

Background: *Platelet Rich Plasma (PRP) is a biological product in the form of autologous plasma produced from the centrifugation process of blood specimens and has a platelet concentration above normal values. Things that can affect the results of PRP are blood collection, centrifugation speed, centrifugation time and temperature and the use of anticoagulants.* ***Aims:*** *The purpose of this study was to determine the average and see the effect of sodium citrate anticoagulant and Acid Citrate Dextrose Formula A anticoagulant by using a shelf life of 0 hours, 4 hours and 8 hours in PRP on platelet count.* ***Methods:*** *This type of research is a pseudo-experiment with a Statistic Group Comparison research design. The number of samples in this study were 6 people. PRP research data on platelet count was processed statistically with the General Linear Model (GLM) method.* ***The Results:*** *The average data of PRP on platelet count using sodium citrate anticoagulant with a shelf life of 0 hours 533.333 cell/µL; 4 hours 527.167 cell/µL; 8 hours 190.167 cell/µL. Average data of PRP on platelet count using ACD-A anticoagulant with a shelf life of 0 hours 986.333 cell/µL; 4 hours 976.500 cell/µL; 8 hours 634.833 cell/µL. The data results for 0 hour and 4 hour shelf life on anticoagulants Sodium Citrate and ACD-A have a Sig value of 0.646 > 0.05 while 8 hour shelf life has a Sig value of 0.000 < 0.05.* ***Conclusion:*** *No effect of 0 hour and 4 hour shelf life while there is an effect of 8 hour shelf life and there is an effect of anticoagulants Sodium Citrate and ACD-A in PRP on platelet count.*

Keywords: *Acid Citrate Dextrose Formula A, Shelf-life, Sodium Citrate, Platelet count on Platelet Rich Plasma*

1. Introduction

Platelet Rich Plasma (PRP) is a biological product in the form of autologous plasma produced from centrifugation of blood samples and has a platelet concentration higher than normal values. Factors that can affect PRP results include blood collection, centrifugation speed, centrifugation time and temperature, and the use of anticoagulants [1]. Sodium Citrate is recommended by the International Committee for Standardization in Haematology (ICSH) and the International Society for Thrombosis and Haematology as a coagulation test because it can maintain blood clotting factors. Based on CLSI, Sodium Citrate anticoagulant can maintain platelet response for 4 hours [2]. Anticoagulant Acid Citrate Dextrose

Formula A (ACD-A) is an anticoagulant that can maintain platelet viability for up to 6 hours and can maintain platelet structural integrity in PRP3. One of the ingredients of ACD-A anticoagulant is dextrose, where dextrose is a nutrient for blood cells so that it can increase the survival and lifespan of blood cells [4]. There are still discrepancies between the results of laboratory tests and the clinical status of patients. Among other things, delays in sample delivery, shift changes between laboratory staff, large numbers of patients, inadequate equipment conditions and improper sample handling which can affect the number of platelets in PRP [5].

According to Yuliandari's research shows that the length of storage for 0 hours, 24 hours and 48 hours using Sodium Citrate anticoagulant, the platelet count in PRP will decrease [6]. The purpose of this study was to determine the average anticoagulant Sodium Citrate 3.2% and ACD-A using a storage time of 0 hours, 4 hours and 8 hours in PRP on platelet count. Knowing whether there is an effect of time and the effect of the type of anticoagulant in PRP on platelet count.

2. Research method

This study is a type of pseudo-experimental research, namely, using shelf-life treatment with anticoagulants Sodium Citrate 3.2% and Acid Citrate Dextrose Formula A (ACD-A) in Platelet Rich Plasma (PRP) on platelet count. The research subjects used were 6 normal blood subjects according to the inclusion and exclusion criteria. Inclusion criteria, namely: willing to participate and sign informed consent, blood is not lysed and lipemic, blood in the tube has no clot and is in good health.

Exclusion criteria, namely suffering from diseases that affect platelet count (dengue fever, thrombocytosis, anemia), had been hospitalized at least a week before blood collection, for women during menstruation.

The time and location of the study was conducted from April to May 2024 at the Hematology Laboratory of the Health Polytechnic of the Ministry of Health Bandung.

