VARIATION OF ANTICOAGULANT AND STORAGE TIME ON PRP QUALITY

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ABSTRACT . Different procedures of PRP preparation can affect the platelet count in PRP. Platelet count is one of the factors that determines the quality of PRP. This study aimed to describe the number of platelets based on variations in anticoagulants and storage time in PRP. This study was an experimental study with a pre-post test only control group design. PRP was processed from whole blood from 9 respondents. Whole blood is collected in a tube containing Sodium Citrate, EDTA, and ACD-A anticoagulants. Each tube will be processed into PRP through 2 stages of centrifugation. The number of platelets in PRP was counted immediately (0 hour) and counted after 24 hours of storage. The results showed that PRP processed using EDTA had the highest platelet count compared to Sodium Citrate and ACD-A at 0 hour of storage, but decreased at 24 hours of storage. The number of PRP platelets in ACD-A and Sodium Citrate tubes increased after 24 hours of storage, but the best platelet counts were found in ACD-A tubes. The use of anticoagulants in the manufacture of PRP and the storage time of PRP resulted in varying PRP platelet counts. This study must be carried out further in order to determine the concentration of growth factors based on the use of anticoagulants and the storage time of PRP.

Keywords: Anticoagulants, Platelets count, PRP, Storage

1 INTRODUCTION

Platelet rich plasma (PRP) has been applied in various fields of medicine because of its role in tissue regeneration and enhancing the healing process (Reddy et al., 2018). PRP is an autologous blood product that has a platelet count above normal values. Platelets have alpha granules which contain various growth factors which play an important role in the regeneration process (Everts et al., 2020).

PRP contains 94% platelets, 5% red blood cells and 1% white blood cells (Molina et al., 2018). The number of platelets in PRP can reach 1,000,000 cells/ µL in 5 mL of plasma (Chorążewska et al., 2017). The quality of PRP is influenced by the number of platelets. The higher the number of platelets, the more growth factors will be released (Yuliandari, 2020). PRP preparation procedures can affect PRP platelet counts, such as the use of anticoagulants. Administration of anticoagulants is used to prevent coagulation by binding to calcium (Sari, 2023). Several researchers use the anticoagulants sodium citrate, EDTA and ACD-A in the procedure for making PRP (do Amaral et al., 2017; Clarissa et al., 2019; Alam, 2022; Abdulla et al., 2022).

PRP is usually applied directly to tissue damage, but some people save it first for various reasons. It is necessary to pay attention to storing PRP to avoid disrupting platelet function (Kim et al., 2018). The storage temperature of 4^0C aims to maintain platelet stability, such as preventing aggregation and maintaining platelet metabolism (Queen et al., 2014). Temperature, anticoagulant and storage duration can affect platelet counts (Diyanti et al., 2017). Based on this, research was conducted on "Variation of anticoagulant and storage time on PRP quality "

2 RESEARCH METHODS

This study was an experimental study conducted at the John Paul II Health Academy's Hematology Laboratory in Pekanbaru. The design used in this study was a pre-post test only control group design. The number of platelets in PRP was counted before storage (0 hours) and after storage (24 hours). The sample used was whole blood from 9 respondents who met the inclusion criteria and agreed to informed consent. The inclusion criteria in this study were respondents who were healthy, had no history of smoking, alcohol intake and diseases related to platelet count (Alkady et al., 2020). The sample size is calculated based on the Federer formula.

Whole blood from each respondent will be collected in a tube containing the anticoagulants Na-citrate, EDTA and ADC-A. Whole blood from each tube will be processed into PRP through 2 centrifugation stages. The first centrifugation at 1000 rpm for 5 minutes and the second centrifugation at 3000 rpm for 5 minutes (Karina et al., 2019). The plasma and buffy coat resulting from the first centrifugation were transferred to an empty tube, and then carried out a second centrifugation. The upper third of the second centrifugation result is discarded and the remainder is called PRP (Rizal et al., 2020). PRP produced from each group immediately counted the number of platelets (0 hour), then PRP was stored in the refrigerator at 4°C for 24 hours. The number of PRP platelets in each tube was recounted after 24 hours of storage.

The platelet count was calculated using a hematology analyzer. PRP in each group was first homogenized and then examined with a hematology analyzer, and the results would appear on the screen. Platelet count data in each group will be analyzed descriptively and presented in the form of pictures and graphs.

3 RESULTS AND DISCUSSION

This study aimed to describe the number of platelets based on variations in anticoagulants and duration of storage in PRP. The preparation of PRP came from 9 whole blood samples obtained from research respondents. Platelet count data in PRP are presented in table 1 and figure 1.

Table 1. Platelet count of PRP based on anticoagulant variations and storage time

Sample	Platelet count of PRP (x 10^3 cell/µL)					
	Sodium Citrate		EDTA		ACD-A	
	0 hour	24 hours	0 hour	24 hours	0 hour	24 hours
1	331	600	807	537	453	836
2	402	690	837	508	577	707
3	464	829	653	548	581	1336
4	519	733	756	605	474	1060
5	580	700	860	608	600	930
6	464	773	828	556	591	780
7	620	800	804	616	900	1280
8	478	870	880	637	670	822
9	416	852	822	710	557	1180
Mean	475	761	805	592	600	992
SD	89,29	87,98	67,08	61,50	130,00	230,35

