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EVALUATION OF PLATELET RICH PLASMA (PRP) PREPARATION PROCEDURE

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ABSTRACT

The success of PRP therapy in repairing tissue damage is influenced by the PRP preparation procedure. Currently, there's no standardization of PRP preparation procedures, and various techniques are used, such as the use of anticoagulants and different centrifugation speeds. This study aimed to evaluating the PRP preparation procedures based on the centrifugations steps (single and double centrifugation) and the use of anticoagulants variation (sodium citrate, EDTA and ACD-A). This study was an experimental study and used blood samples from respondents. The selected respondents must meet inclusion and exclusion criteria. The treatment groups in this study were the single centrifugation group and the double centrifugation group. Each group will be divided into 3 subgroups with different anticoagulant usage (sodium citrate, EDTA and ACD-A). Statistical analysis results showed a significant difference in the mean platelet count in the sodium citrate , EDTA, ACD-A groups with single and double centrifugation steps. Evaluation of platelet preparation procedures in this study, a higher platelet count was obtained, specifically in the sodium citrate group (494 x 10^3 cells/µL), EDTA group (829.4 x 10^3 cells/µL), and ACD-A group (607.1 x 10^3 cells/µL), compared to single centrifugation in the sodium citrate group (354.8 x 10^3 cells/µL), EDTA group (408.1 x 10^3 cells/µL), and ACD-A group (390.6 x 10^3 cells/µL). The highest platelet count in PRP was achieved with the preparation procedure using EDTA as the anticoagulant with double centrifugation. Further research is necessary to evaluate PRP preparation procedures regarding the concentration of growth factors present in PRP.
Keywords: *Anticoagulants; Centrifugation; PRP*

INTRODUCTION

Platelet Rich Plasma (PRP) therapy is a relatively new treatment in the field of medicine and holds potential in the healing process. PRP therapy was first used in open-heart surgery and later in wound healing for maxillofacial surgery (Lansdown & Frontier, 2017). PRP therapy has continued to evolve and is now widely utilized in various medical fields such as dentistry, orthopedics, neurosurgery, ophthalmology, and aesthetics (Karina et al., 2019). Recent research indicates that PRP has the potential to regenerate damaged pancreatic tissue in diabetes patients (El Tahawy et al., 2017), and PRP therapy can lower blood glucose levels in diabetic mice (Yuliandari et al., 2022). PRP is an autologous plasma product processed from fresh whole blood and contains a high concentration of platelets. The normal platelet count in blood ranges from 150,000 cells/µl to 350,000 cells/µl (Kasper et al., 2016), but PRP has platelet concentrations of up to 1,000,000 cells/µl in 5 mL of plasma (Chorążewska et al., 2017).

Platelets have alpha granules that contain various growth factors and cytokines that play a role in the proliferation, regeneration, and differentiation of damaged tissues (Rattanasuwan et al., 2018). These growth factors include Transforming Growth Factor-beta (TGF-β), Platelet-Derived Growth

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Factor (PDGF), Insulin-like Growth Factor (IGF), Connective Tissue Growth Factor (CTGF), Epidermal Growth Factor (EGF), Basic Fibroblast Growth Factor (BFGF), and Vascular Endothelial Growth Factor (VEGF) (Hasan & Kumar, 2020). Growth factors in PRP are the primary components responsible for controlling, regulating, and accelerating the healing process (Rofi'I, 2012).

The success of PRP therapy in the healing process is influenced by the PRP preparation technique. Platelets break apart easily and are difficult to count (Anwari et al., 2020). Several studies have prepared PRP using different procedures. Double centrifugation is one such method in PRP preparation, involving speeds of 1600 rpm and 2000 rpm for 10 minutes (El Tahawy et al., 2017). Other studies have utilized single centrifugation and yielded higher platelet concentrations compared to the PRP group with double centrifugation (Bhatia et al., 2016). Chen et al (2019) mentioned that excessive centrifugation speed can lead to platelet damage, and the optimal centrifugation time is 5-10 minutes. Alkady et al (2020) stated that double centrifugation at low speeds results in a higher platelet count compared to single centrifugation.

Several studies have also used different anticoagulants in the PRP preparation procedure. The use of EDTA in PRP preparation results in a higher platelet concentration compared to citrate dextrose (ACD) and sodium citrate anticoagulants (do Amaral et al., 2016). Another study employed ACD-A as an anticoagulant in PRP preparation and achieved the highest platelet concentration compared to sodium citrate anticoagulant (Clarissa et al., 2019). In a study by Alam (2022), EDTA was used in PRP preparation for treating scalp hair loss. Abdulla (2022) utilized sodium citrate in the PRP preparation procedure.

Currently, there is no standardization of PRP preparation procedures, and many techniques are used, including variations in centrifugation speed, the use of anticoagulants, the duration of centrifugation rounds, and the number of centrifugation cycles. PRP preparation procedures in clinical studies are highly inconsistent, with most not providing sufficient information to enable the use of PRP procedures (Chen et al.,2018). Based on this, a study was conducted to evaluate the platelet-rich plasma (PRP) preparation procedures.