Data collection in this study used primary data, which was obtained from the examination of platelet count in Platelet Rich Plasma (PRP) against the length of storage using anticoagulants Sodium Citrate 3.2% and Acid Citrate Dextrose Formula A (ACD-A), which was examined by automatic method using a hematology analyzer.

Data were processed using statistical tests. If the data is normally distributed, then use the Gineral Linear Model (GLM) test -repeated measures. However, if all variables are not normally distributed or there is one that is not normally distributed, use the Friedman and Wilcoxson tests.

The study has submitted an application for ethical review to the Health Research Ethics

Commission (KEPK) of the Health Polytechnic of the Ministry of Health Bandung with No. 24/KEPK/EC/IV/2024.,

3. Results and Discussion

A. Results

Platelet Rich Plasma (PRP) using anticoagulants Acid Citrate Dextrose Formula A (ACD-A) and 3.2% Sodium Citrate with a shelf life of 0 hours, 4 hours and 8 hours with 6 research subjects using Hematology Analyzer. Data obtained from PRP examination results on platelet count.

Table 1. PRP Examination Results

Subject	Platelet Count (Cells/µL)					
	ACD-A			Sodium Citrate		
	0 hours	4 hours	8 hours	0 hours	4 hours	8 hours
1	997.000	989.000	649.000	541.000	536.000	198.000
2	995.000	986.000	645.000	545.000	538.000	195.000
3	998.000	985.000	647.000	543.000	537.000	196.000
4	976.000	966.000	620.000	524.000	518.000	182.000
5	978.000	968.000	626.000	522.000	515.000	184.000
6	974.000	965.000	622.000	525.000	519.000	186.000

Table 1 shows that each time has decreased. by treating the shelf life of 0 hours, 4 hours and 8 hours and using ACD-A anticoagulant and 3.2% Sodium Citrate.

Table 2: Average PRP Examination Results

	Average value ±SD		
	0 hours	**4 hours**	**8 hours**
ACD-A Avarage	986.333 ± 11.43 865.867	976.500 ± 11.26	634.833 ± 13.53
Sodium Citrate Avarage	533.333 ± 10.71 416.899	527.167 ± 10.87	190.167 ± 6.94

In Table 2, the mean and standard deviation of the results of PRP examination of platelet counts in each different variable are obtained. In the table, it can be seen that from each different variable, the average value of PRP examination on platelet count is different. From Table 1, the Shapiro-Wilk normality test was carried out, because the data processed was less than 50 data. This test is to determine whether it is normally

distributed or not.

Table 3. Normality Test Results of PRP Examination

Data Group				
Anticoagulants	**shelf life**	**Sig.**	**Results**	**Conclusion**
	0 Hours	0,056	p>0,05	Normal Distribution
ACD-A	**4 Hours**	0,062	p>0,05	Normal Distribution
	8 Hours	0,083	p>0,05	Normal Distribution
	0 Hours	0,070	p>0,05	Normal Distribution
Sodium Citrate	**4 Hours**	0,059	p>0,05	Normal Distribution
	8 Hours	0,215	p>0,05	Normal Distribution

Sig>0,05 data berdistribusi normal, Sig< 0,05 data tidak berdistribusi normal

The table above all data variables in this study have a Sig>0.05 value, so it can be concluded that all variables in this study are normally distributed. The test can be carried out using the General Linear Model (GLM) - Repeated Measures test to determine whether there is an effect of 0 hours, 4 hours, 8 hours of storage time and whether there is an effect of ACD-A anticoagulant and 3.2% Sodium Citrate.

Table 4. Test *General Linear Model* (GLM)-*Repeated Measures.*

Data Group		Sig	Results	Conclusion
Anticoagulants	**ACD-A vs Sodium Citrate**	0,000	Sig<0,05	There is an influence
	Sodium Citrate vs ACD-A	0,000	Sig<0,05	There is an influence
Length of Deposit	**0 hours vs 4 hours**	0,646	Sig>0,05	There is no effect
	0 hours vs 8 hours	0,000	Sig<0,05	There is an influence
	4 hours vs 8 hours	0,000	Sig<0,05	There is an influence

Sig>0,05 there is no effect Sig<0,05 there is an influence

In Table 4, the significant value of PRP on platelet count using ACD-A anticoagulant and 3.2% sodium citrate obtained Sig value 0.000 < 0.05, so there is an effect, so it can be concluded that there is an effect of the type of anticoagulant in PRP on platelet count. While the significant value of PRP on platelet count with a shelf life of 0 hours and 4 hours obtained a Sig value of 0.646> 0.05, then there is no effect, so it can be concluded that there is no effect of shelf life of 0 hours and 4 hours on PRP on platelet count. The significant value of PRP on platelet count with a shelf life of 8 hours obtained a Sig

value of 0.000 <0.05, so there is an effect, so it can be concluded that there is an effect of a shelf life of 8 hours on PRP on platelet count.