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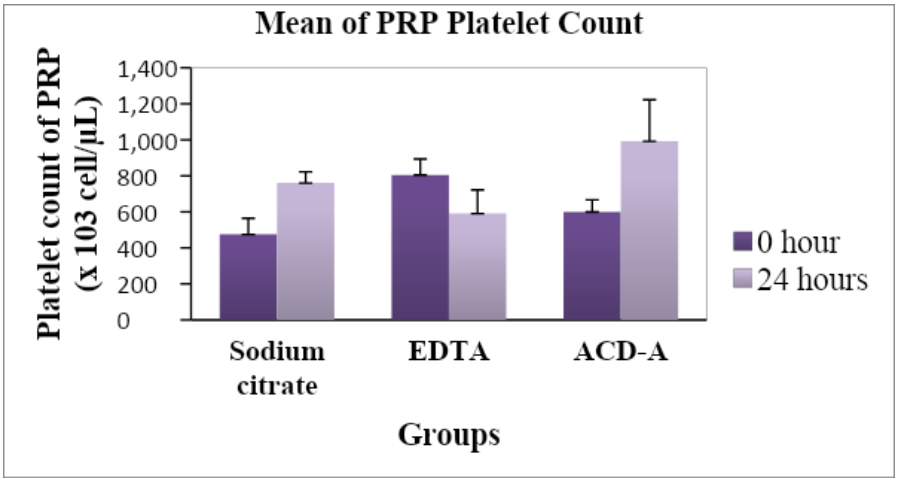

Fig. 1. Mean of PRP platelet count based on anticoagulant variation and storage time. The Mean of PRP platelet count immediately counted (0 hour) in the Sodium Citrate, EDTA and ACD-A groups respectively was 475 x 10^3 ± 89.29 x 10^3 /µL; 805 x 10^3 ± 67.08 x 10^3 /µL; 600 x 10^3 ± 130.00 x 10^3 /µL. The Mean of PRP platelet stored for 24 hours in the sodium citrate, EDTA and ACD-A groups respectively was 761 x 10^3 ± 87.98 x 10^3 /µL; 592 x 10^3 ± 61.50 x 10^3 /µL; 992 x 10^3 ± 230.35 x 10^3 /µL.

The number of platelets greatly determines PRP quality. The use of anticoagulants and storage of PRP is one of the factors that determine the number of platelets in PRP. In this study, the use of EDTA anticoagulant with 0 hour storage had the highest platelet count 805 x 10^3 /µL compared to sodium citrate and ACD-A anticoagulants. This study in accordance with do-Amaral's et al. (2017) study which found that the highest platelet count was in the anticoagulant EDTA compared to sodium citrate and ACD-A. Some researchers say that EDTA is considered unsuitable for PRP preparation. However, research by Aizawa et al. (2020) says that EDTA can maintain the same level of PDGF-BB as ACD-A.

After PRP storage for 24 hours, the platelet count in the EDTA group decreased to 592 x 103/µL. It is thought that PRP platelet activation occurred in the EDTA group so that the platelets burst and released growth factors and the number of platelets decreased compared to 0 hours of storage. Zhang et al. (2019) stated that EDTA causes swelling and activation of platelets. Other researchers also argue that EDTA causes structural changes in platelets and disrupts platelet viability (Rosyidah et al., 2022). EDTA is an effective anticoagulant, but causes structural, biochemical and functional damage to human platelets (Sachs et al., 2022).

The ACD-A group in this study showed the highest platelet count in 24 hour storage compared to Sodium citrate and EDTA. Some researchers recommend the use of the anticoagulant ACD-A in the PRP preparation procedure. ACD-A is able to maintain the intraplatelet signal transduction mechanism during PRP formulation and is able to maintain platelet function for a longer period of time (Anitua et al., 2016).

Hua et al. (2015) stated that ACD-A is optimal for use in PRP preparation because it can maintain platelet viability at a high level. The use of ACD-A is associated with higher platelet concentrations when compared with the use of sodium citrate (Kraus et al., 2018). ACD-A contains dextrose which will provide nutrition so that it can increase platelet viability in PRP (Clarissa et al., 2019). ACD-A can maintain platelet

morphology and function better than other anticoagulants and has no side effects on platelets (Aizawa et al., 2020). ACD-A works by binding to calcium and preventing coagulation proteins from initiating the coagulation cascade. EDTA anticoagulant is not recommended because it can damage the platelet membrane so that growth factors come out prematurely (Dhurat & Sukesh. 2014).

The number of PRP platelets stored for 24 hours in this study increased in the ACD-A and sodium citrate groups. The results of this study are different from the study of Kim et al. (2018) who found that the number of PRP platelets remained stable from the first day to 7 days of storage in the refrigerator at 4°C. The increased number of platelets after 24 hours in the sodium citrate and ACD-A groups was suspected because the anticoagulants needed time to inhibit platelet aggregation. In contrast to the EDTA group, which had a higher platelet count at 0 hour of storage, but decreased at 24 hours of storage. This is reinforced by the statement of Aizawa et al. (2020) that EDTA is more efficient in inhibiting platelet aggregation, resulting in a higher platelet count compared to ACD-A (Aizawa et al., 2020). Proper storage temperature and duration are important considerations for optimizing the effect of PRP for clinical use (Kim et al., 2018).

4 CONCLUSION AND RECOMMENDATION

This study showed that use of anticoagulants in PRP produce and the storage time of PRP results in varying numbers of PRP platelets. The best number of platelets at 0 hour of storage was found in EDTA tubes. The number of PRP platelets in ACD-A and sodium citrate increased after 24 hours of storage, but the best platelet count was found in the ACD-A tube. This study must be carried out further in order to determine the concentration of growth factors based on the use of anticoagulants and the storage time of PRP. The use of anticoagulants in the manufacture of PRP and the storage time of PRP must be reconsidered to optimize its effect on the healing process.

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