This study aimed to evaluating the PRP preparation procedures based on the centrifugations steps (single and double centrifugation) and the use of anticoagulants variation (sodium citrate, EDTA and ACD-A). The platelet concentration in each PRP group will be measured using a hematology analyzer. The research results are expected to establish a standard for PRP preparation procedures, resulting in PRP with a platelet concentration 3–6 times higher than the normal value of platelets in whole blood.

METHODS

This research was carried out at the Hematology Laboratory of the John Paul II Pekanbaru Health Academy. This research is a type of experimental research with a post-test only design. Each group was given the anticoagulants sodium citrate, EDTA and ADC-A. The samples used came from the blood of male respondents who met the inclusion and exclusion criteria.

Blood sampling is carried out by experts after obtaining informed consent. Venous blood was taken using a wing needle connected to a BD vacutainer tube containing the anticoagulants sodium citrate, EDTA and ACD-A. Blood that had been collected in tubes containing anticoagulants was divided into 2 groups, namely the single centrifugation group and the double centrifugation group.

In the procedure for making PRP, venous blood containing various anticoagulants in a single centrifugation group will be centrifuged at a speed of 3000 rpm for 5 minutes (Karina et al., 2019). Plasma and Buffy coat resulting from a single centrifugation were separated and transferred

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to empty tubes without anticoagulant. The upper third is discarded, and the remainder is PRP resulting from a single centrifugation with preparation using various anticoagulants (Rizal et al., 2020). The double centrifugation group will undergo the first stage of centrifugation at a speed of 1000 rpm for 5 minutes and the second centrifugation will be carried out at a speed of 3000 rpm for 5 minutes (Karina et al., 2019). The results of the first stage of centrifugation will show 3 layers, namely the red blood cell layer at the bottom of the tube, the middle layer (Buffy coat and leukocytes), and the top layer is plasma fluid. The top layer and middle layer were transferred into an empty tube without anticoagulant, then the second stage of centrifugation was carried out. The results of the second stage of centrifugation will show 2 layers, namely the plasma layer on top and pellet deposits on the bottom layer. The upper third is discarded and the remainder is PRP resulting from double centrifugation with preparation using various anticoagulants (Rizal et al., 2020).

Calculation of platelet counts was carried out using an automatic method using a hematology analyzer. Each PRP in each group is first homogenized by turning the tube slowly, then examined with a hematology analyzer and the results will appear on the screen. Platelet count data in each group will be analyzed using SPPS and presented in the form of tables and graphs.

RESULTS

The results of the normality test on the number of platelets for each group showed a p value > 0.05, which means that all data were normally distributed. Platelet count data in each group was then analyzed using the paired t test and one-way ANOVA test. The results of the paired t-test analysis for each group of sodium citrate, EDTA and ACD-A obtained p-value <0.05. This means that there is a significant difference in the average PRP that was centrifuged once (single centrifugation) and that which was centrifuged twice (double centrifugation) in each group. Data on platelet counts are presented in Table 1 in the form of mean ± SD.

Table 1. Mean number of platelets in the sodium citrate, EDTA and ACD-A groups processed using single centrifugation and double centrifugation

Mean PRP platelet count ± SD (x 10^3 sel/µL)		
Anticoagulant	**single centrifugation**	**double centrifugation***
Sodium citrate	354,8 ± 74,7	494 ± 98,3
EDTA	408,1 ± 70,7	829,4 ± 61,3
ACD-A	390,6 ± 71,2	607,1 ± 55,1

Note: * there is a significant difference in the number of PRP platelets in each anticoagulant group processed using double centrifugation versus single centrifugation

Based on Table 1, it can be seen that PRP in the sodium citrate, EDTA and ACD-A groups which were processed using double centrifugation had the highest platelet count compared to single centrifugation. Platelet count data from double centrifugation was then analyzed using the One-way ANOVA test. The results of the one-way ANOVA test are presented in Table 2.

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Table 2. Results of one-way ANOVA test on PRP in the sodium citrate, EDTA and ACD-A groups processed using double centrifugation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	582340200000.000	2	291170100000.000	53.092	.000
Within Groups	148075300000.000	27	5484270370.370		
Total	730415500000.000	29			

Based on the results of the one-way ANOVA test analysis, it was found that the p value <0.05. This means that there is a significant difference in the mean number of platelets in the three groups (sodium citrate, EDTA and ACD-A) which were processed using double centrifugation. The number of platelets in this study is presented in graphical form in Figure 1.