B. Discussion

After conducting research and testing the General Linear Model (GLM)-Repeated Measures by looking for the effect of shelf life and type of anticoagulant in PRP on platelet counts presented in table 4, it is known that the data results for shelf life of 0 hours and 4 hours have a Sig value of 0.646> 0.05, that there is no effect of shelf life of 0 hours and 4 hours on PRP on platelet counts. Because it is in accordance with the stability of the anticoagulant used, namely 3.2% Sodium Citrate anticoagulant can maintain platelet response for 4 hours while Acid Citrate Dextrose Formula A (ACD-A) anticoagulant can maintain platelet viability up to 6 2.3. The results of the 8-hour shelf life data have a Sig value of 0.000 <0.05, that there is an effect of 8-hour shelf life on PRP on platelet count and from the results of the study it was found that the average number per hour decreased, but still within the limits of normal values.

The decrease in platelet count in PRP is thought to be due to platelet instability and platelet resistance during storage. Storage can cause platelet aggregation which causes the release of granules in platelets, because platelets have properties that easily agglutinate (agglutination) and properties that are easily broken (lysis) so that platelets cannot be counted when checking platelet counts in PRP using a haematology analyzer [7]. In addition, the anticoagulant Sodium Citrate 3.2% with ACD-A has a different blood ratio, namely 1:9 for the anticoagulant Sodium Citrate 3.2% and 1:5 for the anticoagulant ACD-A [8]. This study uses the speed of gravity with units of rpm. This can result in a sedentary increase, meaning that there is an increase and a decrease in platelet count.

Based on table 4, it is known that the data results for the type of anticoagulant have a Sig value of 0.000 <0.05, so there is an influence on the variable type of anticoagulant in PRP on platelet count. In table 2, the average ACD-A anticoagulant with 3.2% Sodium Citrate is very different, namely 865,867/µL and 416,899/µL so that it can be seen that there is a significant difference where the number of PRP platelets in ACD-A anticoagulant is more.

This is due to the dextrose content of 24.5 g/L in ACD-A. Where dextrose is a nutrient for blood cells so that it can increase the survival and lifespan of blood cells. ACD-A can maintain platelet morphology (size) and functionality (activation and retention of growth factors) better than other anticoagulants used for PRP preparation. As ACD-A has a lower chelating ability

compared to EDTA and has no detectable side effects on platelets, leukocytes or other cells involved in tissue regeneration [9]. The citrate content of ACD serves to prevent coagulation by chelating ionised calcium present in the blood to form a non-ionised calcium citrate complex. Anticoagulant Sodium Citrate 3.2% is an anticoagulant used for the evaluation of platelet diagnostic while ACD-A anticoagulant is the choice for platelet collection by apheresis. Sodium Citrate and ACD-A solutions have differences in terms of pH, Sodium Citrate has a pH of 7.8 while ACD-A has a pH of 4.9. In addition, the concentration of citrate ions in ACD-A is 15.6 mg/mL while Sodium Citrate is 24.4 citrate ions/mL. The use of ACD showed better effectiveness in preventing platelet aggregation [10].

According to the research of Clarissan, et al, platelet counts were examined in PRP using sodium citrate and ACD-A tubes immediately, the data obtained were analysed using the Paired T-Test test, so it can be concluded that there is a statistical effect on PRP on platelet counts with samples that are immediately examined [4]. After being analysed using the General Linear Model (GLM) - Repeated Measure test, the results of Sig <0.0, so it can be concluded that there are significant differences in PRP using ACD-A and 3.2% sodium citrate anticoagulants.

4. Conclusion

Based on the results of the research conducted, it can be concluded that: There is no effect of 0 hour and 4 hour storage time and there is an effect of 8 hour storage time on PRP on platelet count. There is an effect of anticoagulant type in PRP on platelet count.

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