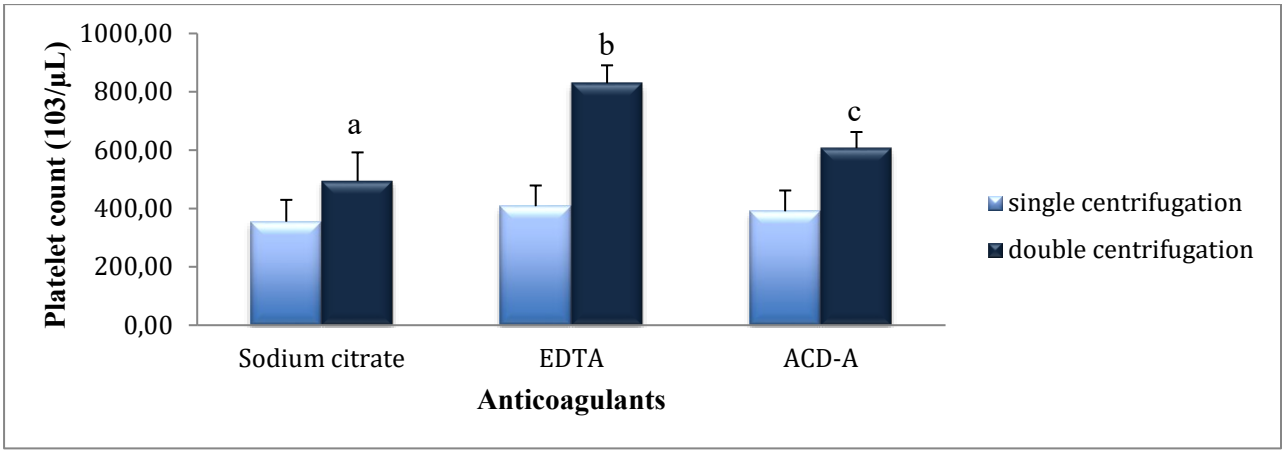

Figure 1. Number of PRP platelets in the sodium citrate, EDTA and ACD-A groups processed using single centrifugation and double centrifugation. a, b, c show significant differences in the number of PRP platelets in the groups (sodium citrate, EDTA and ACD-A) processed using double centrifugation.

DISCUSSION

Platelets in PRP generally range from 3 to 5 times higher than whole blood or a minimum number of 300,000 to 1,000,000 platelets/µL (Anitua et al., 2016). Platelet count is one of the factors that influences the quality of PRP. Based on the results of this study, PRP processed using double centrifugation obtained a higher platelet count, namely the sodium citrate group 494 x 10^3 cells/µL, EDTA 829.4 x 10^3 cells/µL, ACD-A 607.1 x 10^3 cells/µL compared to single centrifugation in the sodium citrate group 354.8 x 10^3 cells/µL, EDTA 408.1 x 10^3 cells/µL, ACD-A 390.6 x 10^3 cells/µL. This research is in line with de Pochini et al (2016), PRP obtained using a single centrifugation protocol showed the lowest platelet count concentration compared to double centrifugation. Klutu et al (2013), said that PRP obtained through a double centrifugation protocol had a higher platelet count than single centrifugation.

Centrifugation is used to separate blood components based on their density gradient. The purpose of centrifugation in the PRP preparation process is to separate platelets from other cell components. Platelets are the lightest component, followed by white blood cells, and red blood cells are the

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heaviest. In this study, single centrifugation was not good enough to separate platelets from other cell components, so the platelet count was lower compared to single centrifugation. Double centrifugation or second stage centrifugation in the PRP preparation procedure aims to separate platelets from plasma, so that the number of platelets obtained is better than single centrifugation (Shin et al., 2017).

Another factor that influences the number of platelets in PRP is the use of anticoagulants. Various anticoagulants are used in the preparation of PRP. In clinical applications and basic research, the use of anticoagulants needs to be reviewed (Hua et al., 2015). Most researchers recommend the use of sodium citrate and ACD-A in the PRP preparation process and agree not to use EDTA because these agents can damage platelet membranes (Rachita & Sukesh, 2014). However, based on the results of this study, the highest number of PRP platelets was in the EDTA group with double centrifugation, namely 829.4 x 10^3 cells/µL compared to the sodium citrate and ACD-A groups. This research is in line with Astuti et al (2018), the number of PRP platelets in the EDTA anticoagulant group was higher, namely 1193.41 x 103 cells/µL compared to citric acid anticoagulant 871.22 x 103 cells/µL. Research by doAmaral et al (2016), the use of EDTA in PRP preparation resulted in the highest platelet count compared to sodium citrate and ACD-A. This study is inversely proportional to Singh's (2018) study, the platelet count was higher in the ACD-A group, when compared with EDTA and sodium citrate. Aizawa et al (2020) said that EDTA is more efficient than ACD-A in inhibiting platelet aggregation in PRP. EDTA can simplify the preparation of well-suspended PRP and maintain PDGF-BB levels at the same level as ACD-A. EDTA should be reconsidered as a promising alternative to citrate-based anticoagulants for PRP preparation.

CONCLUSION

Different procedures in PRP preparation can produce varying platelet count results. The highest platelet count in PRP was obtained by a preparation procedure using EDTA anticoagulant with double centrifugation. This research must be carried out further to evaluate the PRP preparation procedure on the concentration of growth factors contained in PRP. The right procedure in PRP preparation will produce quality PRP.

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